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Emerging and re-emerging viral infections: epidemiology, pathogenesis and new methods for control and prevention

Edited by
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Emerging and re-emerging viral infections: epidemiology, pathogenesis and new methods for control and prevention

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Table of contents

07 Editorial: Emerging and re-emerging viral infections: epidemiology, pathogenesis and new methods for control and prevention
Jawhar Gharbi, Giovanni Rezza and Manel Ben M'hadheb

10 Assessing disparities in medical students' knowledge and attitude about monkeypox: a cross-sectional study of 27 countries across three continents
Samar Abd ElHafeez, Assem Gebreal, Mohammad Adnan Khalil, Naglaa Youssef, Malik Sallam, Abdelhamid Elshabrawy, Suzan Abdel-Rahman, Amira Saad Mahboob, Saja Yazbek, Eman H. Elbanna, Riddhi Adhyaru, Zarin Nudar Rodoshi, Yap Siew Kih, Huda Jawad, Evangelos Kolotouros, Arkadiusz Jaworski, Ghadah AlQarni, Mohammad Gablan, Alexandra Condurat, Ahmed El-Sayed Said Nour Elden, Oumayma Bennani, Kamna Rawat, Areeba Ismail, Yasser Al-Hajj, Nafisa M. K. Elehamer, Jasleen Nagi, Habtamu Admassu, Saja Hassan Al Asaad, Ruthwik Duvuru, Olaoluwaposi Ogunlana, Bandar Alosaimi and Ramy Mohamed Ghazy

24 Determinants of sexually transmitted infections among female sex workers in Ethiopia: a count regression model approach
Feyiso Bati Wariso, Jemal Ayalew, Ammar Barba, Birra Bejiga Bedassa, Gemechu Gudeta Ebo, Jaleta Bulti Tura, Mohammed Rameto, Wudinesh Belete Belihu, Derbachew Asfaw, Minilik Demissie Amogne, Lemessa Negeri, Sileshi Lulseged and Saro Abdella Abrahim

35 Coinfection and superinfection in ICU critically ill patients with severe COVID-19 pneumonia and influenza pneumonia: are the pictures different?
Ziying Chen, Qingyuan Zhan, Linna Huang and Chen Wang

48 Serum HBV RNA: a promising biomarker for blood product safety screening and enhanced diagnostic efficiency in chronic hepatitis B virus infection
Sulan Yu, Yanjuan Guo and Chunxiao Zhang

52 Epidemiological and clinical characteristics of COVID-19 reinfection during the epidemic period in Yangzhou city, Jiangsu province
Yin Wang, Jie Liang, Huimin Yang, Liguo Zhu, Jianli Hu, Lishun Xiao, Yao Huang, Yuying Dong, Cheng Wu, Jun Zhang and Xin Zhou

60 Impact of vaccination and non-pharmacological interventions on COVID-19: a review of simulation modeling studies in Asia
Karan Thakkar, Julia Regazzini Spinardi, Jingyan Yang, Moe H. Kyaw, Egemen Ozbilgili, Carlos Fernando Mendoza and Helen May Lin Oh

75 An integrated public health response to an outbreak of Murray Valley encephalitis virus infection during the 2022–2023 mosquito season in Victoria
Maxwell Braddick, Helen M. O'Brien, Chuan K. Lim, Rebecca Feldman, Cathy Bunter, Peter Neville, Christopher R. Bailie, Grace Butel-Simoes, Min-Ho Jung, Aidan Yuen, Nicole Hughes and N. Deborah Friedman

85 Phase III, randomized, double-blind, placebo-controlled clinical study: a study on the safety and clinical efficacy of AZVUDINE in moderate COVID-19 patients
Sávio Bastos de Souza, Paula Gebe Abreu Cabral, Renato Martins da Silva, Raul Ferraz Arruda, Sheila Passos de Figueiredo Cabral, Arícia Leone Evangelista Monteiro de Assis, Antônio Brazil Viana Junior, Wim Maurits Sylvain Degrave, Aline dos Santos Moreira, Cléber Glória Silva, Junbiao Chang and Pingsheng Lei

98 The epidemiology of *Aedes*-borne arboviral diseases in Zhejiang, Southeast China: a 20years population-based surveillance study
Jiangping Ren, Zhiping Chen, Feng Ling, Ying Liu, Enfu Chen, Xuguang Shi, Song Guo, Rong Zhang, Zhen Wang and Jimin Sun

110 SARS-CoV-2 excretion kinetics in nasopharyngeal and stool samples from the pediatric population
Haifa Khemiri, Mariem Gdoura, Samar Ben Halima, Henda Krichen, Cesare Cammà, Alessio Lorusso, Massimo Ancora, Adriano Di Pasquale, Asma Cherni, Henda Touzi, Amel Sadraoui, Zina Meddeb, Nahed Hogga, Radhia Ammi, Henda Triki and Sondes Haddad-Boubaker

120 External quality assessment for laboratories in pan-India ILI/SARI surveillance for simultaneous detection of influenza virus and SARS-CoV-2
Varsha Potdar, Neetu Vijay, Veena Vipat, Sheetal Jadhav, Nivedita Gupta and Neeraj Aggarwal

127 Genomic evolution of BA.5.2 and BF.7.14 derived lineages causing SARS-CoV-2 outbreak at the end of 2022 in China
Wentao Zhu, Xiaoxia Wang, Yujin Lin, Lvfen He, Rui Zhang, Chuan Wang, Xiong Zhu, Tian Tang and Li Gu

135 "Low-risk groups" deserve more attention than "high-risk groups" in imported COVID-19 cases
Wanshan Zheng, Ying Tan, Zedi Zhao, Jin Chen, Xiaomei Dong and Xiongfei Chen

143 Temporary impact on medical system and effectiveness of mitigation strategies after COVID-19 policy adjustment in China: a modeling study
Chitin Hon, Jingyi Liang, Ruihan Chen, Zhijie Lin, Yangqianxi Wang, Wei He, Ruibin Liu, Jiaxi Sun, Qianyin Li, Lixi Liang, Minyi Zhang, Zichen Chang, Yinqiu Guo, Wenting Zeng, Tie Liu and Arlindo L. Oliveira

153 Beyond SARS-CoV-2: epidemiological surveillance of respiratory viruses in Jalisco, Mexico
Isaac Murisi Pedroza-Uribe, Natali Vega Magaña, José Francisco Muñoz-Valle, Marcela Peña-Rodriguez, Ahtziri Socorro Carranza-Aranda, Rocío Sánchez-Sánchez, Alberto Anthony Venancio-Landeros, Octavio Patricio García-González, Jacob Jecsan Zavala-Mejía, Moisés Ramos-Solano, Oliver Viera-Segura and Mariel García-Chagollán

164 Enabling countries to manage outbreaks: statistical, operational, and contextual analysis of the early warning and response system (EWARS-csd) for dengue outbreaks
Mikaela Schlesinger, Franklyn Edwin Prieto Alvarado, Milena Edith Borbón Ramos, Maquins Odhiambo Sewe, Corinne Simone Merle, Axel Kroeger and Laith Hussain-Alkhateeb

176 Assessing the impact of COVID-19 on epidemiological changes of severe pediatric respiratory syncytial virus infections in Malaysia
Chee Mun Chan, Asrul Abdul Wahab and Adli Ali

185 The potential risks of equine serum therapy in transmitting new infectious diseases: lessons from a post-pandemic era
Manuela B. Pucca and Ana Lucia Camphora

189 Impact of normalized COVID-19 prevention and control measures on lower respiratory tract infection pathogenesis in hospitalized children
Yuan Feng, Huaixiao Zhang, Bo Zhang, Yinfei Zhou and Haibin Yuan

197 "Disease X" and prevention policies
Muhammad Haidar Zaman, Nawab Ali and Muhammad Ilyas

200 Case report: Neglected subacute thyroiditis: a case following COVID-19 vaccination
Shuai Yang, Ting Guan, HuanYi Yang, YiRong Hu and Yan Zhao

206 Current knowledge of human Mpox viral infection among healthcare workers in Cameroon calls for capacity-strengthening for pandemic preparedness
Alex Durand Nka, Yagai Bouba, Joseph Fokam, Aude Christelle Ka'e, Jeremiah Efakika Gabisa, Nadia Mandeng, Delors Jacques Toumansie Mfonkou, Chenwi Collins Ambe, Marie-Laure Mballa Mpouel, Tatiana Djikeussi, Boris Kevin Tchounga, Derrick Tambe Ayuk Ngwese, Debimeh Njume, Sonia Emmanuelle Mbala Nomo, Ezechiel Ngoufack Jagni Semengue, Armand Tiotsia Tsapi, Bernadette Bomgning Fokou, Ingrid Koster Simo Kamdem, Michel Carlos Tommo Tchouaket, Désiré Takou, Willy Pabo, Samuel Martin Sosso, Erick Tandi, Linda Esso, Georges Alain Etoundi Mballa, Anne-Cecile Zoung-Kanyi Bissek, Halle-Ekane Gregory Edie, Nicaise Ndembi, Vittorio Colizzi, Carlo-Federico Perno and Alexis Ndjolo

218 Diagnostic performance of rapid antigen testing for SARS-CoV-2: the COVid-19 AntiGen (COVAG) extension study
Christoph Wertenauer, Alexander Dressel, Eberhard Wieland, Hans-Jörg Wertenauer, Helmine Braitmaier, Anna Straub, Stefan Helfert, Nicolas Lützner and Winfried März

235 Algorithms for predicting COVID outcome using ready-to-use laboratorial and clinical data
Alice Aparecida Lourenço, Paulo Henrique Ribeiro Amaral, Adriana Alves Oliveira Paim, Geovane Marques-Ferreira, Leticia Gomes-de-Pontes, Camila Pacheco Silveira Martins da Mata, Flávio Guimarães da Fonseca, Juan Carlos González Pérez and Jordana Grazziela Alves Coelho-dos-Reis

249 Antimicrobial resistance in intensive care patients hospitalized with SEPSIS: a comparison between the COVID-19 pandemic and pre-pandemic era
Katia Falasca, Luigi Vetrugno, Paola Borrelli, Marta Di Nicola, Claudio Ucciferri, Alessandra Gambi, Magdalena Bazydlo, Giorgia Taraschi, Jacopo Vecchiet and Salvatore Maurizio Maggiore

259 Molecular network characteristics and drug resistance analysis of 392 newly reported MSM HIV/AIDS cases in Chongqing, China
Chongyang Bai, Tianyu Tan, Long Li, Rongrong Lu, Wei Zhang, Ling Ouyang, Guohui Wu and Chao Zhou

268 Recurrent Nipah outbreaks in Kerala: implications for health policy and preparedness
Asuma Ayesha Rahim, Priya Chandran, V. Bindu, Chandini Radhakrishnan, Anitha P. Moorkoth and Lathika Velichapat Ramakrishnan

273 Omicron variant and pulmonary involvements: a chest imaging analysis in asymptomatic and mild COVID-19
Peiben Liu, Kejun Cao, Guanqun Dai, Tingzhen Chen, Yifan Zhao, Hai Xu, Xiaoquan Xu, Quan Cao, Yiyang Zhan and Xiangrong Zuo

282 Global trends in COVID-19 incidence and case fatality rates (2019–2023): a retrospective analysis
Juan Du, Hong-mei Lang, Yan Ma, Ao-wen Chen, Yong-yi Qin, Xing-ping Zhang and Chang-quan Huang

292 Individual risk factors associated with SARS-CoV-2 infection during Alpha variant in high-income countries: a systematic review and meta-analysis
Marta Moniz, Sofia Pereira, Patricia Soares, Pedro Aguiar, Helena Donato and Andreia Leite

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Editorial: Emerging and re-emerging viral infections: epidemiology, pathogenesis and new methods for control and prevention

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Editorial on the Research Topic
Emerging and re-emerging viral infections: epidemiology, pathogenesis and new methods for control and prevention

Viruses are always transmitted directly and/or indirectly from human to human and from animal to human. In host cells, virus replication frequently results in an accumulation of mutations, reassortments, homologous, and heterologous recombinations, contributing to their rapid adaptation to environmental changes, often raising to the emergence of new virus variants or species. These viral characteristics, in addition to spillover events, have resulted recently in an increasing number of outbreaks, epidemics, and pandemics.

Emerging viruses are a very broad category that includes not only newly discovered viruses but also re-emerging variants of known viruses. In the last 20 years, an alarming number of infectious viruses have emerged or re-emerged, presenting great threats to global public health. Ebola, Marburg, and Crimean Congo hemorrhagic fevers, Lassa fever, Dengue fever, Yellow fever (YFV), West Nile fever (WNV), Rift Valley fever (RVF), Nipah and Hendra viruses, Zika virus, Poxvirus, Hepatitis E Virus (HEV), Bunyavirus and Chikungunya vector-borne viruses, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS-CoV), and the most recent coronavirus disease 2019 (COVID-19) are examples of zoonoses that have spread throughout the globe with such a significant impact on public health (1, 2). These viruses continue to cause mass disruption by creating constant threat to public health. The scientific community are always called for a rapid intervention in diagnosing, preventing and treating emerging infections (1, 3). Vaccination is probably the most effective tool in helping the immune system to activate protective responses against pathogens, reducing morbidity and mortality, as proven by historical records and the most recently pandemic situation with the emergence of SARS-CoV-2 virus. Under health emergency conditions, new and development of alternative approaches in antiviral drug and vaccine design are imperative for a rapid and massive vaccination coverage, to manage a disease outbreak and curtail epidemic spread. The emergence and re-emergence of novel pathogens challenging the public health in regards to the development of new diagnostic methods, therapeutics and prevention strategies and maintaining an efficient epidemiological surveillance.

The world remains burdened by high morbidity and mortality diseases and, as exemplified by the recent devastating pandemic of SARS-CoV-2, and new emerging or re-emerging pathogens are likely to spread in the future. In this line, this Research Topic collection, "*Emerging and re-emerging viral infections: epidemiology, pathogenesis and new methods for control and prevention*" provides for researchers a wide range of selected articles presenting the last results of the epidemiology and pathogenesis of a numerous of emerging or re-emerging virus pathogens. It offers an update on current antiviral strategies to manage and control these emerging and re-emerging viruses, including vaccines and antiviral drug discovery.

During the management of this Research Topic collection, we received a considerable number of articles with a good scientific level and sound. We selected the most interesting articles belonging to different categories of papers: One as Case report, 1 as Clinical trial, 1 as Methods, 4 as Opinion, 3 as Review, and finally 20 articles as Original research. All article categories covers the important topics of the collections (Genetic evolution and diversity, diagnosis, antiviral drugs, vaccines…). An important number of articles described the recent pandemic of COVID-19 and presented interesting results regarding the study of the pandemic, the emerged associated virus SARS-CoV-2 and the efficiency of used vaccines. The effectiveness of vaccines categories used during the pandemic of COVID-19 were studied and compared within different populations. Du et al., studied the global trends in COVID-19 incidence and case fatality rates as a retrospective study. They concluded that COVID-19 rates varied significantly by continent and income level. Europe and the Americas faced surges in infections and low case fatality rates. Whereas, Africa experienced low infection rates and higher case fatality rates, with lower-and middle-income nations exceeding case fatality rates in high-income countries over time. The impact of the COVID-19 infection and prevention on lower respiratory tract infection pathogenesis and outcomes was then analyzed by Feng et al.. Study demonstrated that the effects of COVID-19 pandemic on the epidemiological characteristics of respiratory pathogens, which will be beneficial for improving early preventive measures and that intervention encompassing pulmonary and functional rehabilitation exercises, are recommended to improve physical fitness and pulmonary function post-COVID-19.

The genetic evolution and diversity of variants detected of the emerging virus associated to the recent pandemic SARS-CoV-2 were also presented in the articles of Moniz et al., Liu et al., Falasca et al., and Zhu et al., which are involved in this Research Topic. Indeed, Moniz et al., reviewed the individual risk factors associated with SARS-CoV-2 Alpha variant infection in high-income countries indicating the multitude risks factors associated to several pathologies including diabetes, cancer, asthma and cardiovascular diseases. Whereas, Liu et al., suggested that Asymptomatic and symptomatic patients infected with Omicron variant had pulmonary involvements which were not uncommon. Potential risk factors for age stratification, and pulmonary diseases can help clinicians to identify obvious pulmonary involvements in asymptomatic and mildly symptomatic patients infected with Omicron. Falasca et al., presented results of the antimicrobial resistance study during the COVID-19 pandemic and before the pandemic. Authors demonstrated how the COVID-19 pandemic changed the prevalence of sepsis in patients in intensive care. They revealed that the risk factors associated with mortality were APACHE and SOFA scores, age, and, above all, the presence of ESBL-producing bacteria. Despite this, during the pandemic phase, they have observed a significant reduction in the emergence of resistant germs compared to the pre-pandemic phase (Falasca et al.). Finally, Zhu et al., examined the epidemiological, genomic, and evolutionary characteristics of the SARS-CoV-2 genomes from China. They analyzed nearly 20,000 genomes belonging to 17 lineages, predominantly including BF.7.14, DY.2, DY.4, and BA.5.2.48 variants. They identified 43 core mutations in the S gene and 47 core mutations in the ORF1ab gene responsible of the evolution of the virus genome. Their findings provide insights into the genomic characteristics of SARS-CoV-2 genomes in China following the relaxation of the "dynamic zero-COVID" policy and emphasize the importance of ongoing genomic monitoring (Zhu et al.).

Other articles from this Research Topic cover different topics regarding various types of emerging or re-emerging viruses such as arboviruses (Dengue, Murray Valley Encephalitis, Nipah…), Monkeypox virus, Hepatitis viruses, and Human immunodeficiency virus. The review of Braddick et al., supported the integrated public health in response to the outbreaks of mosquito-borne flavivirus, the Murray Valley encephalitis virus (MVEV) infection during mosquito seasons in Victoria, Australia. They concluded that the expanded, climate-informed vector surveillance system detected MVEV in mosquitoes in advance of human cases, acting as an effective early warning system. This informed a one-health oriented public health response including enhanced human, vector and animal surveillance, integrated mosquito management, and health promotion (Braddick et al.). The serum of Hepatits B Virus (HBV) was supposed by Yu et al., to be a promising biomarker for blood product safety screening and enhanced diagnostic efficiency in chronic HBV infection. They suggest that the incorporation of serum HBV RNA detection into clinical practice and the implementation of blood safety precautions helps to a more effective management of chronic HBV infection and moves the aim of HBV eradication closer to realization (Yu et al.). The study conducted by Schlesinger et al., regarding the countries management of Dengue virus outbreaks demonstrated that Migration and/or socioeconomic status are factors that might impact predictive performance and should be further evaluated. Overall early warning and response system (EWARS) performed very well, providing evidence that it should continue to be implemented in countries for outbreak prediction (Schlesinger et al.).

Finally, the concept of a serious global pandemic named "Disease X" was raised and discussed in this Research Topic. Indeed, "Disease X" refers to an unexpected and unknown outbreak of a contagious or infectious disease caused by a "Pathogen X," which is presently unidentified and capable of infecting humans. This pathogen X is most likely a zoonotic virus (4, 5). According to the World Health Organization (WHO), the

”Disease X“ is considered among highly contagious diseases such as Ebola, Zika, and COVID-19 (Zaman et al.). For Scientists, as pathogen X is an elusive pathogen, we are unable to prevent the occurrence of Disease X. However, by implementing preventative measures, we may be able to impede or minimize its transmission and possible health risks. In order to achieve this, a universal scientific protocol for managing and controlling “Disease X” would be required.

Author contributions

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References

1. Trovato M, Sartorius R, D’Apice L, Manco R, De Berardinis P. Viral emerging diseases: challenges in developing vaccination strategies. *Front Immunol.* (2020) 11:2130. doi: 10.3389/fimmu.2020.02130

2. Burrell CJ, Howard CR, Murphy FA. Emerging virus diseases. *Fenner White’s Med Virol.* (2017) 97:217–25. doi: 10.1016/B978-0-12-375156-0.00015-1

3. Hadj Hassine I, Ben M’hadheb M, Almalki MA, Gharbi J. Virus-like particles as powerful vaccination strategy against human viruses. *Rev Med Virol.* (2024) 34:e2498. doi: 10.1002/rmv.2498

4. Simpson S, Kaufmann MC, Glozman V, Chakrabarti A. Disease X: accelerating the development of medical countermeasures for the next pandemic. *Lancet Infect Dis.* (2020) 20:e108–e15. doi: 10.1016/S1473-3099(20)30123-7

5. Tahir MJ, Sawal I, Essar MY, Jabbar A, Ullah I, Ahmed A. Disease X: a hidden but inevitable creeping danger. *Infect Control Hospital Epidemiol.* (2022) 43:1758–9. doi: 10.1017/ice.2021.342

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Assessing disparities in medical students' knowledge and attitude about monkeypox: a cross-sectional study of 27 countries across three continents

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Background and aims: The recent monkeypox (Mpox) outbreak confirmed by the World Health Organization (WHO) underscores the importance of evaluating the knowledge and attitude of medical students toward emerging diseases, given their potential roles as healthcare professionals and sources of public information

during outbreaks. This study aimed to assess medical students' knowledge and attitude about Mpox and to identify factors affecting their level of knowledge and attitude in low-income and high-income countries.

Methods: A cross-sectional study was conducted on 11,919 medical students from 27 countries. A newly-developed validated questionnaire was used to collect data on knowledge (14 items), attitude (12 items), and baseline criteria. The relationship between a range of factors with knowledge and attitude was studied using univariate and multivariate analyses.

Results: 46% of the study participants were males; 10.7% were in their sixth year; 54.6% knew about smallpox; 84% received the coronavirus disease 2019 (COVID-19) vaccine; and 12.5% had training on Mpox. 55.3% had good knowledge of Mpox and 51.7% had a positive attitude towards it. Medical students in their third, fifth, or sixth year high- income countries who obtained information on Mpox from friends, research articles, social media and scientific websites were positive predictors for good knowledge. Conversely, being male or coming from high-income countries showed a negative relation with good knowledge about Mpox. Additionally, a positive attitude was directly influenced by residing in urban areas, being in the fifth year of medical education, having knowledge about smallpox and a history of receiving the coronavirus disease 2019 (COVID-19) vaccine. Receiving information about Mpox from social media or scientific websites and possessing good knowledge about Mpox were also predictors of a positive attitude. On the other hand, being male, employed, or receiving a training program about Mpox were inversely predicting positive attitude about Mpox.

Conclusion: There were differences in knowledge and attitude towards Mpox between medical students in low and high-income countries, emphasizing the need for incorporating epidemiology of re-emerging diseases like Mpox into the medical curriculum to improve disease prevention and control.

KEYWORDS

monkeypox virus, knowledge, attitude, medical students, validated questionnaire

1. Introduction

Monkeypox (Mpox) is a zoonotic infection caused by the monkeypox virus belonging to the genus *Orthopoxovirus* (1). The first human case of Mpox was identified in 1970 in the Democratic Republic of Congo. Since then, there have been multiple outbreaks and sporadic cases, mostly occurring in Central and West Africa (2, 3). The first cases of Mpox reported outside Africa occurred in the United States of America in 2003 (4). The number of cases has increased dramatically and the virus has spread to many other countries (5), such as the United Kingdom (UK) (6), Israel (7), and Singapore (8). In May 2022, the World Health Organization (WHO) confirmed the first Mpox outbreak outside endemic regions involving different continents worldwide (9). The WHO reported that, as of May 3, 2023, there have been more than 87,000 laboratory-confirmed cases and 130 deaths identified from 111 countries around the world (10).

Mpox is mainly transmitted via contact with respiratory secretions, infected skin lesions, or contaminated materials (11). The incubation period of Mpox is usually between 6 to 13 days, although it can last up to 21 days (12). The spectrum of Mpox disease ranges from mild to severe and can even be fatal (13). Mpox is characterized by a febrile prodrome lasting 1–4 days, accompanied by symptoms such as headache and fatigue. This is followed by the centrifugal development of deep, well-circumscribed maculopapular, vesicular, pustular, and finally crusted scab lesions. The lesions last approximately 1–3 days at each stage and progress simultaneously (14, 15). Unlike smallpox, lymphadenopathy may develop before or during the appearance of the Mpox rash (16). Mpox can cause several complications, including vomiting and diarrhea, conjunctivitis, corneal scarring, sepsis, encephalitis, bronchopneumonia, and permanent pitted scarring secondary to bacterial superinfection (17). Standard hygienic practices, vaccination against smallpox, and the use of an antiviral agent known as Tecovirimat are effective measures for the management and control of Mpox (2).

The increasing incidence of Mpox highlights the importance of its prevention, early detection, and rapid response. WHO has stated that one of the challenges in preventing the re-emergence of Mpox is a lack of knowledge of the disease, particularly among healthcare professionals (HCPs) (18). This may hinder control programs such as vaccination, especially in highly impacted countries (19, 20). Enhancing the ability of HCPs to identify cases and improve patient management is therefore an essential feature of surveillance systems for Mpox (21). Specially trained medical doctors ought to be familiar with the epidemiology of Mpox to promptly detect, report and treat new cases and prevent its spread. However, prior studies have indicated that HCPs and general practitioners have little knowledge of Mpox (22–32).

As future HCPs, assessing the knowledge and attitude of medical students towards emerging diseases like Mpox is crucial, as they could influence the general population's perception about a range of diseases and improve public awareness about their preventive measures. Few studies have been conducted on students in health schools to assess their knowledge and attitude toward Mpox (33–38). None of these studies have compared the knowledge and attitude of medical students between high- and low-income countries. Moreover, only one study has validated a questionnaire to assess knowledge among a small sample size of 37 participants (35). Therefore, the aim of this study is to develop and validate a questionnaire to assess the knowledge and attitude of medical students towards Mpox, including a comparison of low-income and high-income countries.

2. Materials and methods

The Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) was followed for conducting and disseminating our study (39).

2.1. Study design and settings

A multinational cross-sectional study was conducted. The co-author (AG) was responsible for recruiting collaborators from a proportionate number of countries representing the four regions of the world via the Global Researcher Club—an international, voluntary, and non-profit scientific research community. Collaborators from 75 countries expressed their willingness to participate in the study, but ultimately collaborators from 43 countries were enrolled. The final list included 27 countries that were able to collect data from the required sample size. These countries were then categorized according to their Gross National Income (GNI) into low-income, lower-middle-income, high-middle income and high-income countries (40).

2.2. Study phases

This study was conducted in two phases:

2.2.1. Phase 1: development and validation of the questionnaire to assess the knowledge and attitude of medical students towards Mpox

A group of the research team who are experienced in questionnaire development and validation held four meetings to develop the questionnaire. This phase included the following steps:

2.2.1.1. Identification of constructs and items

The existing literature and previously published questionnaires related to Mpox were reviewed and an item pool was developed, to be included under the knowledge and attitude constructs (33–38).

2.2.1.2. Development of the items to be included under each construct

The initial questionnaire was developed in English; it consisted of 42 items, divided into 30 items for the knowledge scale and 12 items for the attitude scale.

2.2.1.3. Expert evaluation

An expert panel, consisting of five investigators (one methodologist, one healthcare professional, one tropical medicine professional, and two language professionals) assessed the questionnaire for clarity and determined whether the identified items covered the defined constructs to ensure face and content validity. After three meetings, the panel agreed to remove eight items from the knowledge scale as they overlapped with other items. The second version of the questionnaire, consisting of 34 items, was used to assess its psychometric properties. Subsequently, all 43 collaborators from the enrolled countries were invited to review the pre-final copy of the questionnaire and provide feedback.

2.2.1.4. Pilot testing and cognitive interviews

A pilot test of the pre-final questionnaire was carried out. Trained members of the research team from 10 randomly selected countries (Egypt, Algeria, Ethiopia, Qatar, the United States of America, China, Pakistan, Greece, the UK, and Romania) conducted cognitive interviews with 50 intended respondents (five from each country) to evaluate their understanding of the items, readability, syntax, wording, cultural appropriateness and clarity.

2.2.1.5. Testing the questionnaire's psychometric properties

A sample of 500 medical students was identified to test the reliability and validity of the pre-final version of the questionnaire. The recruitment took place between August 1st and August 15th, 2022. Participants were enrolled from Egypt, Morocco, the United Arab Emirates, Yemen, Georgia, Greece, Nigeria, Tanzania, Malaysia, and Pakistan.

2.2.1.6. Final questionnaire and score interpretation

The final version of the questionnaire was in English and divided into three sections. The first section focused on collecting socio-demographic data such as age, sex, country, residence, educational year, and work status. It also included questions about the participant's knowledge of smallpox, their history of coronavirus disease 2019 (COVID-19) vaccination, their history of chickenpox disease, their experience of receiving training programs related to Mpox, and their sources of information about Mpox. The second section consisted of 14 items with a choice of three answers ("yes", "no", or "uncertain") to evaluate the knowledge of medical students regarding Mpox. This section covered different aspects of Mpox such as the pathogen, mode of transmission, clinical picture, and preventive measures. Finally, the third section assessed the attitude of medical students towards Mpox on 12 items, each having five response options based on a Likert scale ranging from "strongly disagree" to "strongly agree".

The knowledge items in section two were scored as follows: zero for "no", one for "uncertain", and two for "yes". The maximum score for the knowledge section was 28, with a higher score indicating better knowledge. The attitude questions in section three were scored using a five-point Likert scale as follows: one point for "strongly disagree", two points for "disagree", three points for "neutral", four points for "agree", and five points for "strongly agree". The maximum score on the attitude section was 60, a high score indicating a more positive attitude. Negatively worded questions were reverse-scored to ensure consistency with positively worded statements.

2.2.2. Phase 2: assessment of the knowledge and attitude of medical students about Mpox

2.2.2.1. The sample size for phase 2

There was a wide variation in the knowledge and attitude levels of medical students about Mpox based on the previous literature (33–38). Using EPI-Info version 7.2 software, we assumed that 50% of the medical students had good knowledge or a positive attitude about Mpox, with a 5% accepted degree of precision and a power of 80%. Based on these assumptions, the minimum required sample size was 384 participants from each country.

2.2.2.2. Sampling technique and data collection for phase-2

The final version of the questionnaire was uploaded on Google Forms and distributed via QR code flyers or online through various social media platforms (including Facebook, WhatsApp, emails, Telegram, and Twitter) to medical students in the selected countries from September 1 to December 15, 2022. Collaborators were asked to collect the data using the same techniques to minimize information bias. Each collaborator was responsible for sharing the questionnaire with medical student groups in his or her country. A convenience snowball sampling method was used to reach the required sample size of medical students from each country by asking participants to assist the collaborators in identifying further potential research participants and distributing the questionnaire accordingly. Students were eligible for inclusion if they were enrolled in public medical schools before their internship year; those from other paramedical schools were excluded from the study.

2.3. Statistical analysis

2.3.1. Psychometric evaluation of the questionnaire

2.3.1.1. Construct validity

Construct validity represents the 'extent to which an instrument assesses a construct of concern and is associated with evidence that measures other constructs in that domain and measures specific real-world criteria' (41). Construct validity was determined using structural, factorial, and criterion-related validity (42).

Exploratory factor analysis (EFA) was used to determine the factor structure of the questionnaire and to identify the underlying factors/constructs of our set of 34 items (43).

Before performing the EFA, factorability was assessed using both the Kaiser–Meyer–Olkin index (KMO) test and Bartlett's test of sphericity. The KMO statistics ranged from 0 to 1, with values closer to 1 denoting greater adequacy of factor analysis. Bartlett's test of sphericity determines whether the variables are correlated in an identity matrix; a significant value of p associated with this test (e.g., < 0.05) indicates that factorial analysis can be used (44).

To perform the EFA, principal component analysis with varimax rotation was used (45). The number of factors to be retained was determined by the eigenvalue (>1) criteria, parallel analysis, and a scree plot (46, 47). The identification of a group of questionnaire items belonging to a "factor" was achieved through a process of "factor loading". Question items with factor loadings (cut-off value of 0.40) were associated with a distinct factor. All items with communalities less than 0.5 were deleted from the final version of the questionnaire (43).

2.3.1.2. Criterion-related validity

Convergent validity was assessed by analyzing the item-to-total scores of the scale correlation. Discriminant validity was assessed by calculating the heterotrait-monotrait ratio of correlations (HTMT). If the HTMT value was below 0.90, discriminant validity was established (48).

2.3.1.3. Reliability analysis

Cronbach's α was calculated for the questionnaire and the scales to assess internal consistency. As a rule of thumb, Cronbach's α of 0.70 to 0.80 is considered respectable for a scale for research use and an alpha of more than 0.80 is considered very good (49).

2.3.2. Data management

Quantitative variables were summarized as mean ± standard deviation (SD) for normally distributed data or median [interquartile range (IQR)] for non-normally distributed data. The data distribution was checked using visual identification of a normal distribution by QQ plot. Qualitative variables were presented as percentages and frequencies.

Knowledge and attitude scores were categorized according to the median values. Participants who scored above the median value of 20 were considered to have good knowledge, while those who scored below or equal to the median were considered to have poor knowledge. Similarly, participants who scored above the median value of 47 were considered to have a positive attitude, while those who scored below or equal to the median were considered to have a negative attitude.

Independent *t*-tests were used to compare normally distributed data and the chi-square test to determine the categorical variables between knowledge and attitude categories. For correlation analysis, Spearman's rho test was used. To identify predictors of good knowledge and positive attitude, multilevel logistic regression models were used due to the hierarchical structure of the data (medical students nested within different countries). Two separate models were produced—one for knowledge and one for attitude—with random intercept and slope. Explanatory variables were categorized as country-level (higher level) and medical students level (lower level). Country-level variables included country income classification. Medical student variables included age, sex, education level, place of residence, work status, history of chickenpox, history of COVID-19 vaccine knowledge about smallpox, training programs on Mpox, and sources of information regarding Mpox. Additionally, medical students' knowledge of Mpox was included as an explanatory variable in the attitude model. The maximum likelihood with the Laplace approximation method was used to estimate the effect of different explanatory variables on the probability of good knowledge and positive attitude. Model fit was assessed using log-likelihood and intraclass correlation (ICC) was computed to measure clustering within groups. The likelihood ratio test indicated a significant difference after adding random effects to the intercept models. We also calculated I^2 "within-cluster" which indicates how much of the total variance is due to within-cluster heterogeneity. A multilevel logistic regression model was more appropriate for estimating the clustered observations in each country. After accepting the

assumption of heterogeneity of odds across the countries, the model was conducted with the random intercept and added the explanatory variables. The likelihood ratio test was used to assess the significance of the random slope for each variable. The random slope of knowledge of smallpox, the history of chickenpox disease, and the history of COVID-19 vaccine intake were then added to the models. Adding the random slopes for these variables improved the models, as their effects differed significantly across countries. Odds ratios (OR) and 95% confidence intervals (CI) were used to present data, and statistical analyses were performed using STATA version 13 and R packages (lme4) (50). All variables with $p < 0.05$ were considered significant predictors.

2.4. Ethical considerations

The Ethics Committee of the Faculty of Medicine, Alexandria University, Egypt (IRB No. 00012098) approved the study, following the International Ethical Guidelines for Epidemiological Studies.

3. Results

3.1. Phase 1: psychometric evaluation of the developed questionnaire to assess the knowledge and attitude about Mpox among medical students.

The mean age of the 500 medical students who participated in this phase was 21.6 ± 2.1 years, 39.4% were males and 27.8% were in their third year of medical school.

3.1.1. Construct validity

Exploratory factor analysis: the KMO measure of sampling adequacy was 0.83, which was above the recommended value of 0.60, and Bartlett's test of sphericity was highly significant ($p < 0.001$). To determine the number of factors to be retained from the EFA, parallel analysis and a scree plot (Supplementary Figure S1) were performed. The scree plot indicated that five factors should be retained. An EFA was conducted using the five-factor model, which included three subscales assessing knowledge and two subscales assessing attitude. Principal component analysis with varimax rotation was used to calculate the factor loadings of the 34 items in the questionnaire. Eight items with communalities less than 0.5 or low factor loadings were deleted, resulting in 26 items being included in the final EFA model with factor loadings greater than or equal to 0.4 (Supplementary Table S1).

Criterion-related validity: all questionnaire items were found to have a significant correlation with the total score on each scale ($p < 0.001$) indicating good convergent validity (Supplementary Table S2). Furthermore, the HTMT correlation coefficient between the five subscales was 0.32, indicating adequate discriminant validity between the subscales.

3.1.2. Reliability analysis

The internal consistency of both the knowledge and attitude scales was found to be satisfactory, with an overall Cronbach's α of 0.79. The Cronbach's α value for the knowledge scale was 0.74, while for the attitude scale, it was 0.79 (Supplementary Table S2).

3.2. Phase 2: assessment of knowledge and attitude among the medical students about Mpox

3.2.1. Participants' characteristics

Figure 1 displays the 27 countries that were included in the final analysis, with a total of 11,919 medical students. The participants had an average age of 21.7 ± 2.2 years, and 45.6% were males. Among the respondents, 21.8% were in their fourth year of medical school, 18.9% were in their third year, and 10.7% were in their sixth year. The majority (84.0%) lived in urban areas and 18% had part-time jobs. More than half of the participants (54.6%) had knowledge of smallpox, while 84.0% received COVID-19 vaccine. Among the participants, 40.9% gave a history of chickenpox infection, while 41.8% were uncertain. Only 12.5% received training programs on Mpox; the main sources of information on Mpox were social media (73.7%), scientific websites (50.6%), and friends (43.5%) (Table 1).

3.2.2. Knowledge about monkeypox

The total knowledge score ranged from 12.7 in Bangladesh to 23.1 in South Africa (Supplementary Figure S2A). The median (IQR) total knowledge score was 20 (16–23). Medical students demonstrated good knowledge on statements where "yes" was the positive answer, such as Mpox being a viral disease (84.7%), the importance of reporting Mpox symptoms to local health authorities to prevent further transmission (75.4%), and skin rashes being a clinical manifestation of Mpox (72.4%). However, 40.8% were uncertain about the availability of a licensed Mpox vaccine at the time of the study and 32.7% were unsure about Mpox outbreaks in 2022 being related to homosexuality (Table 2).

Overall, 55.3% of medical students had good knowledge. The lowest percentage of good knowledge was found among medical students from upper-middle-income countries (47.7%), followed by high-income countries (51.8%), while medical students from low-income countries had the highest proportion of good knowledge (53%) (Figure 2A).

3.2.3. Attitude towards monkeypox

The total attitude score ranged from 40.2 in Georgia to 52.1 in Algeria (Supplementary Figure S2). The median (IQR) of the total attitude score was 47 (42–50). About two-thirds (64.3%) showed strong agreement with, "I should learn more about Mpox"; 59.3% strongly agreed that "Mpox disease prevention and control measures should be adequately available"; and 57.6% strongly agreed that, "Healthcare workers should be tested when they are in contact with someone infected". Conversely, almost one-fourth strongly disagreed that, "I can visit any family members or friends who are infected with Mpox", and "I do not trust the information about diseases from scientific experts", while 21.3 strongly disagreed that, "I worry that Mpox disease is an attempt to reduce the size of the global population" (Table 3).

Overall, a positive attitude was observed in 51.7% of the study participants. The lowest percentage of positive attitude was among medical students from high-income countries (29.4%), followed by upper-middle-income countries (38.9%). The highest percentage was among medical students from low-income countries (59.5%) (Figure 2B).

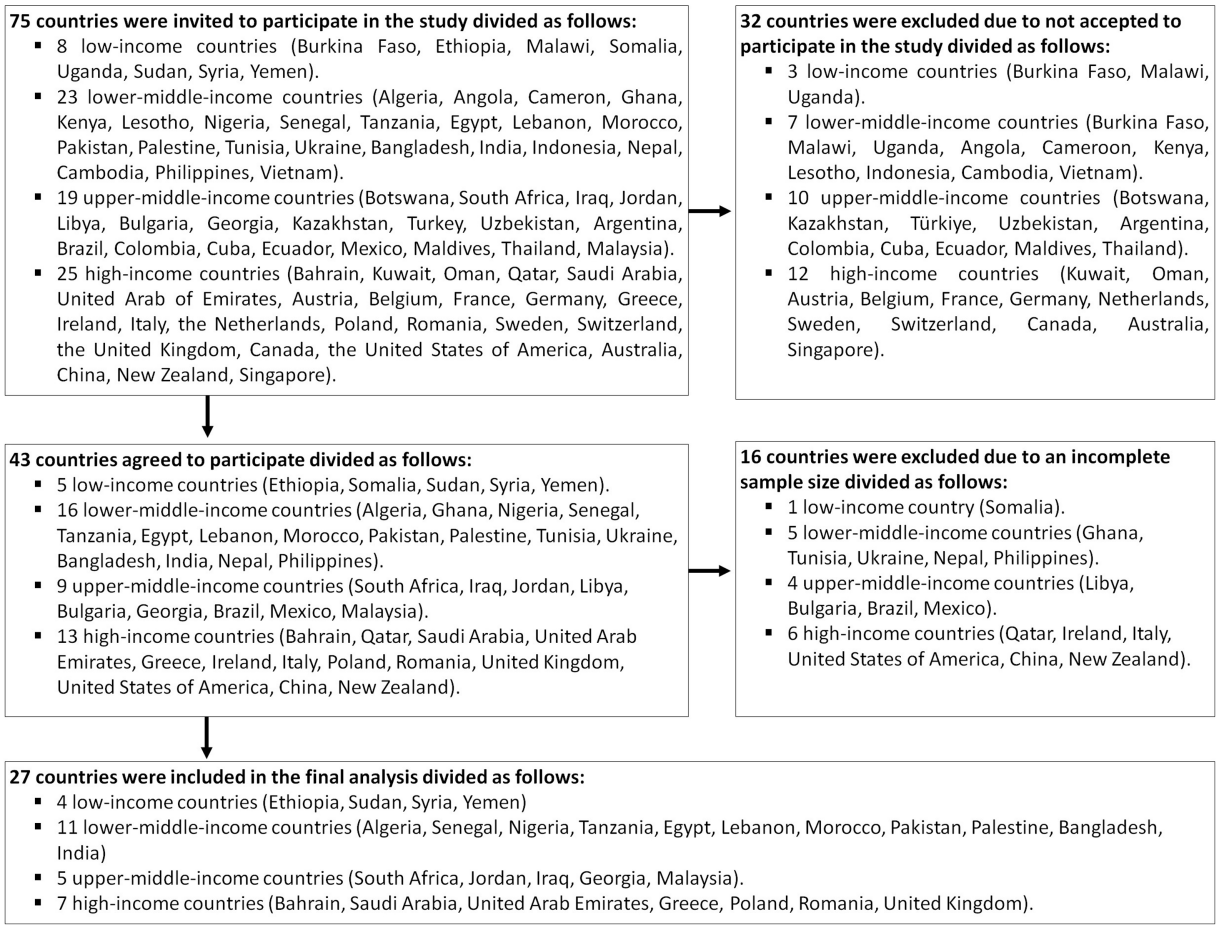

FIGURE 1
Flowchart of the included countries to assess the knowledge and attitude of medical students about human monkeypox.

3.2.4. Association between baseline criteria and knowledge and attitude about Mpox

The mean age of participants who had good knowledge or positive attitude (21.8 years) was significantly higher than those who had either poor knowledge or negative attitude (21.6 years). Fourth-year medical students had the highest proportion of good knowledge (22.2%) and positive attitude (23.2%). Students residing in urban areas had higher scores of good knowledge and positive attitude than others (85.1% vs. 14.9%, $p = 0.003$) and (87.6% vs. 12.4%, $p < 0.001$). In addition, students who were not working had significantly greater good knowledge and positive attitude compared to their peers (79.4% and 81.1%, respectively). Medical students who had knowledge about smallpox had higher positive attitude about Mpox compared to those who did not (57.8% vs. 42.2%, $p < 0.001$). COVID-19 vaccination was associated with a better attitude about Mpox (85.3% vs. 14.7%, $p < 0.001$). History of chickenpox infection was significantly associated with good knowledge and positive attitude about Mpox. Similar findings were observed among respondents from low-middle-income countries; they had both the good knowledge and positive attitude. Social media as a source of information was significantly associated with the highest proportion of good knowledge and positive attitude; 82.8 and 79.1% had good knowledge and positive attitude, respectively (Table 4).

3.2.5. Predictors of knowledge and attitude about Mpox

The study findings suggest that ICC values were 0.18 for the knowledge model with only a random intercept and 0.20 for the knowledge model with both a random intercept and random slope. I^2 "within-cluster" which indicates how much of the total variance that is due to within-cluster heterogeneity. After considering the sampling variability, I^2 equals 75.6% for the knowledge model. Medical students in their third year were 28% more likely to report good knowledge (OR: 1.28; 95%CI: 1.10–1.50) than those in their first year. Similarly, those in their fifth year were 45% more likely to report good knowledge (OR: 1.45; 95%CI: 1.20–1.76) and those in their sixth year were 44% (OR: 1.44; 95%CI: 1.14–1.83) more likely to report good knowledge, compared to their first-year counterparts. Furthermore, medical students who received information about Mpox from friends (OR: 1.23, 95%CI: 1.12–1.34), social media (OR: 1.67, 95%CI: 1.51–1.84), research articles (OR: 1.40; 95%CI: 1.27–1.54), and scientific websites (OR: 1.33; 95%CI: 1.21–1.46) were more likely to demonstrate good knowledge compared to those who did not receive information. However, male students had a 16% (OR: 0.84; 95%CI: 0.77–0.91) lower probability of having good knowledge about Mpox than female medical students, and those from high-income countries

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TABLE 1 Baseline characteristics of the study medical students (*N*=11,919).

Characteristics	*n*	%
Age		
(Mean ± standard deviation)	21.7 ± 2.2	
Sex		
Male	5,432	45.6
Female	6,487	54.4
Country (%)		
Algeria	405	3.4
Bangladesh	388	3.3
Bahrain	461	3.9
Egypt	385	3.2
Ethiopia	399	3.3
Georgia	386	3.2
Greece	440	3.7
India	458	3.8
Iraq	426	3.6
Jordan	564	4.7
Lebanon	420	3.5
Malaysia	483	4.1
Morocco	573	4.8
Nigeria	402	3.4
Pakistan	745	6.3
Palestine	385	3.2
Poland	390	3.3
Romania	388	3.3
Saudi Arabia	385	3.2
Senegal	391	3.3
South Africa	385	3.2
Syria	439	3.7
Sudan	408	3.4
Tanzania	385	3.2
United Arab Emirates	480	4.0
United Kingdom	477	4.0
Yemen	471	4.0
Educational year		
First year	1719	14.4
Second year	2080	17.5
Third year	2248	18.9
Fourth year	2602	21.8
Fifth year	1996	16.7
Sixth year	1274	10.7
Place of residence		
Urban/city	10011	84.0
Rural	1908	16.0
Employment status		
Not-working	9178	77.0
Employed (part-time job)	2148	18.0
Employed (full time job)	593	5.0
Knowledge of smallpox		
Yes	6512	54.6
No	5407	45.4
Vaccinated against COVID-19		
Yes	10011	84.0
No	1908	16.0
History of chickenpox disease		
Yes	4880	40.9
No	4977	41.8
Uncertain	2062	17.3
Receiving training programs about monkeypox		
Yes	10428	12.5
No	1491	87.5
Source of information about monkeypox[a]		
Family members		
Yes	4500	37.8
No	7419	62.6
Friends		
Yes	5183	43.5
No	6736	56.5
Social media		
Yes	8788	73.7
No	3131	62.7
Research articles		
Yes	4451	37.3
No	7468	62.7
Scientific websites		
Yes	6033	50.6
No	5886	49.4

[a]Mutually non-exclusive.

were 51% (OR: 0.49; 95%CI: 0.24–0.99) less likely to have good knowledge compared to students from low-income countries (Table 5).

Regarding the attitude of medical students towards Mpox, the ICC for the model with random intercept is 0.11 and 0.17 in the case of the model including both a random intercept and random slope. The I^2 equals 81.3% for the attitude model. Medical students residing in urban/city areas were 35% (OR: 1.35; 95%CI: 1.20–1.53) more likely to have a positive attitude towards Mpox compared to those who lived in rural areas, and those in the fifth year of medical education had a 23% (OR: 1.23; 95%CI: 1.01–1.49) higher

TABLE 2 Knowledge of medical students about monkeypox (*N*=11,919).

Components of knowledge scale	Yes	Uncertain	No
1. Human monkeypox is a viral disease	84.7	10.2	5.1
2. Monkeypox is a re-emerging disease	54.4	28.3	17.4
3. Monkeypox is easily transmitted from animal-to-human through direct contact	53.9	25.8	20.3
4. Blood-borne transmission of monkeypox is possible	45.3	35.3	19.4
5. Monkeypox can be transmitted through eating food	32.7	31.1	36.2
6. Monkeypox outbreaks in 2022 were noted to be related to homosexuality	41.2	32.7	26.2
7. Skin rashes are one of clinical manifestations of monkeypox disease	72.4	13.7	14.0
8. Avoiding contact with wild animals (alive or dead) is essential to prevent further monkeypox transmission	60.7	23	16.4
9. Monkeypox could be prevented by cooking meat properly	43.0	31.9	25.1
10. Avoiding contact with any objects that have been in contact with sick animal can prevent spread of disease	62.9	22.1	15.0
11. Avoiding contact with any person that has a rash can prevent the spread of disease	67.1	19.8	13.1
12. Avoiding contact with any object that has been in contact with sick person can prevent spread of disease	66.8	20.4	12.8
13. Reporting symptoms of monkeypox to local health authorities is important to prevent further disease transmission	75.4	13.5	11.1
14. There was a licensed monkeypox vaccine available at the time of this study	35.1	40.8	24.1
Knowledge score, median (IQR)	20 (16–23)		

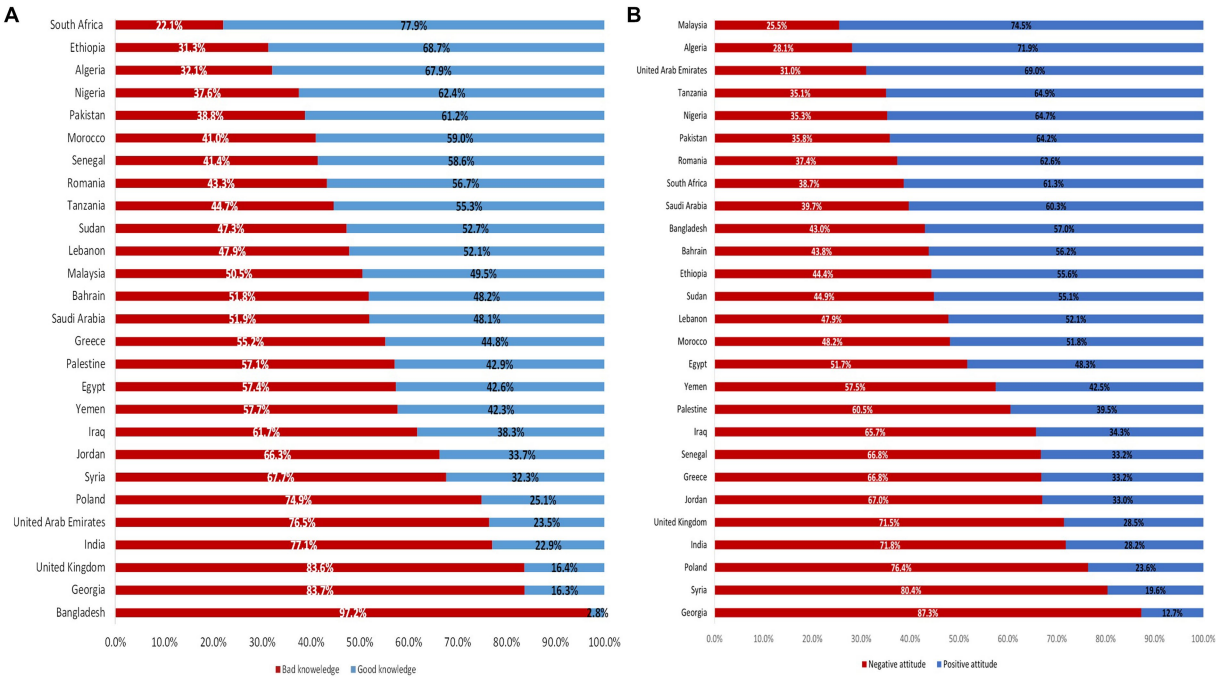

FIGURE 2
Differences between worldwide regions (low-income countries, lower-middle-income countries, upper-middle-income countries, high-income countries) in the knowledge (Figure 2A) and attitude (Figure 2B) of medical students about human monkeypox.

probability of exhibiting a positive attitude than those in the first year. Moreover, study participants who had knowledge about smallpox were 27% (OR: 1.27; 95% CI: 1.01–1.60) more likely to show a positive attitude towards Mpox. Similarly, medical students who had received the COVID-19 vaccine had a 53% (OR: 1.53; 95%CI: 1.19–1.96) higher probability of having a positive attitude towards Mpox compared to those who did not receive the vaccine. Additionally, those who received information about Mpox from social media (OR: 1.29; 95% CI: 1.17–1.42) or scientific websites (OR: 1.21; 95% CI: 1.09–1.33) were more likely to exhibit a positive attitude towards Mpox compared to those who did not receive such information. Medical students with a good knowledge of Mpox were almost three times (OR: 2.96; 95%CI: 2.71–3.23) more likely to exhibit a positive attitude compared to those with poor knowledge. Conversely, male medical students were 28% (OR: 0.72; 95% CI: 0.66–0.78) less likely to exhibit a positive attitude towards

TABLE 3 Attitude of medical students about monkeypox (*N*=11,919).

Components of attitude scale	Strongly agree	Agree	Neutral	Disagree	Strongly disagree
1. I should learn more about monkeypox disease	64.3	27.5	6.4	1.3	0.4
2. I worry that monkeypox disease can be transmitted to my country	39.0	32.5	21.0	5.9	1.6
3. Monkeypox disease prevention and control measures should be adequately available	59.3	29.2	10.1	1.2	0.2
4. Traveling to monkeypox disease-infected countries should be restricted	38.1	28.0	22.3	9.4	2.3
5. I should take monkeypox vaccine if it is available	47.0	29.0	18.4	4.4	1.2
6. Health care workers should be tested when they are in contact with someone infected	57.6	28.9	11.0	2.1	0.4
7. I can visit any family members or friends who are infected with monkeypox	14.5	14.1	20.2	27.7	23.5
8. I should take more hygienic preventive measures due to monkeypox disease	49.7	32.8	14.1	2.9	0.6
9. All people with a skin rash should be tested for monkeypox	27.4	27.3	25.8	14.2	5.4
10. I worry that monkeypox will become a new pandemic, and its impact will be like COVID-19	33.1	27.9	22.3	13.1	3.6
11. I do not trust the information about diseases from scientific experts	11.2	14.4	17.7	33.0	23.7
12. I worry that monkeypox disease is an attempt to reduce the size of global population	17.5	16.8	21.8	22.7	21.3
Attitude score median (IQR)	47 (43–51)				

Mpox compared to females. Furthermore, medical students who were part-time employees (OR: 0.86; 95% CI: 0.77–0.98) or full-time employees (OR: 0.66; 95% CI: 0.54–0.82) were less likely to report a positive attitude towards Mpox. Finally, medical students who received training on Mpox were also 26% (OR: 0.74; 95%CI: 0.64–0.85) less likely to exhibit a positive attitude towards Mpox (Table 5).

4. Discussion

This study evaluated the knowledge and attitude of 11,919 medical students from 27 low-income and high-income countries towards Mpox. In addition, the study identified factors affecting their level of knowledge and attitude about Mpox. Medical students in their third, fifth, or sixth years who accessed Mpox information from social media and scientific websites demonstrated a higher likelihood of possessing good knowledge. Conversely, being male or originating from high-income countries were linked to lower levels of knowledge regarding Mpox. Additionally, a positive attitude was directly influenced by factors such as residing in urban areas, being in the fifth year of medical education, having knowledge of smallpox, and receiving the COVID-19 vaccine. Receiving Mpox information from social media or scientific websites, along with having a solid understanding of Mpox, were also predictive of a positive attitude. On the other hand, being male, employed, or participating in a Mpox training program were inversely associated with a positive attitude towards Mpox.

The study's findings in relation to the level of knowledge about Mpox among participants are consistent with the limited number of similar studies conducted globally. Previous findings reported disparities in knowledge about Mpox among medical students that varied from 6.3% in Pakistan (37), 22.8% in the United Arab Emirates (UAE) (35), 26% in Jordan (33), 28% in Saudi Arabia (34), and above 40% among pre-clinical and clinical dental students in Malaysia (38). This highlights the significance of this study in adding to the existing literature on Mpox knowledge among medical students and the need for more comprehensive and targeted educational efforts to improve disease prevention and control.

The diversity of the study's results regarding the knowledge level of medical students about Mpox was analyzed. Participants from high-income countries had a low level of good knowledge about Mpox compared to low-income countries. One probable explanation for such gaps in the understanding of this growing disease is the lack of coverage of emerging viral infections, including Mpox in the country's school health curriculum (33). Education and understanding of diseases found in those countries are sometimes relatively poor because the perception of the danger of infectious diseases' importation and endemicity is low (51). Another explanation could be that the health of the population in low-income countries is threatened by the double burden of lifestyle-associated diseases and new and existing infectious diseases. Students in low-income countries may also have limited access to advanced medical resources, which forces them to rely on their knowledge and skills to manage different health problems, leading to a better understanding of the disease. This is the reason why there are continuous updates to medical education to train future healthcare workers in dealing with new health challenges (52).

For that, medical schools and health organizations need to consider the observed difference in knowledge levels about Mpox among medical students from different regions. They should work on developing targeted educational programs to improve knowledge and awareness of the disease. This could include incorporating Mpox into the medical curriculum, organizing training sessions, and promoting research on the disease in high-income countries. These strategies could enhance disease prevention and control globally, and lead to better preparedness in the face of future outbreaks.

A higher level of medical education, receiving information about Mpox from social media followed by research articles, scientific websites, and friends were found to impact the level of knowledge. These results find support in previous studies. For example, in the UAE and Pakistan, receiving information about Mpox was a strong determinant of good knowledge (35, 37). The re-emergence of Mpox globally has emphasized the need for different media to prioritize risk

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TABLE 4 Association between students' characteristics and their knowledge and attitude about monkeypox based on univariate analysis.

Baseline criteria	Knowledge			Attitude		
	Poor	Good	*p*-value	Negative	Positive	*p*-value
Age (mean ± standard deviation)	21.6 (2.2)	21.8 (2.2)	0.002	21.6 (2.2)	21.8 (2.2)	0.001
Sex (%)			<0.001			<0.001
Male	47.4	43.4		50.4	40.5	
Female	52.6	56.6		49.6	59.5	
Educational year (%)						
First year	15.4	13.2	<0.001	14.9	13.9	<0.001
Second year	18.8	15.7		19.7	15.0	
Third year	18.7	19.0		19.0	18.7	
Fourth year	21.6	22.2		20.5	23.2	
Fifth year	15.4	18.4		15.5	18.1	
Sixth year	10.0	11.5		10.4	11.0	
Place of residence (%)						
Urban/city	83.1	85.1	0.003	80.6	87.6	<0.001
Rural	16.9	14.9		19.4	12.4	
Employment status (%)						
Part-time job	19.2	16.6	<0.001	20.6	15.2	<0.001
Full-time job	5.8	4.0		6.2	3.6	
Not working	75.0	79.4		73.1	81.1	
Knowledge of smallpox (%)						<0.001
Yes	55.2	53.9	0.16	51.7	57.8	
No	44.8	46.1		48.3	42.2	
History of COVID-19 vaccine intake (%)						<0.001
Yes	83.8	84.2	0.62	82.8	85.3	
No	16.2	15.8		17.2	14.7	
History of chickenpox disease (%)						
Uncertain	19.0	15.2	<0.001	20.1	14.3	<0.001
Yes	39.3	43.0		39.1	42.9	
No	41.7	41.8		40.8	42.8	
Receiving training programs about monkeypox (%)						<0.001
Yes	11.8	13.3	0.02	14.8	10.0	
No	882.2	86.7		85.2	90.0	
Country classification (%)						
Low-income countries	13.4	15.6	<0.001	16.0	12.7	<0.001
Low-middle income countries	38.1	45.6		37.7	45.3	
High-middle income countries	19.5	17.9		20.6	17.0	
High-income countries	28.9	20.9		25.7	25.0	
Source of information (%)						
Family members						0.32
Yes	38.1	37.3	0.39	37.3	38.2	
No	61.9	62.7		62.7	61.8	
Friends						<0.001
Yes	41.0	46.6	<0.001	40.2	47.0	
No	59.0	53.4		59.8	53.0	

(Continued)

TABLE 4 (Continued)

Baseline criteria	Knowledge			Attitude		
	Poor	Good	*p*-value	Negative	Positive	*p*-value
Social media						<0.001
Yes	66.4	82.8	<0.001	68.7	79.1	
No	33.6	17.2		31.3	20.9	
Research articles						<0.001
Yes	33.1	42.7	<0.001	35.8	39.0	
No	66.9	57.3		64.2	61.0	
Scientific websites						<0.001
Yes	45.2	57.3	<0.001	47.0	54.5	
No	54.8	42.7		53.0	45.5	

communication for zoonotic diseases using non-stop daily updates. This is expected to improve the public's knowledge and awareness regarding Mpox (35). In contrast, male students showed low awareness about Mpox compared to female students. Findings from previous studies have also shown better knowledge among females (33, 35). Medical schools and health officials need to address the multifactorial nature of the drivers that explain the gender effect on knowledge level by tailoring the provided information. It is also worth noting that receiving information about Mpox from social media, research articles, scientific websites, and friends has an effect on the level of knowledge and highlights the importance of diverse sources of information in promoting awareness and understanding of emerging diseases. This suggests that health officials and educators should consider using multiple channels of communication to ensure that information about emerging diseases reaches as many people as possible, including medical students and healthcare professionals.

There is a dearth of studies that investigate the attitude of medical students toward Mpox. In Saudi Arabia, about 45% of medical students agreed that Mpox could transmit to their country (34), while the majority (~90%) of pre-clinical and medical students in Malaysia had a positive attitude toward Mpox (38).

A positive attitude towards Mpox accompanied a high level of knowledge and was also predicted by achieving a high academic level, living in an urban/city environment, having knowledge about smallpox, having received the COVID-19 vaccine, and receiving information from social media or scientific websites. Conversely, male students who were employed or received training about Mpox had a negative attitude. It was speculated that this difference may be observed if assessed at the country level, as the difference might be due to women's rights in each country and the extent to which they are allowed to be involved in education (53). Receiving training boosts medical students' confidence and increases trust in their ability to combat the epidemic using available prevention and control measures. They are less likely to panic about new emerging diseases (54, 55). This important finding implies that educational programs and interventions aimed at improving knowledge and attitude towards Mpox need to take into account these determinants and tailor their approach accordingly. For example, such programs could use social media or scientific websites to disseminate information about Mpox to medical students. Additionally, the programs could target male students who are employed or have received training about Mpox with specific interventions to improve their attitude towards the disease.

To conclude, further research should prioritize continuous education and awareness-raising programs, along with developing strategies to address the factors that affect knowledge and attitude regarding the Mpox pandemic. This can be achieved by listening to physicians' concerns and integrating public and health perspectives into policy and program development. It is crucial to note that a lack of knowledge about the disease can negatively impact vaccination acceptability and adherence to public health intervention strategies. Therefore, it is necessary to prioritize the education of the public and healthcare professionals to promote successful disease prevention and control.

4.1. Strengths and limitations

The strengths of this study were the use of a validated questionnaire to assess the knowledge and attitudes of the study participants to ensure the internal validity of the study findings. Also, we included a large sample of medical students from 27 countries across three continents, which may have enhanced the external validity of the study findings. However, this study has several limitations that should be considered when interpreting the findings. The study relied on self-reported data, which can be subject to information bias, and the sample was a convenience sample rather than a probabilistic sample. The use of electronic surveys and specific platforms may have excluded certain groups of students who did not have access to these platforms. Additionally, the timing of the survey, which was conducted during the COVID-19 pandemic, may have influenced the students' claimed knowledge levels and might not accurately reflect the extent of instructional information supplied through university courses. In addition, cultural, religious, economic, and political differences across the study population may have influenced the knowledge and attitude of individuals towards Mpox. The study attempted to reduce this heterogeneity and ensure internal validity, external validity, and standardization of the study findings by taking the following steps. First, the use of a validated questionnaire to assess

TABLE 5 Predictors of knowledge and attitude of medical students about monkeypox based on multilevel logistic regression models.

Predictors	Knowledge	Attitude
	Odds ratio (OR) (95%CI), *p*-value	Odds ratio (OR) (95%CI), *p*-value
Age (1 year)	0.97 (0.94–1.00), 0.06	0.99 (0.96–1.02), 0.51
sex		
Female (reference group)	1	1
Male	0.84 (0.77–0.91), <0.001	0.72 (0.66–0.78), <0.001
Educational year		
First year (reference group)	1	1
Second year	1.15 (0.99–1.34), 0.06	0.89 (0.76–1.03), 0.13
Third year	1.28 (1.10–1.50), 0.002	1.08 (0.92–1.26), 0.37
Fourth-year	1.13 (0.96–1.34), 0.14	1.15 (0.97–1.37), 0.10
Fifth year	1.45 (1.20–1.76), <0.001	1.23 (1.01–1.49), 0.04
Sixth year	1.44 (1.14–1.83), 0.002	1.19 (0.94–1.51), 0.14
Place of residence		
Rural (reference group)	1	1
Urban/city	1.06 (0.94–1.20) 0.32	1.35 (1.20–1.53), <0.001
Employment status		
Not employed (reference group)	1	1
Employed (part-time job)	0.90 (0.79–1.01), 0.08	0.86 (0.77–0.98), 0.02
Employed (full time job)	0.84 (0.68–1.03), 0.09	0.66 (0.54–0.82), <0.001
Knowledge of smallpox		
No (reference group)	1	1
Yes	0.97 (0.76–1.25), 0.84	1.27 (1.01–1.60), 0.04
History of COVID-19 vaccine intake		
No (reference group)	1	1
Yes	1.17 (0.97–1.42), 0.10	1.53 (1.19–1.96), <0.001
History of chickenpox disease		
No (reference group)	1	1
Uncertain	0.90 (0.69–1.19), 0.47	0.81 (0.65–1.00), 0.05
Yes	1.20 (0.95–1.52), 0.12	1.10 (0.94–1.27), 0.22
Receiving training programs about monkeypox		
No (reference group)	1	1
Yes	0.96 (0.83–1.10), 0.56	0.74 (0.64–0.85), <0.001
Country classification		
Low-income countries (reference group)	1	1
Low-middle income countries	1.11 (0.58–2.13), 0.75	0.98 (0.54–1.78), 0.94
High-middle income countries	0.60 (0.28–1.26), 0.18	0.75 (0.37–1.51), 0.41
High income countries	0.49 (0.24–0.99), 0.04	0.81 (0.42–1.57), 0.53
Source of information about monkeypox (no is the reference group)		
Family members	0.96 (0.87–1.05), 0.38	0.98 (0.89–1.08), 0.73
Friends	1.23 (1.12–1.34) <0.001	1.09 (0.99–1.19), 0.08
Social media	1.67 (1.51–1.84) <0.001	1.29 (1.17–1.42), <0.001
Research articles	1.40 (1.27–1.54) <0.001	1.05 (0.95–1.15), 0.36
Scientific websites	1.33 (1.21–1.46) <0.001	1.21 (1.09–1.33), <0.001
Knowledge		
Bad knowledge (reference group)	—	1
Good knowledge		2.96 (2.71–3.23), <0.001

the knowledge and attitude of a large sample of medical students. It was tested by participants from 10 countries to ensure it was valid across different cultures and social backgrounds. Second, medical students who knew about infectious diseases distributed the same questionnaire and respondents completed it themselves. This ensured that all participants interpreted the questions in the same way and provided standardized responses. Finally, a large number of responses from 27 countries was collected across three continents to enhance the external validity of the study findings.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

This study was approved by the Ethics Committee of the Faculty of Medicine, Alexandria University, Egypt (IRB No. 00012098), following the International Ethical Guidelines for Epidemiological Studies. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

SA: conceptualization, methodology, validation, formal analysis, data curation, writing—original draft, writing—review and editing, visualization, and supervision. AG: validation, investigation, resources, data curation, writing—review and editing, visualization, and project administration. NY: validation, writing—review and editing, and resources. MS, BA, and MK: review and editing. AbE and SA-R: formal analysis and data curation. AM, SY, and EE: resources and validation. RA, ZR, YK, HJ, EK, AJ, GA, MG, AC, AhE, OB, KR, AI, YA-H, NE, JN, HA, SA, RD, and OO: validation and investigation. BA and RMG: conceptualization, methodology, formal analysis, writing—original draft, and writing—review and editing. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1192542/full#supplementary-material

References

1. Gong Q, Wang C, Chuai X, Chiu S. Monkeypox virus: a re-emergent threat to humans. *Virol Sin*. (2022) 37:477–82. doi: 10.1016/j.virs.2022.07.006

2. Beer EM, Rao VB. A systematic review of the epidemiology of human monkeypox outbreaks and implications for outbreak strategy. *PLoS Negl Trop Dis*. (2019) 13:e0007791. doi: 10.1371/journal.pntd.0007791

3. Breman JG, Kalisa R, Steniowski MV, Zanotto E, Gromyko AI, Arita I. Human monkeypox, 1970–79. *Bull World Health Organ*. (1980) 58:165–82.

4. Multistate outbreak of monkeypox—Illinois, Indiana, and Wisconsin, 2003. *JAMA*. (2003) 290:30–1. doi: 10.1001/jama.290.1.30

5. Ghazy RM, Hammad EM, Hall MA, Mahboob A, Zeina S, Elbanna EH, et al. How can imported monkeypox break the borders? A rapid systematic review. *Comp Immunol Microbiol Infect Dis*. (2023) 92:101923. doi: 10.1016/j.cimid.2022.101923

6. Vaughan A, Aarons E, Astbury J, Balasegaram S, Beadsworth M, Beck CR, et al. Two cases of monkeypox imported to the United Kingdom, September 2018. *Euro Surveill*. (2018) 23:1800509. doi: 10.2807/1560-7917.ES.2018.23.38.1800509

7. Erez N, Achdout H, Milrot E, Schwartz Y, Wiener-Well Y, Paran N, et al. Diagnosis of imported monkeypox, Israel, 2018. *Emerg Infect Dis*. (2019) 25:980–3. doi: 10.3201/eid2505.190076

8. Ng OT, Lee V, Marimuthu K, Vasoo S, Chan G, Lin RTP, et al. A case of imported monkeypox in Singapore. *Lancet Infect Dis*. (2019) 19:1166. doi: 10.1016/s1473-3099(19)30537-7

9. World Health Organization. Monkeypox. (Accessed May 19, 2022). Available at: https://www.who.int/news-room/fact-sheets/detail/monkeypox.

10. World Health Organization. (2023). 2022–2023 Mpox (monkeypo NOTEREF _Ref138070920 \f [1]x) outbreak: global trends. (Accessed March 03, 2023) Available at: https://worldhealthorg.shinyapps.io/mpx_global/.

11. Vaughan A, Aarons E, Astbury J, Brooks T, Chand M, Flegg P, et al. Human-to-human transmission of monkeypox virus, United Kingdom, October 2018. *Emerg Infect Dis*. (2020) 26:782–5. doi: 10.3201/eid2604.191164

12. Reynolds MG, Yorita KL, Kuehnert MJ, Davidson WB, Huhn GD, Holman RC, et al. Clinical manifestations of human monkeypox influenced by route of infection. *J Infect Dis*. (2006) 194:773–80. doi: 10.1086/505880

13. Damon IK. Status of human monkeypox: clinical disease, epidemiology and research. *Vaccine*. (2011) 29:D54–9. doi: 10.1016/j.vaccine.2011.04.014

14. McCollum AM, Damon IK. Human monkeypox. *Clin Infect Dis*. (2013) 58:260–7. doi: 10.1093/cid/cit703%JClinicalInfectiousDiseases

15. Al Awaidy ST, Khamis F, Sallam M, Ghazy RM, Zaraket H. Monkeypox (mpox) outbreak: more queries posed as cases soar globally. *Sultan Qaboos Univ Med J*. (2023) 23:1–4. doi: 10.18295/squmj.8.2022.046

16. Ježek Z, Szczeniowski M, Paluku K, Mutombo M. Human monkeypox: clinical features of 282 patients. *J Infect Dis*. (1987) 156:293–8. doi: 10.1093/infdis/156.2.293

17. Ježek Z, Grab B, Szczeniowski M, Paluku K, Mutombo M. Clinico-epidemiological features of monkeypox patients with an animal or human source of infection. *Bull World Health Organ*. (1988) 66:459–64.

18. World Health Organization. WHO Informal consultation on monkeypox 2017 (2018). Available at: https://apps.who.int/iris/bitstream/handle/10665/272620/WHO-WHE-IHM-2018.3-eng.pdf

19. Ghazy RM, Okeh DU, Sallam M, Hussein M, Ismail HM, Yazbek S, et al. Psychological antecedents of healthcare workers towards monkeypox vaccination in Nigeria. *Vaccines*. (2022) 10:2151. doi: 10.3390/vaccines10122151

20. Ghazy RM, Yazbek S, Gebreal A, Hussein M, Addai SA, Mensah E, et al. Monkeypox vaccine acceptance among Ghanaians: a call for action. *Vaccines*. (2023) 11:240. doi: 10.3390/vaccines11020240

21. Bass J, Tack DM, McCollum AM, Kabamba J, Pakuta E, Malekani J, et al. Enhancing health care worker ability to detect and care for patients with monkeypox in the Democratic Republic of the Congo. *Int Health*. (2013) 5:237–43. doi: 10.1093/inthealth/iht029

22. Harapan H, Setiawan AM, Yufika A, Anwar S, Wahyuni S, Asrizal FW, et al. Knowledge of human monkeypox viral infection among general practitioners: a cross-sectional study in Indonesia. *Pathog Glob Health*. (2020) 114:68–75. doi: 10.1080/20477724.2020.1743037

23. Bates BR, Grijalva MJ. Knowledge, attitudes, and practices towards monkeypox during the 2022 outbreak: an online cross-sectional survey among clinicians in Ohio, USA. *J Infect Public Health*. (2022) 15:1459–65. doi: 10.1016/j.jiph.2022.11.004

24. Riccò M, Ferraro P, Camisa V, Satta E, Zaniboni A, Ranzieri S, et al. When a neglected tropical disease goes global: knowledge, attitudes and practices of Italian physicians towards monkeypox, preliminary results. *Trop Med Infect Dis*. (2022) 7:135. doi: 10.3390/tropicalmed7070135

25. Alshahrani NZ, Algethami MR, Alarifi AM, Alzahrani F, Alshehri EA, Alshehri AM, et al. Knowledge and attitude regarding monkeypox virus among physicians in Saudi Arabia: a cross-sectional study. *Vaccines*. (2022) 10:2099. doi: 10.3390/vaccines10122099

26. Hasan M, Hossain MA, Chowdhury S, Das P, Jahan I, Rahman MF, et al. Human monkeypox and preparedness of Bangladesh: a knowledge and attitude assessment study among medical doctors. *J Infect Public Health*. (2023) 16:90–5. doi: 10.1016/j.jiph.2022.11.032

27. Alsanafi M, Al-Mahzoum K, Sallam M. Monkeypox knowledge and confidence in diagnosis and management with evaluation of emerging virus infection conspiracies among health professionals in Kuwait. *Pathogens*. (2022) 11:994. doi: 10.3390/pathogens11090994

28. Sallam M, Al-Mahzoum K, AB A-T, Alkurtas M, Mirzaei F, Kareem N, et al. Assessing healthcare workers' knowledge and their confidence in the diagnosis and management of human monkeypox: a cross-sectional study in a middle eastern country. *Healthcare*. (2022) 10. doi: 10.3390/healthcare10091722

29. Riad A, Drobov A, Rozmarinová J, Drapáčová P, Klugarová J, Dušek L, et al. Monkeypox knowledge and vaccine hesitancy of Czech healthcare workers: a health belief model (HBM)-based study. *Vaccine*. (2022) 10:2022. doi: 10.3390/vaccines10122022

30. Salim NA, Septadina IS, Permata M, Hudari H. Knowledge, attitude, and perception of anticipating 2022 global human monkeypox infection among internal medicine residents at Palembang Indonesia: an online survey. *J Kedokt dan Kesehat*. (2022) 9:253–62. doi: 10.32539/JKK.V9I3.18799

31. Bilgin S, Dost B, Terzi O, Koksal E, Kaya C, Ustun YB, et al. Evaluation of knowledge and attitudes of anesthesiology specialists and residents toward patients infected with the Monkeypox disease: a national survey study. *Surg Infect*. (2022) 23:880–6. doi: 10.1089/sur.2022.301

32. Malaeb D, Sallam M, Salim NA, Dabbous M, Younes S, Nasrallah Y, et al. Knowledge, attitude and conspiracy beliefs of healthcare workers in Lebanon towards monkeypox. *Trop Med Infect Dis*. (2023) 8:81. doi: 10.3390/tropicalmed8020081

33. Sallam M, Al-Mahzoum K, Dardas LA, Al-Tammemi AB, Al-Majali L, Al-Naimat H, et al. Knowledge of human monkeypox and its relation to conspiracy beliefs among students in Jordanian health schools: filling the knowledge gap on emerging zoonotic viruses. *Medicina*. (2022) 58:924. doi: 10.3390/medicina58070924

34. Alshahrani NZ, Mitra S, Alkuwaiti AA, Alhumam MN, Altmimi SMB, Alamri MHM, et al. Medical students' perception regarding the re-emerging monkeypox virus: an institution-based cross-sectional study from Saudi Arabia. *Cureus*. (2022) 14:8:e28060. doi: 10.7759/cureus.28060

35. Jairoun AA, Al-Hemyari SS, Abdulla NM, El-Dahiyat F, Shahwan M, Hassan N, et al. Awareness and preparedness of human monkeypox outbreak among university student: time to worry or one to ignore? *J Infect Public Health*. (2022) 15:1065–71. doi: 10.1016/j.jiph.2022.08.015

36. Swed S, Alibrahim H, Bohsas H, Amir Rais M, Shoib S, Khair Eldien Jabban Y, et al. Monkeypox awareness among the Syrian doctors and medical students: a cross sectional study. *Int J Med Stud*. (2023) 10:S206. doi: 10.5195/ijms.2022.1772

37. Kumar N, Ahmed F, Raza MS, Rajpoot PL, Rehman W, Khatri SA, et al. Monkeypox cross-sectional survey of knowledge, attitudes, practices, and willingness to vaccinate among university students in Pakistan. *Vaccine*. (2023) 11:97. doi: 10.3390/vaccines11010097

38. Lin G, Tan W, Chan D, Ooi K, Hashim H. Monkeypox awareness, knowledge, and attitude among undergraduate preclinical and clinical students at a Malaysian dental school: an emerging outbreak during the COVID-19 era. *Asian Pac J Trop Med*. (2022) 15:461–7. doi: 10.4103/1995-7645.359787

39. Bolignano D, Mattace-Raso F, Torino C, D'Arrigo G, ElHafeez SA, Provenzano F, et al. The quality of reporting in clinical research: the CONSORT and STROBE initiatives. *Aging Clin Exp Res*. (2013) 25:9–15. doi: 10.1007/s40520-013-0007-z

40. The World Bank. *World Bank country and lending groups*. (2022). Available at: https://datahelpdesk.worldbank.org/knowledgebase/articles/906519-world-bank-country-and-lending-groups.

41. EA D. Validity and reliability in social science research. *Educ Res Perspect*. (2011) 38:105–23. doi: 10.2146/ajhp070364

42. Boateng GO, Neilands TB, Frongillo EA, Melgar-Quiñonez HR, Young SL. Best practices for developing and validating scales for health, social, and behavioral research: a primer. *Front Public Health*. (2018) 6:149. doi: 10.3389/fpubh.2018.00149

43. Ockey GJ. Exploratory factor analysis and structural equation modeling. *The companion to language assessment* (2013) 1224–1244.

44. Samuels P *Advice on exploratory factor analysis*. (2017).

45. Coughlin KB. *An analysis of factor extraction strategies: a comparison of the relative strengths of principal axis, ordinary least squares, and maximum likelihood in research contexts that include both categorical and continuous variables*. University of South Florida (2013).

46. Field A, Miles J, Field Z. *Discovering statistics using R*. Sage Publications (2012).

47. Yong AG, Pearce S. A beginner's guide to factor analysis: focusing on exploratory factor analysis. *Tutor Quant Methods Psychol*. (2013) 9:79–94. doi: 10.20982/tqmp.09.2.p079

48. Henseler J, Ringle CM, Sarstedt M. A new criterion for assessing discriminant validity in variance-based structural equation modeling. *J Acad Mark Sci*. (2015) 43:115–35. doi: 10.1007/s11747-014-0403-8

49. Cronbach LJ. Coefficient alpha and the internal structure of tests. *Psychometrika*. (1951) 16:297–334. doi: 10.1007/BF02310555

50. Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *J Stat Softw*. (2015) 67:1–48. doi: 10.18637/jss.v067.i01

51. Mackey TK, Liang BA, Cuomo R, Hafen R, Brouwer KC, Lee DE. Emerging and reemerging neglected tropical diseases: a review of key characteristics, risk factors, and the policy and innovation environment. *Clin Microbiol Rev*. (2014) 27:949–79. doi: 10.1128/cmr.00045-14

52. Celletti F, Buch E, Samb B. Medical education in developing countries In: K Walsh, editor. *Oxford textbook of medical education*: Oxford University Press (2013).

53. Galasso V, Pons V, Profeta P, Becher M, Brouard S, Foucault M. Gender differences in COVID-19 attitudes and behavior: panel evidence from eight countries. *Proc Natl Acad Sci U S A*. (2020) 117:27285–91. doi: 10.1073/pnas.2012520117

54. Lönnbro J, Nylén K, Wallerstedt SM. Developing professional confidence in the art of prescribing-a randomized controlled study on structured collegial discussions during internship. *Eur J Clin Pharmacol*. (2019) 75:687–96. doi: 10.1007/s00228-018-02619-4

55. McNair R, Griffiths L, Reid K, Sloan H. Medical students developing confidence and patient centredness in diverse clinical settings: a longitudinal survey study. *BMC Med Educ*. (2016) 16:176. doi: 10.1186/s12909-016-0689-y

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Determinants of sexually transmitted infections among female sex workers in Ethiopia: a count regression model approach

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Background: Sexually transmitted infections (STIs) remain a major public health problem worldwide, with the burden of these infections being high among female sex workers (FSWs), who are often not aware of their infection status. This study aimed to determine the factors that are associated with the number of STIs among FSWs in Ethiopia.

Methods: A cross-sectional bio-behavioral study involving respondent-driven sampling (RDS) was conducted among 6,085 FSWs in 16 towns in Ethiopia. The hurdle Poisson regression model was fitted using STATA Version 16.2. The incident rate ratio and adjusted odds ratio with a 95% confidence interval were employed to show the strength and direction of the association. A p-value of ≤ 0.05 was used as a threshold for statistical significance.

Results: At least one STI was identified in 1,444 (23.64%) of the FSWs. Age group 35–49 years [IRR = 2.32; 95% CI (1.43, 3.74)], forced first sex [IRR = 1.32; 95% CI (1.01, 1.74)], condom breakage [IRR = 1.32; 95% CI (1.01, 1.74)], and a history of depression [IRR = 1.55; 95% CI (1.12, 2.18)] increase the number of STIs. FSWs aged 25–34 years [AOR = 2.99; % CI (2.54, 3.52)] and 35 = 59 years [AOR = 8.05; % CI (6.54, 9.91)], who were selling sex for 5–10 years [AOR = 1.30; 95% CI (1.1, 1.55)], and above 11 years [AOR = 1.21; 95% CI (1.03, 1.43)] were more likely to get STIs.

Conclusion: STIs are common in Ethiopia. The covariates age, educational status, monthly income, condom failure, age at the first sexual encounter, and long duration of sexual practice are significant predictors of STIs. Health interventions among FSWs need to include awareness generation about the prevention and control of STIs and address the determinants identified in this analysis.

KEYWORDS

serology, epidemiology, sexually transmitted infections, female sex workers, hurdle poison regression model

Highlights

- *What is already known on this topic:* The prevalence of STIs among FSWs varies across different countries and is determined by various sociodemographic factors, which are not known in Ethiopia.
- *What this study adds:* STIs are highly prevalent among FSWs in Ethiopia, and factors such as age, educational status, monthly income, and condom use determine their occurrence.
- *How this study might affect research, practice, or policy:* Awareness generation on the high prevalence and the determinants need to be considered in health policy and strategy formulation and further research on the prevention and control of STIs among FSWs.

Introduction

Sexually transmitted infections (STIs) are common and constitute public health concerns globally (1, 2). They include viral infections such as human immunodeficiency virus (HIV), hepatitis B virus (HBV), and hepatitis C virus (HCV), as well as other bacterial STIs, specifically syphilis. According to the World Health Organization's (WHO) estimate, more than 1 million people are newly infected worldwide with STIs each day (2). This is equivalent to 374 million people infected per year, of which 96 million are in Africa. Globally, more than 4.5 million people contract HIV and viral hepatitis each year, and among adults aged 15 to 49, 7.1 million new cases of syphilis are identified (2–4).

STIs remain a major public health problem in Africa (5, 6). The Global Burden of Disease (GBD) (1) estimates that the age-standardized incidence rate (ASIR) for STIs is 9,535 per 100,000 person-years, with the highest rate estimated at 19,973 per 100,000 person-years in 2019 being in sub-Saharan Africa (SSA). STIs constitute the second-leading cause of mortality and disability-adjusted life years (DALYs) in low- and middle-income countries, particularly among those aged 20–24 years (3). Globally, over 2.3 million people died as a result of STIs (4) in 2021, which also accounted for the increased number of years of life lost (1). According to a study conducted in Botswana, DALYs increased with time due to HIV and other STIs (7).

STIs also increase the risk of cancer and account for 13% of global cancer incidence, with the highest rate in SSA in 2018 (4). HBV and HCV are the most common primary causes of cancer (8). STIs increase medical costs (9), are drug resistant, and are associated with an array of maternal and neonatal morbidities (3, 10), as well as a stigma among FSWs (11).

STIs are prevalent, particularly among key populations, including adolescents, young adults, and FSWs (4), owing to the high probability of co-infections, overlapping routes of transmission, and common health determinants. FSWs bear the greatest burden of STIs (4) because of their sexual behavior, which puts them at higher risk of acquiring the infection (1, 12); their vulnerability to violence and having the most limited access to health and social services exacerbate the problem (4).

The prevalence of STIs among FSWs varies by country and across different studies (11, 13–16) ranging from 13.3% (14) to 43.2% (11). In Ethiopia, 23% of FSWs reported having one or more STIs (15). The prevalence of STI co-infection in FSWs also varies across studies from different countries (14, 17–19). The prevalence of HIV and syphilis co-infection ranges across studies from 1.09 (14) to 43% (17), and HCV and HBV were reported in 40 and 2% of FSWs with HIV/AIDS worldwide, respectively.

The prevalence of STIs and co-infections is high among FSWs (9, 18, 20). Several factors determining the occurrence of STIs among FSWs have been reported, including age, lower education level (21), and unemployment (21, 22). Factors such as frequent unsafe intercourse with various sex workers (21, 23), current drug use, inconsistent condom use (24), HIV stigma (22), previous exposure to violence (25), lack of access to treatment, and the ability to pay for services (25) are among the most frequently identified determinants.

Concurrent multiple STIs occurring in individuals are often associated with having multiple sexual partners and can be a source of an STI epidemic among FSWs (26). In this population group, there is variation in STI co-infection prevalence and the associated determinants in different settings (14, 18, 19). Indeed, there is a need for further studies on the drivers of the number of STIs. The suggested STI categories (zero STI and at least one STI) in the data count used in previous studies might exhibit excess zero counts (no STI), and excluding the zero counts increases the possibility of biased estimates (27). In addition, counting data as non-negative might be over-dispersed and contain excess zeros, making data analysis complex (28). Therefore, in this analysis, we aimed to determine the factors that are associated with the number of STIs among FSWs in Ethiopia using robust statistical methods.

Methods

Study design, setting, and population

This study was a cross-sectional bio-behavioral study among FSWs conducted in 16 towns in Ethiopia between December 2019 and May 2020. The target towns include Adama, Addis Ababa, Arba Minch, Bahir Dar, Kombolcha/Dessie, Dilla, Dire Dawa, Gambella, Gonder, Harar, Hawassa, Jimma, Logia/Semera, Mizan, Nekemite, and Shashemane. We conducted respondent-driven sampling (RDS) on 6,085 FSWs aged 15 years and older who had received money or other benefits from selling sex to four or more people in the previous 30 days and had lived or worked in the surveyed town for at least the last month.

Sampling and data collection

To recruit the participants, we employed respondent-driven sampling (RDS), a technique with benefits well-documented in previous reports (29, 30). As an initial step, 5 to 12 initial study participants referred to as "seeds" were selected from each study town. The seed participants were informed about the study, consent was obtained from each, and each participant was provided with three coupons for recruiting three eligible participants from her social network. All newly recruited participants were given three coupons as was done for the initial seeds to invite additional study participants. The data were collected through an anonymous

interview administered by the study team in a private room using an Open Data Kit (ODK) electronic data management system with built-in skip patterns and logical validations.

Study variables

The outcome variable in this study was the total number of STIs per FSW, categorized as zero STI, one STI, two STIs, three STIs, and four STIs. The independent sociodemographic variables included participants' age, the age at first sex sale, educational and married status, average monthly income, and the duration of sex work. Behavioral factors included alcohol and drug use, condom breakage, desired or forced first sex, HIV knowledge, and depression status.

The depression level was computed using the Patient Health Questionnaire (PHQ9) assessment tool (31). Participants with scores 0–4 were labeled as "non-minima", 5–9 as "mild", 10–14 as "moderate", 15–19 as "moderately severe," and 20–27 as "severe" depression severity levels.

Alcohol dependence level was computed using the alcohol use disorder identification test (AUDIT) (31). Drinking levels with scores 0–8 were labeled "social drinking", 9–13 "harmful or hazardous drinking" for females, and 13–40 "alcohol dependence" severity levels.

Compressive HIV knowledge was computed from the four prevention and treatment and three misunderstanding knowledge questions. Respondents with seven true answers were labeled "had comprehensive knowledge" and otherwise "did not have comprehensive knowledge".

The testing procedure and quality control

The study used whole blood to test for HIV, HBV, HCV, and syphilis using a rapid diagnostic kit. HIV testing was done using the national algorithm, which included three rapid tests: assay 1 (STAT-PAK (HIV1/2, USA), assay 2 (ABONE, HIV1/2/O Tri-Line Device, Hangzhou, China), and assay 3 (SD Bioline, HIV1/2, USA). According to the algorithm, those who tested positive for all three were considered HIV-positive.

Hepatitis B surface antigen (HbsAg) detection was performed by the Virucheck HbsAg test kit manufactured in India. A one-step test for HbsAg detects the presence of HbsAg in serum or plasma specimens. Hepatitis C was screened using the Flaviscreen PLUSTM Test Kit produced in India. Flaviscreen is a rapid, third-generation, two-site sandwich immunoassay for the detection of total antibodies specific to the hepatitis C virus. It utilizes the principle of agglutination of antibodies or antisera with the respective antigen in the immunochromatography format.

Syphilis was screened using the Syphicheck-WB Screen and Confirm Assay produced in Kerala, India. Syphicheck is a rapid, qualitative immunoassay for the detection of antibodies to *Treponema pallidum*. It utilizes the principle of agglutination of antibodies in immunochromatography format. The interpretation of HBV, HCV, and Syphilis test results was according to the manufacturer's guide using the test kit insert.

The study staff received training to ensure the safety and effectiveness of the testing techniques, and standard operating procedures were followed throughout the process, including specimen collection, transportation, testing, and storage. The temperature was monitored while transporting the specimens, and invalid test results were repeated.

Method of data analysis

Data were collected on tablet computers using the ODK software, exported to MS Excel, cleaned, and imported into STATA Version 16 for analysis. The RDS recruitment process (Tree of recruitment), RDS assumption assessment, and RDS weight generation were all carried out using the RDS package inbuilt into R statistical software (30, 32). Homophily and convergence, two common assumptions in RDS, were checked in HIV status, consistent condom usage, and FSW type and met the RDS criteria. The RDS weights were exported to STATA using the RDS-II function and merged with the entire dataset for further analysis. Descriptive statistics such as the crude and RDS-adjusted frequency, mean, and standard deviation were computed using RDS-II as a weighting variable. Univariable analysis was conducted to select potential risk factors to be considered in the final multivariable analysis using a modest level of significance ($\alpha = 0.25$).

The Poisson, negative binomial, zero-inflated Poisson, zero-inflated negative binomial, hurdle Poisson, and hurdle negative binomial models were employed. The Poison regression model is a baseline count model for count data in which the variance of the dependent variable is equivalent to its mean (33). The deviance and Pearson's chi-square statistic values corresponding to their degree of freedom were used to test the presence of over-dispersion after fitting the Poisson regression model. In this case, the mean and variance were 0.27 and 0.53, respectively; thus, the assumption is violated, indicating that the data were dispersed. Then, a negative binomial regression model, the extension of the Poisson regression model was fitted to handle the problem of over-dispersion in the dataset (33). However, count data often exhibit an excess number of zeros (one cause of over-dispersion) which cannot be accommodated by the Poisson and negative binomial regression models (33). In the presence of zero inflation and over-dispersion, zero-inflated (zero-inflated Poisson and zero-inflated negative binomial) and hurdle models (hurdle Poisson and hurdle negative binomial) were frequently used to fit epidemiological data (27, 28, 33), and they provide a flexible and effective framework for modeling (33). The models have two parts: the first predicts non-zero STI counts (i.e., at least one STI), and the second predicts the zero-hurdle model (zero infections vs. not zero infections) among FSWs.

Hurdle Poisson regression model

The hurdle Poisson regression model has two components: a truncated Poisson component with a rate parameter u, $u > 0$ that models non-zero positive counts, and a logit component with

success probability, π_0, $\pi_0 \geq 0$ that models the probability of zero counts. If the discrete random variable Y_i follows hurdle Poisson distribution, then the hurdle Poisson probability mass function is given as follows:

$$P\left(Y_i = y_i|\pi_0, \mu\right) = \begin{cases} \pi_0\,, & if\ y_i = 0 \\ (1-\pi_0)\,\frac{\exp(-\mu_i)\mu_i{}^{y_i}}{y_i!(1-\exp(-\mu_i))}, & if\ y_i > 0 \end{cases} \quad (1)$$

where $0 \leq \pi_0 \leq 1$, and defined by $\pi_0 = \mathrm{p}\ (\mathrm{y} = 0)$

For the logit part, the conditional mean is given by E $\{p(Y_i = 0/x_i)\} = \pi_0\ (x_i) = \frac{\exp(X_i^{'}\gamma)}{1+\exp(X_i^{'}\gamma)}$. Taking natural logarithms in both sides of the equation, we have as follows:

$$logit(\pi_0) = \log(\frac{\pi_0}{1-\pi_0}) = Z^{'}$$
$$\gamma = \gamma_0 + \gamma_1 X_1 + \gamma_2 X_2 + \ldots\ldots\ldots + \gamma_k X_k \quad (2)$$

where $\mathbf{X} = (1, X_1, X_2, \ldots\ldots\ldots, X_k)^{'}$ is a vector of independent variables, $\gamma = (\gamma_0, \gamma_1, \ldots\ldots\ldots, \gamma_k)^{'}$ is a vector of regression coefficients, and $\log(\frac{\pi_0}{1-\pi_0})$ is the log transformation of the odds of at least one STI.

Similarly, the conditional mean for the truncated Poisson is given by: $E(Y_i/x_i) = u_i = \exp(X_i^{'}\beta)$

Therefore, the truncated Poisson regression model is given by:

$$\log^{(u_i)} = \mathbf{X}^{'}\beta = \beta_0 + \beta_1 X_1 + \beta_2 X_2 + \ldots\ldots + \beta_k X_k \quad (3)$$

where $\mathbf{X} = (1, X_1, X_2, \ldots\ldots\ldots, X_k)^{'}$ and $\beta = (\beta_0, \beta_1, .., \beta_k)^{'}$ are a vector of independent variables and regression coefficients, respectively.

Each model's goodness-of-fit was evaluated using the Akaike Information Criteria (AIC), and rootogram visual assessment (34, 35). Both the count and the zero-inflated parts were analyzed. Finally, a 95% confidence interval (CI) was reported for the incident rate ratio and adjusted odds ratio. A p-value of $\leq$0.05 was used to define statistical significance.

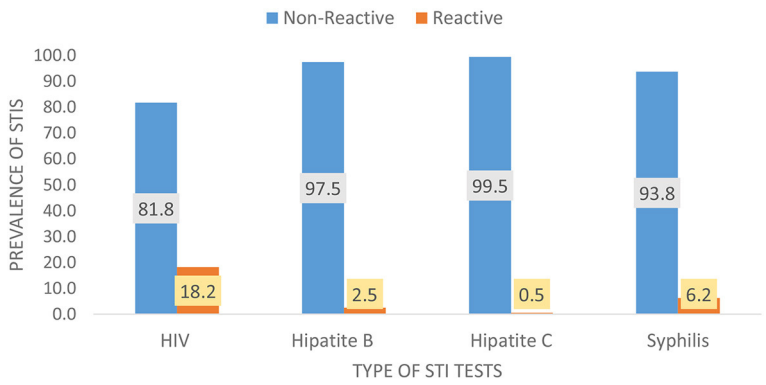

FIGURE 1
Weighted (RDS-adjusted) prevalence for the types of STIs among female sex workers in Ethiopia, bio-behavioral survey 2020.

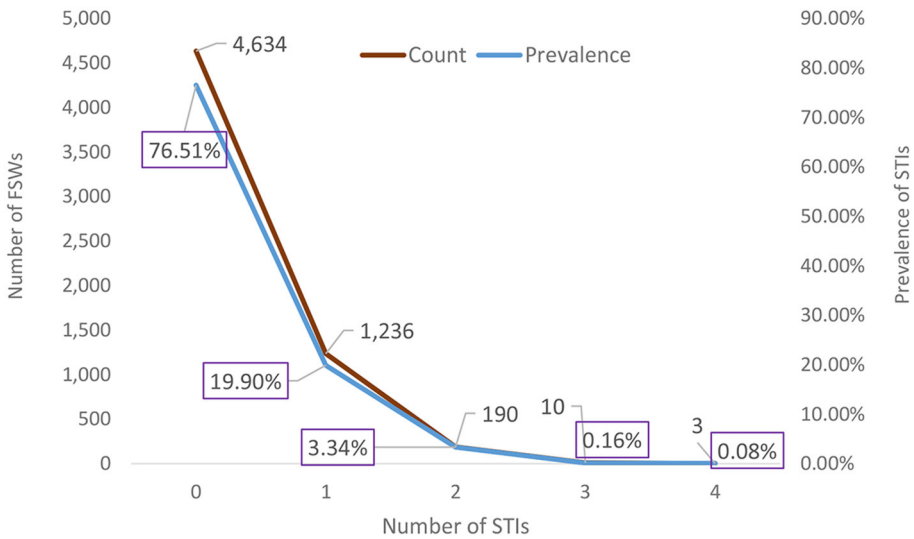

FIGURE 2
Weighted prevalence of the number of STIs among female sex workers in Ethiopia, bio-behavioral survey 2020.

Ethical considerations

Ethiopian Public Health Institutes' Scientific and Ethical Research Office provided ethical clearance for the survey protocol (Ethical approval number: EPHI-IRB-108-2018). Each survey participant gave her consent to be interviewed, have blood specimens taken, and have the biospecimens stored for testing. Individuals who tested positive for STIs were transferred to the nearest or preferred health facility for appropriate clinical care. All collected information including the test results and seed contact information were kept entirely confidential.

Patient and public involvement

Locally available organizations working on HIV prevention interventions, such as the HIV/AIDS Prevention and Control Office (HAPCO), District Health offices, and Drop-in Centers (DICs) were used to identify the initial participants (seeds) of the survey.

The seeds were selected based on the type of sexual worker, age category, and geographic location of the site. An FSW with a known social network was given three coupons so that she could invite her friends or other FSW contacts that were in her network. This approach allowed the study to reach as many eligible FSWs as possible. Finally, the findings of the study were shared with the FSW associations, HAPCO, and District Health offices through officially written letters and documents and using different platforms such as technical working group meetings and workshops.

Results

The magnitude of STIs

Among the 6,085 FSWs involved in the study, 18.2% had HIV, 6.2% had syphilis, 2.5% had HBV, and 0.5% had HCV (Figure 1). Approximately one quarter, 1,439 (23.64%) of them, had at least 1 STI; 1,236 (19.90%) had 1 STI, 190 (3.34%) had 2 STIs, 10 (0.16%) had 3 STIs, and 3 (0.08%) had 4 STIs (Figure 2). The mean number of STIs among the FSWs was 0.27 [95% CI (0.26, 0.29)]. As shown in Tables 1, 2, comparing the mean number of STIs by category of the categorical covariates, on average, a higher number of STIs were observed among FSWs aged 35 years and above (0.64 ± 0.70), those who have a history of condom failure (0.34 ± 0.58), no formal education (0.45± 0.64), have moderate to severe depression (0.36±0.61), and are residing at Jimma (0.36 ± 0.62).

Model selection

The distribution of the number of STIs is skewed to the right, signifying the likelihood of over-dispersion. The zero STIs on the bar charts in Figure 2 are highly selected, suggesting that count data models that account for excess zeros, such as zero-inflated models and hurdle models, would better fit the data of the number of STIs. The hurdle Poisson model has the smallest AIC value and

TABLE 1 Sociodemographic characteristics of female sex workers with sexually transmitted infections, bio-behavioral survey, Ethiopia, 2020 (N = 6,085).

Characteristics	Crude value		Weighted value	
	n	%	Mean	Standard deviation
Study town				
Adama	676	11.1	0.23	0.48
Addis Ababa	1,101	18.1	0.22	0.47
Arba Minch	251	4.1	0.33	0.57
Bahir Dar	372	6.1	0.35	0.56
Kombolcha/Dessie	251	4.1	0.32	0.58
Dilla	251	4.1	0.31	0.57
Dire Dawa	434	7.1	0.32	0.56
Gambella	468	7.7	0.26	0.47
Gonder	250	4.1	0.28	0.51
Harar	242	4	0.29	0.48
Hawassa	522	8.6	0.27	0.57
Jimma	254	4.2	0.36	0.62
Logia/Semera	251	4.1	0.26	0.51
Mizan	255	4.2	0.27	0.53
Nekemite	257	4.2	0.25	0.53
Shashemane	250	4.1	0.26	0.53
Marital status				
Married/cohabitation	231	3.8	0.28	0.54
Divorced/separated/widowed	2,908	47.8	0.38	0.60
Never married	2,946	48.4	0.16	0.42
The main source of income				
Sex work	5,694	93.6	0.27	0.52
Other than sex work	391	6.4	0.38	0.62
Average monthly income from selling sex in USD				
<65	1,778	29.2	0.37	0.60
65 and 150 USD	2,066	34	0.27	0.52
150 to 200 USD	1,175	19.3	0.23	0.48
200 and above USD	1,066	17.5	0.17	0.43
Age at first sex selling				
<20	2,328	38.3	0.18	0.44
20–24	2,348	38.6	0.25	0.51
25+	1,406	23.1	0.46	0.62
Age at first sex				
15 or less	2,430	39.9	0.33	0.56
16–20	3,384	55.6	0.23	0.49
21+	271	4.5	0.31	0.54

(Continued)

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TABLE 1 (Continued)

Characteristics	Crude value		Weighted value	
	n	%	Mean	Standard deviation
Age category				
15–24	2,595	42.6	0.11	0.35
25–34	2,671	43.9	0.32	0.54
35–59	819	13.5	0.64	0.70
Highest educational status attained				
Non-formal education	1,054	17.3	0.45	0.64
Primary school (grades 1–8)	3,560	58.5	0.26	0.51
Secondary school and above	1,471	24.2	0.18	0.44
Year lived as SFWs				
<5 Years	2,307	38	0.18	0.43
5–10 Years	1,556	25.6	0.31	0.54
11+ Years	2,110	36.4	0.35	0.58

is considered the final model (see Table 3). Furthermore, a visual assessment of the fit was made in terms of the rootogram.

Hurdle negative binomial has an inferior fit, with some low numbers over-predicted. The hurdle Poisson model was found to be the best fit for the data based on their respective log-likelihood, AIC, and rootogram (Figure 3).

Factors associated with the number of STIs

The model is divided into two sections (Table 4): the first predicts non-zero counts of STIs (truncated negative binomial with log link), and the second predicts the zero-hurdle model (binomial with logit link) with zero STIs vs. no zero STIs.

Truncated Poisson with log link function

Table 4 shows the results of hurdle Poisson model parameter estimates, incidence rate ratio (IRR), standard error, *P*-values, and 95% CIs for IRR. In the results of a truncated Poisson with a log link function to predict the number of at least one STI found, after controlling for the effect of other characteristics, FSWs' age, condom breakage, a history of moderate to severe depression, and drug use were significant predictors of STIs.

When compared to FSWs under the age of 25 years, those aged 35–49 were approximately 2.3 times [IRR = 2.3; 95% CI (1.43, 3.74)] more likely to experience STIs. FSWs with moderate to severe depression were 1.55 times (IRR = 1.554; 95 % CI: 1.15, 2.18) more likely to have STIs compared to those without depression.

FSWs who were forced to have sex at their first sexual encounter were 1.32 times [IRR = 1.32; 95% CI (1.01, 1.74)] more likely to have STIs compared with those who willingly had sex at their first encounter. When compared to FSWs who had not

TABLE 2 Behavioral and clinical characteristics of female sex workers with sexually transmitted infections, bio-behavioral survey, Ethiopia, 2020 (N = 6,085).

Characteristics	Crude value		Weighted values	
			Mean	Standard Deviation
Level of depression				
Not depressed	2,468	40.6	0.24	0.50
Mild depression	2,525	41.5	0.27	0.51
Moderate to severe depression	1,092	17.9	0.36	0.61
Alcohol drinking (AUDIT)				
Social drinking/not risky	2,594	42.8	0.29	0.56
Harmful or hazardous drinking	1,210	20	0.27	0.51
Alcohol dependence indication	2,257	37.2	0.26	0.50
Chewing khat in the last 30 days				
Never	2,258	37.1	0.28	0.54
Yes	3,827	62.9	0.27	0.52
Alcohol use				
Never	5,385	88.5	0.28	0.53
Yes	700	11.5	0.25	0.53
First sex experience				
Wanted	4,738	77.9	0.25	0.51
Forced	1,347	22.1	0.34	0.59
Changed location of selling sex in the past 6 months				
No	4,572	75.1	0.27	0.52
Yes	1,513	24.9	0.29	0.54
Number of cities worked sex selling in the last 3 years				
Same town	4,933	81.1	0.27	0.52
1 more town	776	12.8	0.27	0.52
2 or more towns	374	6.1	0.28	0.57
Having sex without a condom in the last 30 days (at least once)				
Yes	5,119	84.1	0.31	0.58
No	966	15.9	0.27	0.51
Experienced condom failure in the last 30 days				
No	4,260	70	0.25	0.50
Yes	1,825	30	0.34	0.58
Ever had anal sex				
Never	5,659	93	0.28	0.53
Yes	426	7	0.23	0.49
Number of non-paying partners in the past 6 months				
Never	4,347	71.4	0.28	0.53
Only one	1,404	23.1	0.26	0.53

(Continued)

TABLE 2 (Continued)

Characteristics	Crude value		Weighted values	
			Mean	Standard Deviation
2 and more	334	5.5	0.30	0.50
At least two STI symptoms occurred in the last 12 months				
No	5,083	83.5	0.26	0.52
Yes	1,002	16.5	0.33	0.56
Ever been raped or forced to have sex in the past 12 months				
No	5,314	87.3	0.27	0.53
Yes	771	12.7	0.30	0.53
Number of pregnancies				
0	1,873	30.8	0.16	0.41
1	2,059	33.8	0.25	0.51
2	1,238	20.3	0.34	0.57
3+	915	15	0.47	0.64
Currently pregnant				
No	5,973	98.2	0.28	0.53
Yes	112	1.8	0.15	0.43
History of miscarriage pregnancy				
No	5,471	89.9	0.26	0.52
Yes	614	10.1	0.38	0.59
History of aborted pregnancy				
No	4,809	79	0.27	0.52
Yes	1,276	21	0.29	0.53
Number of clients in the past 6 months				
4–30	2,308	37.9	0.31	0.56
31–90	2,132	35	0.26	0.51
91+	1,645	27	0.24	0.49

experienced condom breakage during sexual intercourse, those who had experienced condom breakage were 1.32 times [IRR = 1.32; 95% CI (1.01, 1.74)] more likely to have STIs.

The estimated frequency of STIs experienced by an FSW was significantly associated with the alcohol dependency indicator. Compared to social drinking/not risky FSWs, those with alcohol dependency signs had a 37.7% (IRR = 0.62; 95% CI: 0.45, 0.86) lower risk of STIs.

Zero-hurdle model (binomial with logit link)

The second predicts the zero-hurdle model (binomial with logit link) with zero STIs vs. no STIs. The zero-hurdle model's estimated adjusted odds ratio (AOR) and 95% CI for the factor change in the odds of experiencing at least one STI are shown in Table 4. After controlling for all other factors in the model, the probability of having an STI was found to be significantly associated with age, education level, average monthly income, depression, age at selling sex, years spent as FSWs, and condom breakage.

From the results of the hurdle model (binomial with logit link), the odds of having STI (at least one STI) were lower by 31% [AOR = 0.69; 95% CI (0.59, 0.81)] and 48% [AOR = 0.52; 95% CI (0.42, 0.64)] for FSWs who had attended primary and secondary school or above, respectively, compared to those who had no formal education. FSWs aged 25–34 and 35–59 years had approximately three times [AOR = 2.99; 95% CI (2.54, 3.52)] and eight times [AOR = 8.051; 95% CI: (6.54, 9.91)] the chances of having at least one STI, respectively, compared to FSWs aged 24 years or younger.

FSWs earning between 65 and 150 USD, 150 to 200 USD, and 200 and above USD had a 30% [AOR = 0.70; 95% CI (0.60, 0.82)], 33% [AOR = 0.68; 95% CI (0.56, 0.82)], and 49% [AOR = 0.51; 95% CI (0.42, 0.63)] lower risk of having at least one STI. Those who began selling first sex between the ages 16–20 years and at the age of 20 or more had a 28% [AOR = 0.72; 95% CI (0.63, 0.83)] and 33% [AOR = 0.67; 95% CI (0.50, 0.91)] lower risk of experiencing at least one STI than those who began selling first sex at the age of 15 years below. FSWs who worked on this business for <5 years were 1.3 [AOR = 1.3; 95% CI (1.1, 1.55)] and 1.21 [AOR = 1.21; 95% CI (1.03, 1.43)] times more likely to have at least one STI.

FSWs with a history of condom breakage were 1.4 [AOR = 1.4; 95% CI (1.21, 1.61)] times more likely to have at least one STI than those without a history of condom breakage. The odds of experiencing at least one STI were 1.21 [AOR = 1.21; 95% CI (1.01, 1.45)] times higher in FSWs who were moderately or seriously depressed compared to those who did not have depression.

Discussion

This study found that 18.2% of the 6,085 FSWs studied had HIV, 6.2% had syphilis, 2.5% had HBV, and 0.5% had HCV. This study found that at least one STI was found in 23.64% of the FSWs, which is consistent with the findings of previous reports from Ethiopia (15), Mexico (16), and Ecuador (13) where at least 17.6% of FSWs were infected with STIs. Our finding could be a higher estimate because the other studies used polymerase chain reaction (PCR) to test for STIs.

The finding among the FSWs in our study is higher than those of the reports from Brazil of 13.3% (14), but lower than the Russian report (11), which showed that 43.2% of participants had at least one STI. This disparity could be due to differences in sociodemographic characteristics, STI diagnostic methods, types, and the number of STIs included in the specific studies.

Age, educational status, and average monthly income, as well as a history of depression, condom breakage, early initiation of sex selling, and living as a sex worker for a long time were associated with the frequency of STIs in our study. This suggests that the country has to make a greater effort to work toward increasing awareness among FSWs as well as the general population and improving prevention, care, and treatment services for STIs among FSWs.

Our study found that FSWs over the age of 30 were more likely to have at least one STI. This finding is comparable to those reported by studies conducted in Namibia (21), the Republic of Congo (29), South Africa (36), Ecuador (13), and Rwanda

TABLE 3 Model selection and comparison of the number of STIs among female sex workers.

Model selection	Poisson	Negative binomial	Zero-inflated Poisson	Zero-inflated negative binomial	Hurdle Poisson	Hurdle negative binomial
AIC	7,139.42	7,141.49	7,146.22	7,148.22	7,093.25	7,095.25
Log-likelihood	−3,522	−3,531	−3,537	−3,537	−3,511	−3,511

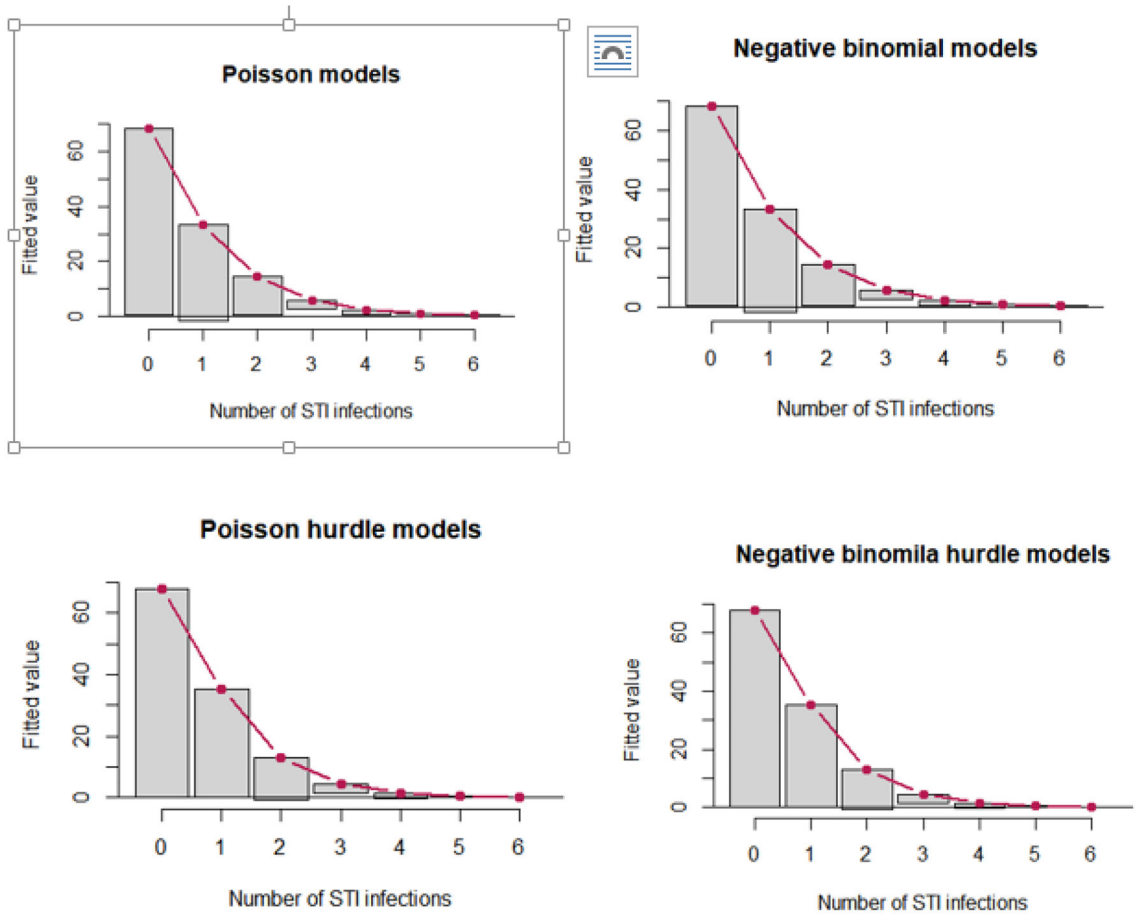

FIGURE 3
Hanging rootograms for count regression models of STIs among female sex workers in Ethiopia, bio-behavioral survey 2020.

(19). Studies show an increase in STIs and co-infections with age, probably due to older FSWs not considering themselves high risk and failing to persuade customers to use condoms (13, 20). This could also be due to the cohort effect, where older women have more chances of acquiring STIs. Consequently, FSWs in this age group could be the major drivers of STIs among FSWs and their clients unless effective and comprehensive programs are implemented. In contrast, a report from Iran indicates that being under the age of 25 is independently associated with increased STIs (37). This disparity could be associated with sociocultural differences between population groups, but it requires further exploration.

Similar to a finding in Rwanda (19), FSWs who were in the business for a long time in our study had higher odds of getting at least one STI. Similarly, those who began selling sex at a young age had the highest odds of having at least one STI, observations also reported from Iran (37). This may be explained by the fact that young sex workers are more likely to report inconsistent condom use and condomless sex with their last clients (38, 39). FSWs who started the business at earlier ages are also more likely to drink alcohol heavily (40) and experience multiple clients each day (41). However, these findings contrast the Rwandan study (38), which found no statistically significant association between sex work starting at earlier ages and STIs.

Our finding shows that, compared to those who had no formal education, the odds of experiencing at least one STI was lower among educated FSWs is consistent with findings from the studies in Rwanda (19), Russia (11), and Namibia (21). As reported by others (21), this could be because those with low educational levels are more likely to be unemployed and engage in risky sexual

TABLE 4 Factors associated with the number of sexually transmitted infections (STIs) among female sex workers, bio-behavioral survey, Ethiopia, 2020.

Characteristics	Count model coefficients (Truncated Poisson with log link)			Zero-hurdle model coefficients (binomial with logit link)			
		Incidence rate ratio	Std. err	95% CI	AOR	Std. err	95% CI
Age	15–25 (ref)						
	25–34	1.45	0.34	0.91, 2.3	2.99	0.25	2.54, 3.52**
	35–59	2.32	0.57	1.43, 3.74*	8.05	0.85	6.54, 9.91**
Educational status	Non-formal education (ref)						
	Primary school (1-8)	0.88	0.13	0.66, 1.17	0.69	0.06	0.59, 0.81**
	Secondary school and above	0.8	0.18	0.52, 1.23	0.52	0.05	0.42, 0.64**
Average monthly income	<65 (ref)						
	65 and 150 USD	0.86	0.13	0.64, 1.16	0.70	0.06	0.60, 0.82**
	150 to 200 USD	0.83	017	0.55, 1.23	0.68	0.07	0.56, 0.82**
	200 and above USD	0.74	0.19	0.44, 1.24	0.51	0.06	0.42, 0.63**
Depression	No depression (ref)						
	Mild depression	0.97	0.16	0.70, 1.34	1.04	0.08	0.90, 1.20
	Moderate to severe	1.55	0.26	1.12, 2.18*	1.21	0.11	1.01, 1.45**
Drug use–AUDIT score	Social drinking/not risky (ref)						
	Harmful/hazardous drinking	0.81	0.14	0.58, 1.15	1.06	0.09	0.89, 1.27
	Alcohol dependency indication	0.62	0.10	0.45, 0.86*	1.06	0.08	0.91, 1.24
First sex forced	Wanted (ref)						
	Forced	1.32	0.19	1.01, 1.74*	1.13	0.09	0.97, 1.32
Age at first sex	≤15 (ref)						
	16–20	0.98	0.13	0.75, 1.28	0.72	0.05	0.63, 0.83**
	≥20	0.74	0.25	0.37, 1.45	0.67	0.10	0.50, 0.91**
Year lived as FSWs	≤5 (ref)						
	5–10	1.03	0.21	0.70, 1.52	1.30	0.11	1.10, 1.55**
	≥11	1.10	0.20	0.76, 1.58	1.21	0.10	1.03, 1.43**
Condom break	No (ref)						
	Yes	1.32	0.18	1.01, 1.74*	1.40	0.09	1.21, 1.61**

*Statistically significant Incidence rate ratio; **Statistically significant Adjusted odds ratio (AOR).

behaviors associated with STIs (42). Similarly, FSWs with low average monthly income were more likely to experience at least one STI in our series, which could be because the sex workers earned less money as they got older and had to do sex work more often with many clients, and the clients decided to use condoms (41).

Condom breakage increases the frequency of STIs and increases the probability of having STIs among FSWs as shown by our study, which identified that those with a history of condom breakage had an increase in the rate of developing at least one STI by 46%. This may be because those experiencing condom breakage were exposed to pornography and used sexual enhancement products (43). The finding is consistent with previous reports for Ethiopia (15) and China (44). Available evidence has also shown that consistent and high levels of condom use among FSWs decrease the incidence of STIs among sex workers as well as the general population.

Strengths and limitations

Overall, the advantage of our study, which was a nationwide survey involving a large sample of FSWs recruited by using the RDS technique from 16 towns across the country, outweighs a venue-based selection approach in terms of obtaining a representative sample. However, as this was a cross-sectional study, temporal relationships between determinants and the outcome cannot be established. In addition, key measures rely on self-report; biases

such as social desirability response bias could have some effect. The rapid test/serological markers we used in this survey do not detect the duration of infections as recent or long-term. In this study, we only considered HIV, HBV, HCV, and syphilis, our results could underestimate the overall prevalence of STIs.

Conclusion

The prevalence of STIs among FSWs is high in Ethiopia. Age, educational status, average monthly income, history of depression, history of condom breakage, early initiations of selling sex, and living as FSWs for a long period of time are identified as independent predictors for developing at least one STI. Targeted STI prevention and control programs need to be improved, with a focus on promoting higher education among women, condom distribution, and the creation of awareness on proper and consistent use, enhancing STI testing, prevention, care and treatment, interventions, and supporting income-generating activities. Further epidemiological research is needed on STIs among FSWs in Ethiopia to determine the magnitude of the problem, which should include a broader list of STIs, confirmatory diagnostic tests, and recency testing.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving human participants were reviewed and approved by PHI-IRB-108-2018 and the Ethiopian Public Health Institute. The participants provided their written informed consent to participate in this study.

Author contributions

FW, SA, JA, SL, and BB conceived the manuscript. FW wrote the first draft and with further contributions from all authors. JA and DA conducted the statistical analysis with support from FW, BB, JT, AB, MR, and SA. JA and DA undertook data management. LN, GE, WB, MA, FW, and SA are principal investigators (PIs). FW was the guarantor of the manuscript, accepted full responsibility for the study, had access to the data, and controlled the decision to publish. All authors contributed to data interpretation, reviewed successive drafts, and approved the final version of the manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Zheng Y, Yu Q, Lin Y, Zhou Y, Lan L, Yang S, et al. Global burden and trends of sexually transmitted infections from 1990 to 2019: an observational trend study. *Lancet Infect Dis.* (2021) 22:541–551. doi: 10.1016/S1473-3099(21)00448-5

2. World Health Organization. *Global health sector strategies on, respectively, HIV, viral hepatitis and sexually transmitted infections for the period 2022-2030.* Geneva: World Health Organization (2022). Available online at: https://www.who.int/publications-detail-redirect/9789240053779 (accessed December 14, 2022).

3. Holmes KK, Bertozzi S, Bloom BR, Jha P, Gelband H, DeMaria LM, et al. Major infectious diseases: key messages from disease control priorities, Third Edition. In: Holmes KK, Bertozzi S, Bloom BR, Jha P, editors *Major Infectious Diseases,* Washington (DC): The International Bank for Reconstruction and Development/The World Bank (2017). doi: 10.1596/978-1-4648-0524-0_ch1

4. World Health Organization. *Global progress report on HIV, viral hepatitis and sexually transmitted infections, 2021: accountability for the global health sector strategies 2016–2021: actions for impact: web annex 2: data methods.* Geneva: World Health Organization (2021). p. 28. Available online at: https://apps.who.int/iris/handle/10665/342813 (accessed March 2, 2022).

5. Lewis DA. HIV/sexually transmitted infection epidemiology, management and control in the IUSTI Africa region: focus on sub-Saharan Africa. *Sex Transm Infect.* (2011) 87:ii10–3. doi: 10.1136/sextrans-2011-050178

6. Gerbase AC, Mertens TE. Sexually transmitted diseases in Africa: time for action. *Afr Health.* (1998) 20:10–2.

7. Hanass-Hancock J, Carpenter B. Trends in health and disability in Botswana. An analysis of the global burden of disease study. *Disabil Rehabil.* (2021) 43:3606–12. doi: 10.1080/09638288.2020.1743774

8. de Martel C, Georges D, Bray F, Ferlay J, Clifford GM. Global burden of cancer attributable to infections in 2018: a worldwide incidence analysis. *Lancet Glob Health.* (2020) 8:e180–90. doi: 10.1016/S2214-109X(19)30488-7

9. Rashti R, Alavian SM, Moradi Y, Sharafi H, Mohamadi Bolbanabad A, Roshani D, et al. Global prevalence of HCV and/or HBV coinfections among people who inject drugs and female sex workers who live with HIV/AIDS: a systematic review and meta-analysis. *Arch Virol.* (2020) 165:1947–58. doi: 10.1007/s00705-020-04716-1

10. Chemaitelly H, Weiss HA, Smolak A, Majed E, Abu-Raddad LJ. Epidemiology of Treponema pallidum, Chlamydia trachomatis, Neisseria gonorrhoeae, Trichomonas vaginalis, and herpes simplex virus type 2 among female sex workers in the Middle East and North Africa: systematic review and meta-analytics. *J Glob Health.* (2019) 9:020408. doi: 10.7189/jogh.09.020408

11. Bernier A, Rumyantseva T, Reques L, Volkova N, Kyburz Y, Maximov O, et al. HIV and other sexually transmitted infections among female sex workers in Moscow (Russia): prevalence and associated risk factors. *Sex Transm Infect.* (2020) 96:601–7. doi: 10.1136/sextrans-2019-054299

12. Peitzmeier SM, Wirtz AL, Peryshkina A, Sherman S, Colantuoni E, Beyrer C, et al. Associations between violence and HIV risk behaviors differ by perpetrator among Russian sex workers. *AIDS Behav.* (2020) 24:812–22. doi: 10.1007/s10461-019-02668-5

13. Llangarí-Arizo LM, Sadiq ST, Márquez C, Cooper P, Furegato M, Zhou L, et al. Sexually transmitted infections and factors associated with risky sexual practices among female sex workers: A cross sectional study in a large Andean city. *PLoS ONE.* (2021) 16:e0250117. doi: 10.1371/journal.pone.0250117

14. Costa Ferreira-Júnior O, Guimarães MD, Damacena GN, de Almeida WD, de Souza-Júnior PR, Szwarcwald CL, et al. Prevalence estimates of HIV, syphilis, hepatitis B and C among female sex workers (FSW) in Brazil, 2016. *Medicine (Baltimore).* (2018) 97:S3–8. doi: 10.1097/MD.0000000000009218

15. Metaferia Y, Ali A, Eshetu S, Gebretsadik D. Seroprevalence and associated factors of human immunodeficiency virus, Treponema pallidum, Hepatitis B virus, and hepatitis C virus among female sex workers in Dessie city, northeast Ethiopia. *BioMed Res Int.* (2021) 2021:6650333. doi: 10.1155/2021/6650333

16. Patterson TL, Strathdee SA, Semple SJ, Chavarin CV, Abramovitz D, Gaines TL, et al. Prevalence of HIV/STIs and correlates with municipal characteristics among female sex workers in 13 Mexican cities. *Salud Publica Mex.* (2019) 61:116–24. doi: 10.21149/8863

17. Ingabire R, Parker R, Nyombayire J, Ko JE, Mukamuyango J, Bizimana J, et al. Female sex workers in Kigali, Rwanda: a key population at risk of HIV, sexually transmitted infections, and unplanned pregnancy. *Int J STD AIDS.* (2019) 30:557–68. doi: 10.1177/0956462418817050

18. Zalla LC, Herce ME, Edwards JK, Michel J, Weir SS. The burden of HIV among female sex workers, men who have sex with men and transgender women in Haiti: results from the 2016 Priorities for Local AIDS Control Efforts (PLACE) study. *J Int AIDS Soc.* (2019) 22:e25281. doi: 10.1002/jia2.25281

19. Mutagoma M, Nyirazinyoye L, Sebuhoro D, Riedel DJ, Ntaganira J. Syphilis and HIV prevalence and associated factors to their co-infection, hepatitis B and hepatitis C viruses prevalence among female sex workers in Rwanda. *BMC Infect Dis.* (2017) 17:525. doi: 10.1186/s12879-017-2625-0

20. Liu H, Dumenci L, Morisky DE, Xu Y, Li X, Jiang B. Syphilis among middle-aged female sex workers in China: a three-site cross-sectional study. *BMJ Open.* (2016) 6:e010420. doi: 10.1136/bmjopen-2015-010420

21. Jonas A, Patel SV, Katuta F, Maher AD, Banda KM, Gerndt K, et al. HIV Prevalence, Risk Factors for Infection, and Uptake of Prevention, Testing, and Treatment among Female Sex Workers in Namibia. *J Epidemiol Glob Health.* (2020) 10:351–8. doi: 10.2991/jegh.k.200603.001

22. Owczarzak J, Phillips SD, Cho W. "Pure" drug users, commercial sex workers and "ordinary girls": gendered narratives of HIV risk and prevention in post-Soviet Ukraine. *Cult Health Sex.* (2018) 20:1171–84. doi: 10.1080/13691058.2017.1421708

23. Kalemi G, Gkioka S, Tsapatsari P, Tzeferakos G, Kandri T, Psarra ML, et al. Stigma and self-esteem: A case of HIV-positive sex-workers. *Psychiatr Psychiatr.* (2017) 28:67–74. doi: 10.22365/jpsych.2017.281.67

24. Mo PKH, Lau JTF, Xin M, Fong VWI. Understanding the barriers and factors to HIV testing intention of women engaging in compensated dating in Hong Kong: The application of the extended theory of planned behavior. *PLoS ONE.* (2019) 14:e0213920. doi: 10.1371/journal.pone.0213920

25. Prakash R, Manthri S, Tayyaba S, Joy A, Raj SS, Singh D, et al. Effect of physical violence on sexually transmitted infections and treatment seeking behaviour among female sex workers in Thane District, Maharashtra, India. *PLoS ONE.* (2016) 11:e0150347. doi: 10.1371/journal.pone.0150347

26. Chemaitelly H, Weiss HA, Calvert C, Harfouche M, Abu-Raddad LJ, HIV. epidemiology among female sex workers and their clients in the Middle East and North Africa: systematic review, meta-analyses, and meta-regressions. *BMC Med.* (2019) 17:119. doi: 10.1186/s12916-019-1349-y

27. Pittman B, Buta E, Krishnan-Sarin S, O'Malley SS, Liss T, Gueorguieva R. Models for analyzing zero-inflated and overdispersed count data: an application to cigarette and marijuana use. *Nicotine Tob Res.* (2018) 22:1390–8. doi: 10.1093/ntr/nty072

28. Bertoli W, Conceição KS, Andrade MG, Louzada F. A new regression model for the analysis of overdispersed and zero-modified count data. *Entropy Basel Switz.* (2021) 23:646. doi: 10.3390/e23060646

29. Navarrete MS, Adrian C, Bachelet VC. Respondent-driven sampling: Advantages and disadvantages from a sampling method. *Medwave.* (2022) 21:e8513. doi: 10.5867/medwave.2022.01.002528

30. Garcini LM, Galvan T, Peña JM, Chen N, Klonoff E. Effectiveness of Respondent-Driven Sampling for Conducting Health Studies Among Undocumented Immigrants at a Time of Heightened Immigration Enforcement. *J Immigr Minor Health.* (2022) 24:102–10. doi: 10.1007/s10903-020-01112-4

31. World Health Organization, US Centers for Disease Control and Prevention, UNAIDS, FHI360. *Biobehavioral survey guidelines for populations at risk for HIV.* Geneva: World Health Organization (2017). p. 208. Available online at: https://apps.who.int/iris/handle/10665/258924 (accessed May 20, 2023).

32. Lee S, Ong AR, Chen C, Elliott M. Respondent Driven Sampling for Immigrant Populations: A Health Survey of Foreign-Born Korean Americans. *J Immigr Minor Health.* (2021) 23:784–92. doi: 10.1007/s10903-020-01077-4

33. Schober P, Vetter TR. Count data in medical research: poisson regression and negative binomial regression. *Anesth Analg.* (2021) 132:1378–9. doi: 10.1213/ANE.0000000000005398

34. Kleiber C, Zeileis A. Visualizing count data regressions using rootograms. *Am Stat.* (2016) 70:296–303. doi: 10.1080/00031305.2016.1173590

35. Green JA. Too many zeros and/or highly skewed? A tutorial on modelling health behaviour as count data with Poisson and negative binomial regression. *Health Psychol Behav Med.* (2021) 9:436–55. doi: 10.1080/21642850.2021.1920416

36. Coetzee J, Jewkes R, Gray GE. Cross-sectional study of female sex workers in Soweto, South Africa: Factors associated with HIV infection. *PLoS ONE.* (2017) 12:e0184775. doi: 10.1371/journal.pone.0184775

37. Moayedi-Nia S, Bayat Jozani Z, Esmaeeli Djavid G, Entekhabi F, Bayanolhagh S, Saatian M, et al. HIV, HCV, HBV, HSV, and syphilis prevalence among female sex workers in Tehran, Iran, by using respondent-driven sampling. *AIDS Care.* (2016) 28:487–90. doi: 10.1080/09540121.2015.1109582

38. Kelly-Hanku A, Weikum D, Badman SG, Willie B, Boli-Neo R, Kupul M, et al. Factors associated with HIV and syphilis infection among female sex workers in three cities in Papua New Guinea: findings from Kauntim mi tu, a biobehavioral survey. *Sex Health.* (2020) 17:311–20. doi: 10.1071/SH19218

39. Parcesepe AM, L'Engle KL, Martin SL, Green S, Suchindran C, Mwarogo P. Early sex work initiation and condom use among alcohol-using female sex workers in Mombasa, Kenya: a cross-sectional analysis. *Sex Transm Infect.* (2016) 92:593–8. doi: 10.1136/sextrans-2016-052549

40. Amogne MD, Agardh A, Abate E, Ahmed J, Asamoah BO. Determinants and consequences of heavy episodic drinking among female sex workers in Ethiopia: A respondent-driven sampling study. *PLoS ONE.* (2021) 16:e0252432. doi: 10.1371/journal.pone.0252432

41. Han L, Zhou C, Li Z, Poon AN, Rou K, Fuller S, et al. Differences in risk behaviours and HIV/STI prevalence between low-fee and medium-fee female sex workers in three provinces in China. *Sex Transm Infect.* (2016) 92:309–15. doi: 10.1136/sextrans-2015-052173

42. Liu H. Egocentric network and condom use among mid-age female sex workers in China: a multilevel modeling analysis. *AIDS Patient Care STDs.* (2016) 30:155–65. doi: 10.1089/apc.2015.0349

43. Bradley J, Rajaram SP, Isac S, Gurav K, Ramesh BM, Gowda C, et al. Pornography, sexual enhancement products, and sexual risk of female sex workers and their clients in Southern India. *Arch Sex Behav.* (2016) 45:945–54. doi: 10.1007/s10508-015-0486-4

44. Guida J, Hu L, Liu H. Sexual behavior with noncommercial partners: a concurrent partnership study among middle-aged female sex workers in China. *J Sex Res.* (2019) 56:670–80. doi: 10.1080/00224499.2018.1434115

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Coinfection and superinfection in ICU critically ill patients with severe COVID-19 pneumonia and influenza pneumonia: are the pictures different?

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Background: Similar to influenza, coinfections and superinfections are common and might result in poor prognosis. Our study aimed to compare the characteristics and risks of coinfections and superinfections in severe COVID-19 and influenza virus pneumonia.

Methods: The data of patients with COVID-19 and influenza admitted to the intensive care unit (ICU) were retrospectively analyzed. The primary outcome was to describe the prevalence and pathogenic distribution of coinfections/ICU-acquired superinfections in the study population. The secondary outcome was to evaluate the independent risk factors for coinfections/ICU-acquired superinfections at ICU admission. Multivariate analysis of survivors and non-survivors was performed to investigate whether coinfections/ICU-acquired superinfections was an independent prognostic factor.

Results: In the COVID-19 (n = 123) and influenza (n = 145) cohorts, the incidence of coinfections/ICU-acquired superinfections was 33.3%/43.9 and 35.2%/52.4%, respectively. The most common bacteria identified in coinfection cases were *Enterococcus faecium, Pseudomonas aeruginosa, and Acinetobacter baumannii* (COVID-19 cohort) and *A. baumannii, P. aeruginosa, and Klebsiella pneumoniae* (influenza cohort). A significant higher proportion of coinfection events was sustained by *Aspergillus* spp. [(22/123, 17.9% in COVID-19) and (18/145, 12.4% in influenza)]. The COVID-19 group had more cases of ICU-acquired *A. baumannii*, *Corynebacterium striatum* and *K. pneumoniae*. *A. baumannii*, *P. aeruginosa*, and *K. pneumoniae* were the three most prevalent pathogens in the influenza cases with ICU-acquired superinfections. Patients with APACHE II ≥18, CD8+ T cells ≤90/μL, and 50 < age ≤ 70 years were more susceptible to coinfections; while those with CD8+ T cells ≤90/μL, CRP ≥120 mg/L, IL-8 ≥ 20 pg./mL, blood glucose ≥10 mmol/L, hypertension, and smoking might had a higher risk of ICU-acquired superinfections in the COVID-19 group. ICU-acquired superinfection, corticosteroid administration for COVID-19 treatment before ICU admission, and SOFA score ≥ 7 were independent prognostic factors in patients with COVID-19.

Conclusion: Patients with COVID-19 or influenza had a high incidence of coinfections and ICU-acquired superinfections. The represent agents of

coinfection in ICU patients were different from those in the general ward. These high-risk patients should be closely monitored and empirically treated with effective antibiotics according to the pathogen.

KEYWORDS

COVID-19, influenza, coinfection, ICU-acquired superinfection, bacteria

Introduction

The coronavirus disease 2019 (COVID-19) outbreak has caused a global health crisis and led to a high rate of critical illness (1). Although the Omicron variant might cause milder cases, mortality increased during the Omicron period, even in a highly vaccinated population (2). Influenza virus infection is also a global public health problem that has caused major morbidity and mortality in many countries (3). Both predispose patients to coinfections and superinfections, especially with bacteria, which could promote severe disease and necessitate timely diagnosis (4–6).

Coinfections in patients with COVID-19 seem uncommon, ranging from 0 to 19% (7–12). However, similar to influenza, superinfections are common in COVID-19, which can follow the initial infection phase or occur during recovery (13). Early recognition of patients with a high risk of coinfections/superinfections is important for the early use of antibiotics and in implementing measures to limit the possibility of superinfection, which may, in turn, reduce mortality, especially in the intensive care unit (ICU). It could even reduce antibiotic resistance caused by the unnecessary use of antibiotics. However, as of March 2023, the risk factors and characteristics related to coinfections/superinfections in COVID-19 and influenza cases in the same ICU have not been described.

Therefore, we aimed to describe the prevalence, pathogenic distribution and clinical characteristics of coinfections/superinfections in patients with COVID-19 and influenza in the same ICU. We also explored the predictive factors of coinfections/superinfections, which might help choose the appropriate application time and variety of antibiotics.

Materials and methods

Study design and patients

This retrospective observational study included patients with severe influenza virus pneumonia from December 1, 2017, to February 28, 2022, and patients with severe COVID-19 from December 1, 2022, to February 28, 2023, admitted to the respiratory ICU (RICU) of China-Japan Friendship Hospital in China. Patients younger than 18 years of age were excluded.

Demographics, clinical data, and results of microbiological examinations were extracted from the electronic medical record management system. Due to the study's retrospective nature, the need for informed consent from the patients or their legal guardians was waived. The study was approved by the institutional ethics committees of China-Japan Friendship Hospital.

The primary outcome in our study was a description of the prevalence and pathogenic distribution of coinfections and ICU-acquired superinfections in patients with COVID-19 and influenza. The secondary outcome was an evaluation of the independent risk factors for coinfections/ICU-acquired superinfections at ICU admission. Multivariate analysis of survivors and non-survivors was performed to investigate whether coinfections/ICU-acquired superinfections was an independent prognostic factor.

Diagnostic criteria

All patients with influenza infection underwent testing using nasopharyngeal swabs or lower respiratory tract (LRT) specimens. Two methods were used for laboratory diagnosis: polymerase chain reaction (PCR) and serological testing (14, 15). Severe influenza virus pneumonia was defined as the presence of influenza infection and severe community-acquired pneumonia (CAP) (16).

SARS-CoV-2 infection was confirmed via viral genome positivity in PCR or antigen, according to the Diagnosis and Treatment of Novel Coronavirus Infection Interim Guidance Report by the National Health Commission of the People's Republic of China (17). Severe COVID-19 was defined as any one of the following: (1) shortness of breath with respiratory rate $\geq$ 30 per minute; (2) pulse oxygen saturation $\leq$ 93% in the resting state; and (3) partial pressure of oxygen/fraction of inspiration oxygen (PaO_2/FiO_2) $\leq$300 mmHg. Critically ill patients satisfied any one of the following criteria: (1) respiratory failure where invasive ventilation is needed, (2) shock, and (3) failure of any other organ and need for ICU care (17).

Definitions

Coinfection was defined as pathogen detection via diagnostic test at the time of or within the first 48 h of ICU admission. If detection occurred $\geq$48 h after ICU admission, the infection was defined as an ICU-acquired superinfections. These tests included cultures of the respiratory tract secretions (sputum, bronchoalveolar lavage fluid, and endotracheal aspiration), multiplex respiratory PCRs performed on a nasopharyngeal swab or on respiratory tract secretions, metagenomic next-generation sequencing (mNGS) for respiratory tract secretions, and urinary antigen test for *Streptococcus pneumoniae*. The final diagnosis of causative agents was made according to the clinical physician expert groups combining imaging and clinical symptoms. Tracheobronchitis was defined as follows: The combination of fever (>38°C) with no other recognizable cause, new or increased sputum production, and a positive tracheal aspirate culture without radiographic evidence of pneumonia (18). Pneumonia was defined by the presence of new or progressive radiographic infiltrate associated with two of the following criteria: (1) Fever, temperature above 38°C; (2) leukocyte count above 10×10^9/L or below 4×10^9/L, and (3) purulent endotracheal

aspirate (19). Fungal infection was diagnosed according to the taskforce report on the diagnosis and clinical management of COVID-19 associated pulmonary aspergillosis (20) and clinical practice guideline for the management of candidiasis: 2016 Update by the Infectious Diseases Society of America (21).

Statistical analysis

Continuous variables are presented as mean ± standard deviation or median (interquartile range) and compared using a *t*-test or Mann–Whitney *U* test. Categorical variables were described using percentages and compared using the chi-square or Fisher's exact tests. All significance tests were two-tailed, and statistical significance was defined as $p \leq 0.05$. Factors associated with coinfections/ICU-acquired superinfections were evaluated using univariate and multivariate analyzes. The multivariate analysis included all variables ($p < 0.1$) from the univariate analysis and the factors reported to be associated with coinfections/ICU-acquired superinfections. All results were analyzed using SPSS for Windows, version 26 (IBM Corp, Armonk, NY, United States).

Results

A total of 123 patients with confirmed COVID-19 during the study period were included. The median age of them was 69 (59–78) years, 99/123 (80.5%) of the patients were male. And 87/123 (70.7%) had underlying diseases (60.2% hypertension, 39.8% diabetes, 11.4% chronic heart failure, 22% chronic renal failure, 13.8% chronic lung disease and 40.7% immunocompromised). The Acute Physiology And Chronic Health Evaluation (APACHE) II and Sequential Organ Failure Assessment (SOFA) scores were 17 (12–23) and 6(4–10), respectively. Among them, 78/123 (63.4%) underwent bronchoscopy to obtain bronchoalveolar lavage fluid (BALF) specimens, mNGS of the BALF was performed for 59/123 (48%) patients.

In total, 145 patients with confirmed influenza were identified during three consecutive influenza seasons from December 2017 to February 2022. The median age was 58 (46–69) years, 95/145 (65.5%) were male, and 82 (56.6%) patients had underlying diseases. The APACHE II and SOFA scores were 19 (14–23) and 7 (4–11), respectively (Supplementary Table 3).

Prevalence and pathogenic distribution of coinfections

Among the 123 patients with COVID-19, 41/123 (33.3%) had coinfections: 27/123 (22%) with bacterial infections, 20/123 (16.3%) with fungal infections, and 6/123 (4.9%) with viral infections. The most common bacteria were *Enterococcus faecium* (9/123, 7.3%), *Pseudomonas aeruginosa* (9/123, 7.3%), *Acinetobacter baumannii* (6/123, 4.9%). The most common gram-positive bacteria were *E. faecium* (9/123, 7.3%), *S. pneumoniae* (5/123, 4.1%) and *Staphylococcus aureus* (5/123, 4.1%), which included 1/5 (20%) case of Methicillin-resistant *S. aureus* (MRSA). The most common gram-negative bacteria were *P. aeruginosa* (9/123, 7.3%), *A. baumannii* (6/123, 4.9%), and *Klebsiella pneumoniae* (5/123, 4.1%). Of these, multidrug-resistant (MDR) organisms accounted for 16.6% (4/24). *Aspergillus* spp. (22/123, 17.9%) and *Candida spp.* (7/123, 5.7%) were the predominant causes of fungal infection; 6/123 (4.9%) patients with COVID-19 were positive for *Human Cytomegalovirus* (CMV) (Table 1; Figure 1).

Among the patients with influenza, the prevalence of coinfection was 35.2% (51/145), similar to those with severe COVID-19 patients (35.2% vs. 33.3%, $p = 0.752$). Among them, 29/145 (20%) pathogens were bacteria, 20/145 (13.8%) were fungi, and 10/145 (6.9%) were viruses. Gram-positive bacteria mainly included *S. aureus* (5/145, 3.4%), which included 2/5 (40%) MRSA. The main gram-negative bacteria detected were *A. baumannii* (14/145, 9.7%), *P. aeruginosa* (10/145, 6.9%), and *K. pneumoniae* (6/145, 4.1%). The gram-negative bacteria were MDR, being more than those in the COVID-19 group (46.2% vs. 16.6%, $p = 0.017$). For fungi, *Aspergillus* spp. (18/145, 12.4%) was the most frequently reported, followed by *Candida spp.* (3/145, 2.1%) and *Rhizopus spp.* (1/145, 0.7%). *CMV* (9/145, 6.2%) was the most common virus. The prevalence of bacteria, fungi and viruses was also similar to that of the COVID-19 group (Table 1; Figure 2).

Prevalence and pathogenic distribution of ICU-acquired superinfections

Overall, 54/123 (43.9%) patients with COVID-19 experienced ICU-acquired superinfections: 50/123 (40.7%) and 15/123 (12.2%) had bacterial and fungal infections, respectively. *A. baumannii* (27/123, 22%) was the most common bacteria, followed by *C. striatum* (23/123, 18.7%), *K. pneumoniae* (8/123, 6.5%), *Escherichia coli* (5/123, 4.1%), and *E. faecium* (5/123, 4.1%). Also, 59.6% of the patients had MDR bacteria infections. For fungi, we mainly found *Aspergillus* spp. (16/123, 13%), *Candida spp.* (5/123, 4.1%) and *Rhizopus spp.* (2/123, 1.6%). There was no ICU-acquired virus superinfection in COVID-19 patients (Table 1; Figure 3).

A total of 76/145 (52.4%) patients had ICU-aqcuired superinfections in the influenza group: most were bacteria (69/145, 47.6%), followed by fungi (13/145, 9.0%) and viruses (19/145, 13.1%). The prevalence of bacteria and fungi were similar to that of the COVID-19 group. *A. baumannii* (41/145, 28.3%), *P. aeruginosa* (31/145, 21.4%), *K. pneumoniae* (17/145, 11.7%), and *Burkholderia cenocepacia* (17/145, 11.7%) were the most prevalent bacteria. The proportions of *P. aeruginosa, Stenotrophomonas maltophilia*, and *B. cenocepacia* were higher than in the COVID-19 group (21.4% vs. 3.3%, $p < 0.001$; 7.6% vs. 0.8%, $p = 0.008$; 11.7% vs. 2.4%, $p = 0.004$, respectively). However, the influenza group had fewer *Corynebacterium striatum* infections than the COVID-19 group (2.1% vs. 18.7%, $p < 0.001$). In addition， 60.2% of the patients had MDR bacteria, similar to the COVID-19 group. The fungal infections included *Candida* spp. (8/145, 5.5%), *Aspergillus spp.* (6/145, 4.1%) and *Pneumocystis jirovecii* (1/145, 0.7%).The influenza group had less *Aspergillus spp.* infection than COVID-19 group (4.1% vs. 13%, $p = 0.008$). ICU-acquired CMV infection accounted for 11.7% (17/145) of causes, which should be of great concern (Table 1; Figure 4).

Risk factors for coinfections

The univariate analysis of patients with COVID-19 showed no significant differences in age, APACHE II, SOFA, and comorbidities on diagnosis between patients with and without coinfections (Supplementary Table 4). Combining the factors reported in the literature associated with coinfection,

TABLE 1 Pathogens in COVID-19 and influenza patients with co-infections and ICU-acquired superinfections.

	Coinfections			ICU-acquired superinfections		
	COVID-19 *N* = 123	Influenza *N* = 145	*p* value	COVID-19 *N* = 123	Influenza *N* = 145	*p*-value
Bacterial infection	27 (22)	29 (20)	0.695	50 (40.7)	69(47.6)	0.255
Gram-positive						
Enterococcus faecium	9 (7.3)	0	0.001	5 (4.1)	1 (0.7)	0.097
Staphylococcus aureus	5 (4.1)	5 (3.4)	1	3 (2.4)	1 (0.7)	0.336
MRSA	1 (20)	2 (40)	1	3 (100)	1 (100)	-
MSSA	0	3 (60)	0.167	0	0	-
Streptococcus pneumoniae	5 (4.1)	0	0.019	1 (0.8)	0	0.459
Streptococcus constellatus	0	0	-	1 (0.8)	0	0.459
Corynebacterium striatum	3 (2.4)	4 (2.8)	1	23 (18.7)	3 (2.1)	<0.001
Enterococcus faecali	3 (2.4)	0	0.095	1 (0.8)	0	0.459
Tropheryma whipplei	3 (2.4)	0	0.095	0	0	-
Streptococcus agalactiae	1 (0.8)	0	0.459	1 (0.8)	0	0.459
Mycobacterium tuberculosis	0	1 (0.7)	1	2 (1.6)	1 (0.7)	0.595
Gram-negtive						
Pseudomonas aeruginosa	9 (7.3)	10 (6.9)	0.894	4 (3.3)	31 (21.4)	<0.001
Acinetobacter baumannii	6 (4.9)	14 (9.7)	0.138	27 (22)	41 (28.3)	0.236
Klebsiella pneumoniae	5 (4.1)	6 (4.1)	0.976	8 (6.5)	17 (11.7)	0.143
Escherichia coli	0	0	0	5 (4.1)	2 (1.4)	0.253
Stenotrophomonas maltophilia	3 (2.4)	1 (0.7)	0.336	1 (0.8)	11 (7.6)	0.008
Achromobacter xylosoxidans	1 (0.8)	1 (0.7)	1	1 (0.8)	1 (0.7)	1
Burkholderia cenocepacia	0	5 (3.4)	0.064	3 (2.4)	17 (11.7)	0.004
Enterobacter cloacae	0	0	–	2 (1.6)	4 (2.8)	0.690
Ralstonia mannitolilytica	0	2 (1.4)	0.502	2 (1.6)	4 (2.8)	0.690
Klebsiella aerogenes	0	0	–	1 (0.8)	0	0.459
Klebsiella oxytoca	0	0	–	0	1 (0.7)	1
Haemophilus influenzae	0	0	–	1 (0.8)	0	0.459
Citrobacter koseri	0	0	–	1 (0.8)	0	0.459
Elizabethkingia meningoseptica	0	0	–	1 (0.8)	0	0.459
Burkholderia polyphagia	0	0	–	0	3 (2.1)	0.252
Acinetobacter picotelli	0	0	–	0	1 (0.7)	1
MDR	4 (16.6)	18 (46.2)	0.017	34 (59.6)	80 (60.2)	0.948
Fungal infection	20 (16.3)	20 (13.8)	0.572	15 (12.2)	13 (9.0)	0.389
Aspergillus spp	22 (17.9)	18 (12.4)	0.210	16 (13)	6 (4.1)	0.008
Candida spp	7 (5.7)	3 (2.1)	0.119	5 (4.1)	8 (5.5)	0.581
Rhizopus spp	0	1 (0.7)	1	2 (1.6)	0	0.210
Pneumocystis jirovecii	3 (2.4)	0	0.095	0	1 (0.7)	1
Viral infection	6 (4.9)	10 (6.9)	0.487	0	19 (13.1)	<0.001
CMV	6 (4.9)	9 (6.2)	0.637	0	17 (11.7)	<0.001
RSV	0	3 (2.1)	0.252	0	2 (1.4)	0.502
Others	0	0	–	0	0	–
Mycoplasma pneumoniae	0	0	–	0	1 (0.7)	1
Chlamydia psittaci	1 (0.8)	0	0.459	0	0	–
Ureaplasma	2 (1.6)	0	0.210	0	0	–

COVID-19, coronavirus disease 2019; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-sensitive *Staphylococcus aureus*; MDR, multiple drug resistant; CMV, human cytomegalovirus; RSV, respiratory syncytial virus.

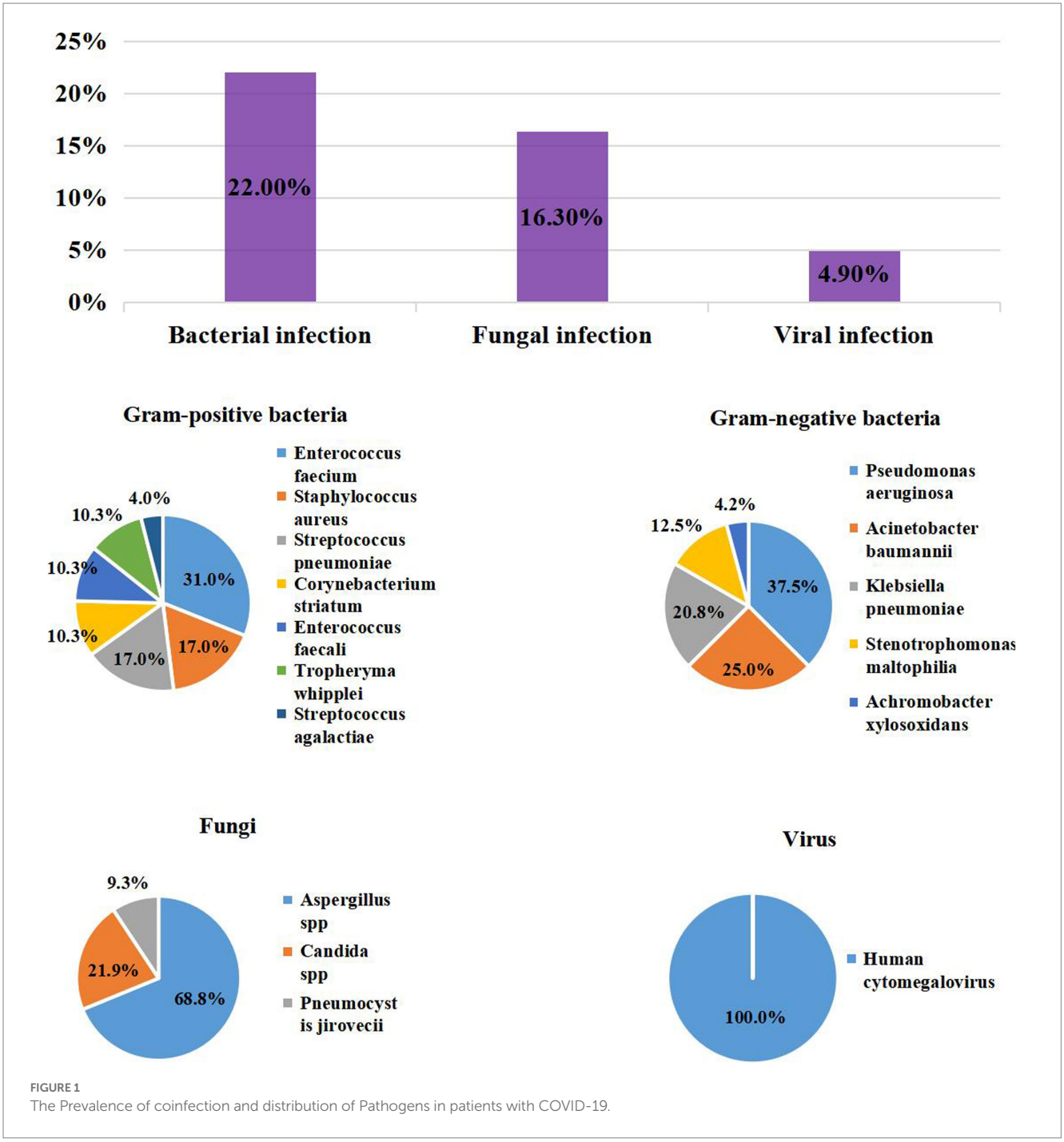

FIGURE 1
The Prevalence of coinfection and distribution of Pathogens in patients with COVID-19.

we conducted a multivariate analysis. APACHE II ≥18 (OR: 2.309; 95%CI: 1.005–5.304; $p = 0.049$), CD8+ T cells ≤90/μL (OR: 2.466; 95%CI: 1.084–5.612; $p = 0.031$), and 50 < age ≤ 70 years (OR: 2.680; 95%CI: 1.183–6.072; $p = 0.018$) were independent risk factors for coinfection (Table 2).

In the influenza cohort, the time from illness onset to ICU admission was longer in patients with coinfections [13 (7–22) vs. 8 (5.5–11.5), $p = 0.001$]. Patients with coinfections had a lower body mass index (BMI) than those without coinfections [23.38 (21.40–25.90) vs. 25.34 (22.49–28.01), $p = 0.012$]. The white blood cell count and prothrombin time were higher in patients with coinfection (Supplementary Tables 4, 5). After multivariate analysis, BMI ≤23.5 kg/m^2 (OR: 2.722; 95%CI: 1.304–5.683; $p = 0.008$) and white blood cell (WBC) count ≥10 × 10^9/L (OR: 2.102; 95% CI: 1.009–4.380; $p = 0.047$) were independent predictive variables for coinfections in patients with influenza (Table 2).

Risk factors of ICU-acquired superinfections

Patients with COVID-19 who developed ICU-acquired superinfections had higher APACHE II [19.5 (14.25–26) vs. 14 (10.75–21.25), $p = 0.004$] and SOFA [7 (5–10) vs. 4 (2.75–8), $p = 0.002$] scores and higher rates of smoking (48.1% vs. 23.2%, $p = 0.004$), dyspnea (100% vs. 84.1%, $p = 0.002$), and hypertension (72.2%

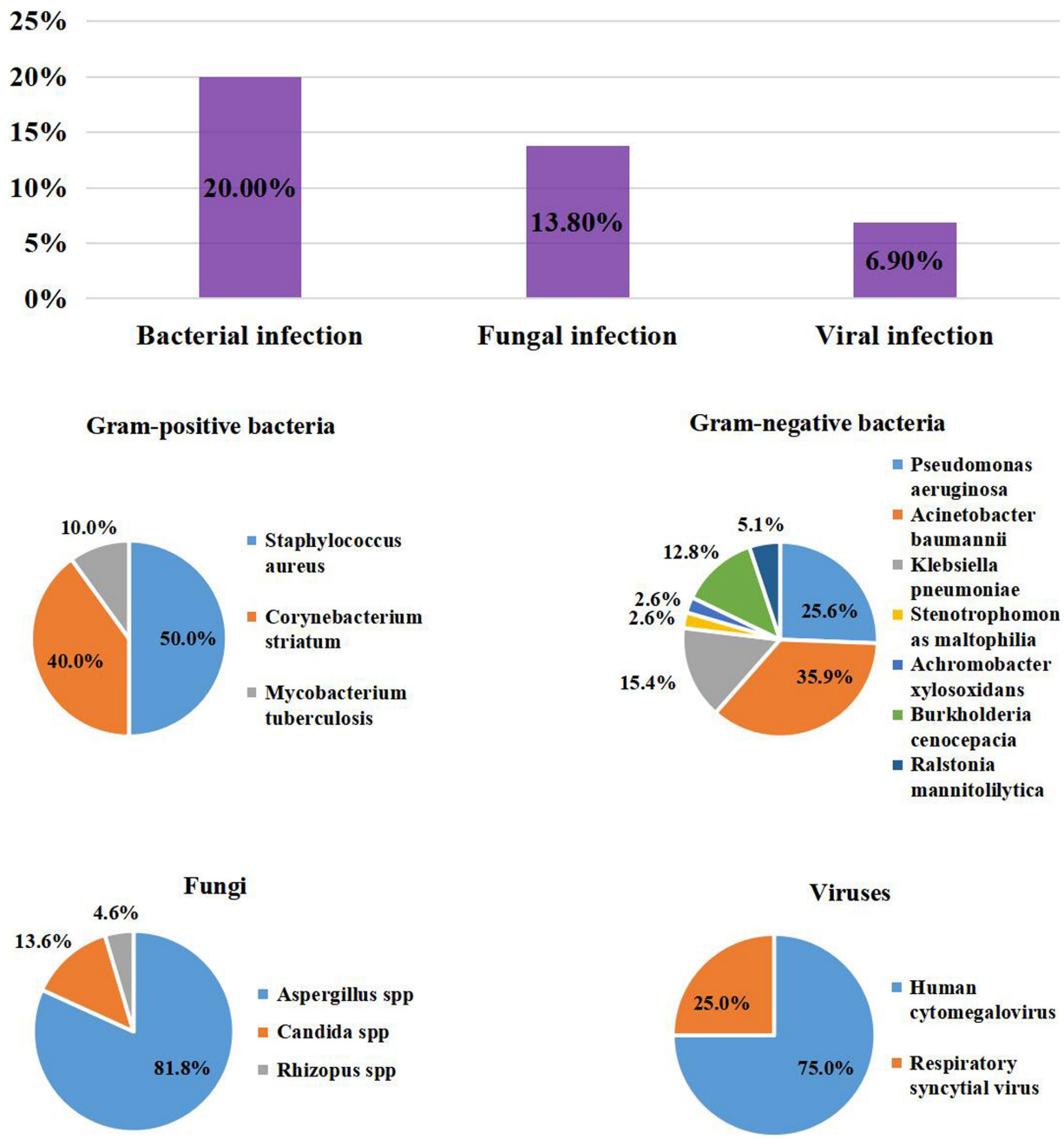

FIGURE 2
The Prevalence of coinfection and distribution of Pathogens in patients with Inluenza.

vs. 50.7%, p = 0.016; Supplementary Table 1). The levels of neutrophils, C-reactive protein (CRP), procalcitonin, interleukin-6 (IL-6), interleukin-8 (IL-8), and D-dimer were higher and, the level of CD8+ T cells was lower in patients with ICU-acquired superinfections than in those without (Supplementary Tables 6, 7). Multivariate analysis showed that CD8+ T cells ≤90/μL (OR: 6.016; 95%CI: 2.270–15.944; p < 0.001), CRP ≥120 mg/L (OR: 4.111; 95%CI: 1.508–11.208; p = 0.006), IL-8 ≥ 20 pg./mL (OR: 3.178; 95%CI: 1.233–8.192; p = 0.017), blood glucose ≥10 mmol/L (OR: 2.843; 95%CI: 1.101–7.341; p = 0.031), hypertension (OR: 2.694; 95%CI: 1.041–6.973; p = 0.041), and smoking (OR: 4.599; 95%CI: 1.723–12.275; p = 0.002) were independent risk factors for ICU-acquired superinfections in the COVID-19 cohort (Supplementary Table 1).

In patients with influenza, we did not find a significant difference in demographic characteristics between patients with ICU-acquired superinfections and those without. However, patients without ICU-acquired superinfections had a higher rate of fever than those with ICU-acquired superinfections (Supplementary Tables 6, 7). However, in the multivariate analysis, WBC ≥10 × 10^9/L(OR: 2.419; 95%CI: 1.175–4.983; p = 0.017), fever (OR: 0.263; 95%CI: 0.084–0.826; p = 0.022), expectoration (OR: 0.328; 95%CI: 0.129–0.835; p = 0.019) and dyspnea (OR 4.190; 95%CI: 1.229–14.291; p = 0.022) were associated with a higher rate of ICU-acquired superinfections in patients with influenza (Supplementary Table 1).

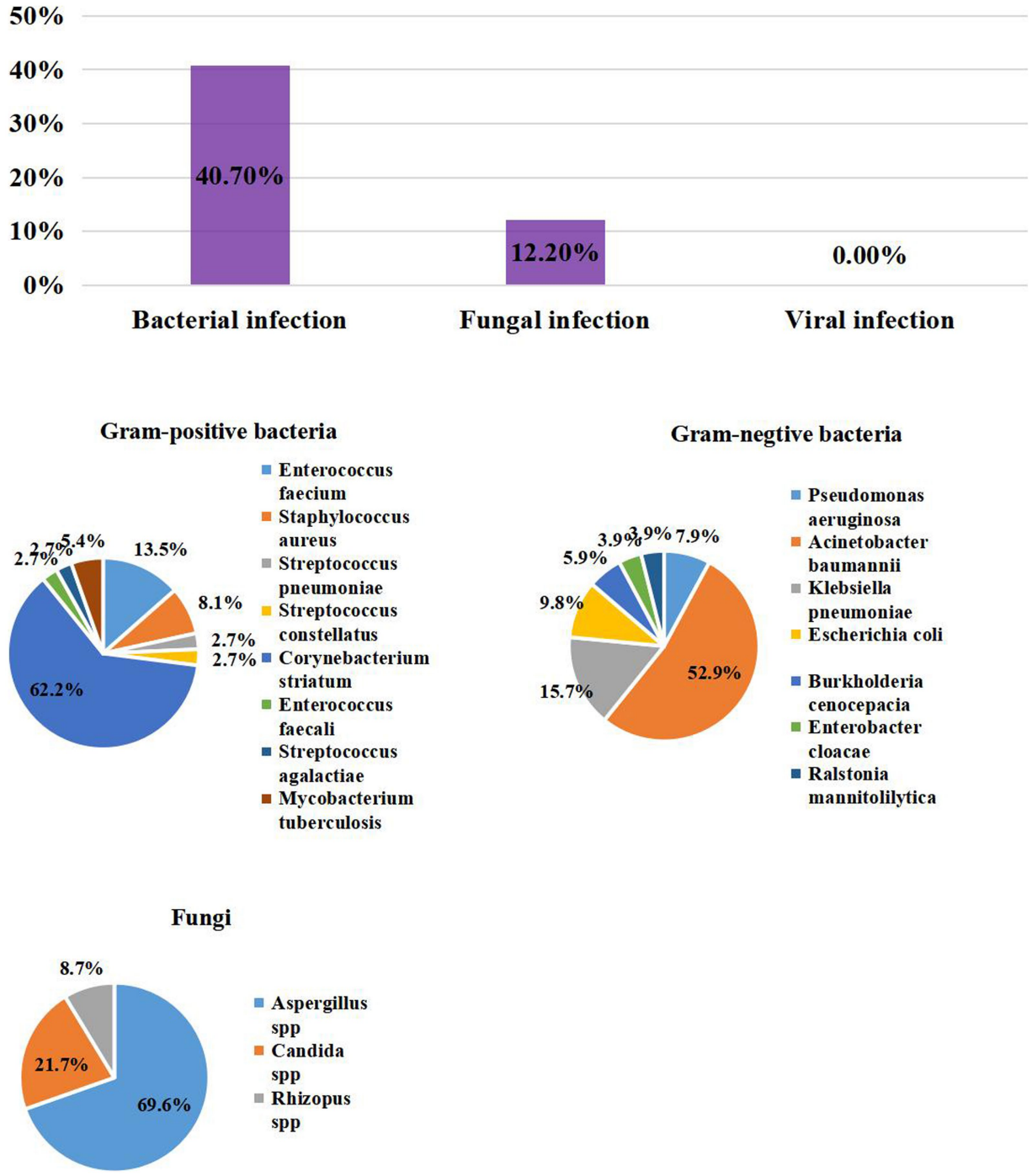

FIGURE 3
The Prevalence of ICU – acquired infection distribution of Pathogens in patients with COVID-19.

Influence of coinfections and ICU-acquired superinfections on treatment and prognosis

In severe COVID-19 pneumonia, the use of antifungal agents (80% vs. 31.6%, $p < 0.001$) was higher in patients with coinfections. Also, the following were significantly higher in patients with coinfections: incidence of acute kidney injury (AKI; 51.4% vs. 20.3%, $p = 0.001$), need for tracheal intubation (80.5% vs. 44.4%, $p < 0.001$), tracheotomy (56.1% vs. 23.8%, $p < 0.001$), extracorporeal membrane oxygenation (20.5% vs. 4.1%, $p = 0.008$), and prone position (61.5% vs. 25.7%, $p < 0.001$). There was a significantly longer duration of intensive positive-pressure ventilation (IPPV) in patients with coinfections [6 (0.25–17) vs. 0 (0–7.5), $p = 0.003$]. However, the treatment and outcomes were similar between patients with and without coinfection in the influenza cohort (Table 3).

The need of tracheal intubation (90.7% vs. 29.4%, $p < 0.001$ and 89.5% vs. 54.4%, $p < 0.001$), the length of IPPV (10.5 days vs. 0 days,

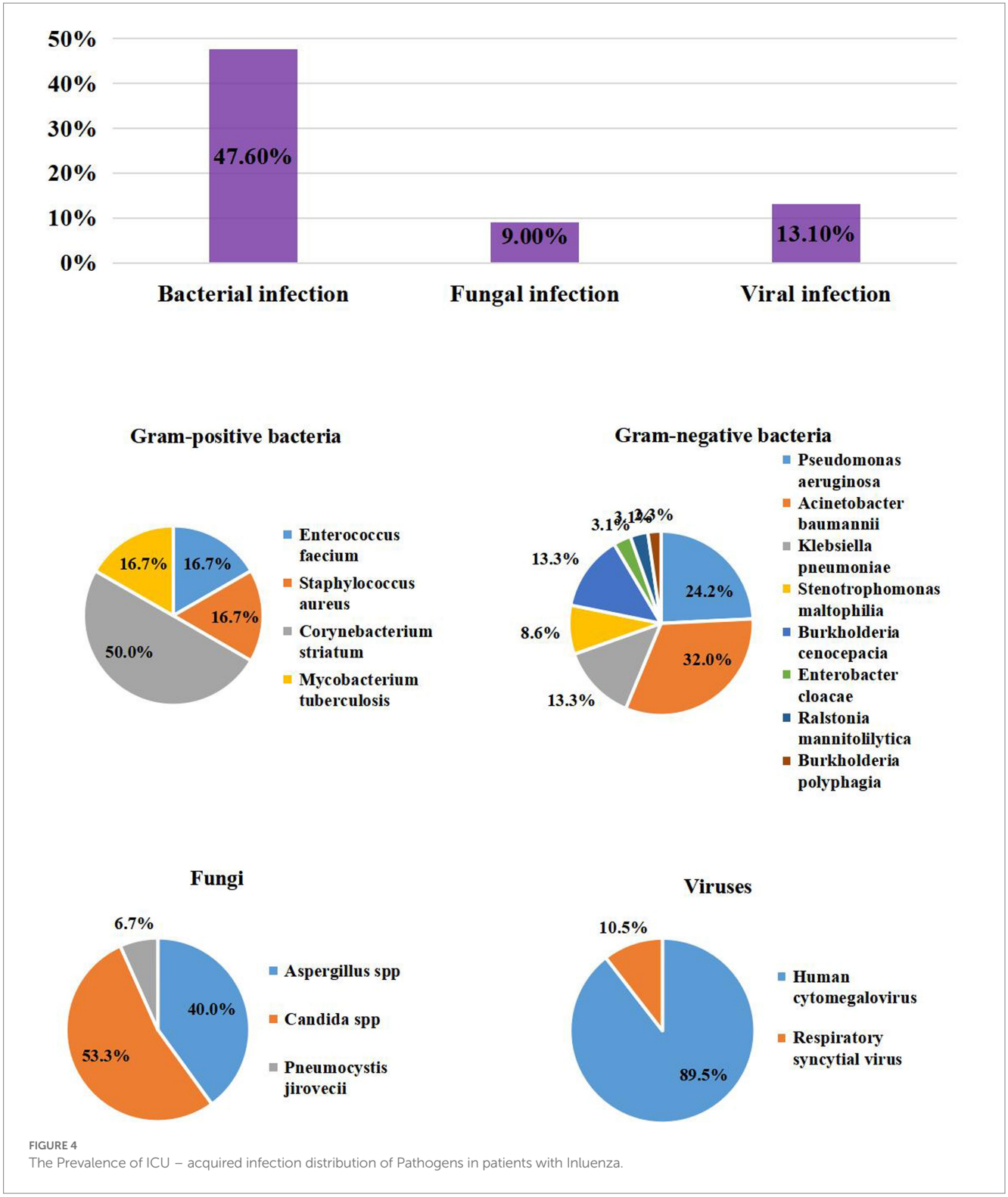

FIGURE 4
The Prevalence of ICU – acquired infection distribution of Pathogens in patients with Inluenza.

$p < 0.001$ and 14 days vs. 5 days, $p < 0.001$), and length of ICU stay (14 days vs. 5.5 days, $p < 0.001$ and 18 days vs. 7 days, $p < 0.001$) were higher in patients with ICU-acquired superinfections in both the COVID-19 and influenza groups. In the COVID-19 group, patients with ICU-acquired superinfections, had higher rates of AKI (50% vs. 14.8%, $p < 0.001$), cardiovascular failure (52.1% vs. 27.9%, $p = 0.010$), gastrointestinal bleeding (34% vs. 16.1%, $p = 0.030$), CRRT (44.9% vs. 15.5%, $p = 0.001$), tracheotomy (66.7% vs. 9%, $p < 0.001$), prone position (62% vs. 19%, $p < 0.001$) and recruitment (11.8% vs. 1.7%, $p = 0.048$; (Table 4)).

In the COVID-19 group, the hospital survival rate was lower in patients with ICU-acquired superinfections (18.5% vs. 56.5%, $p < 0.001$). By comparing survivors and non-survivors, we found that ICU-acquired superinfection (OR: 3.677; 95%CI: 1.518–8.906; $p =$

TABLE 2 Independent risk factors for coinfection of COVID-19 and influenza.

Variable	COVID−19			Influenza		
	p value	OR	95% CI	*p* value	OR	95% CI
APACHEII ≥18	0.049	2.309	1.005–5.304	–	–	–
CD8+ T cell ≤90/μL	0.031	2.466	1.183–6.072	–	–	–
50 < Age ≤ 70 years	0.018	2.680	1.183–6.072	–	–	–
BMI ≤23.5 kg/m^2	–	–	–	0.008	2.722	1.304–5.683
WBC ≥10× 10^9 /L	–	–	–	0.047	2.102	1.009–4.380

APACHE, acute physiology and chronic health evaluation; BMI, body mass index; WBC, white blood cell.

0.004), corticosteroid administration for COVID-19 treatment before ICU admission (OR: 0.317; 95%CI: 0.125–0.805; $p = 0.016$), and SOFA score ≥ 7 (OR: 6.710; 95%CI: 2.536–17.754; $p < 0.001$) were independent prognostic factors (Supplementary Table 2). However, the hospital survival rate was similar regardless of ICU-acquired superinfection in patients with severe influenza virus pneumonia.

Discussion

In this study, the prevalence of respiratory coinfections/ICU-acquired superinfections in the COVID-19 and influenza cohorts were 33.3%/43.9 and 35.2%/52.4%, respectively. Bacteria were isolated more frequently not only in coinfection but also in ICU-acquired superinfections cases. The most common bacteria identified in coinfection cases were *P. aeruginosa*, *E. faecium, and A. baumannii* in patients with COVID-19 and *P. aeruginosa*, *A. baumannii, and K. pneumoniae* in patients with influenza. Besides, a significant higher proportion of coinfection events was sustained by *Aspergillus* spp. The COVID-19 group had more cases of ICU-acquired *A. baumannii, C. striatum* and *K. pneumoniae*. *P. aeruginosa*, *A. baumannii*, and *K. pneumoniae* were the three most prevalent pathogens in the influenza cases with ICU-acquired superinfections. In addition, ICU-acquired superinfection was an independent prognostic factor in the COVID-19 group.

Our study demonstrated a higher proportion of patients with coinfections in the COVID-19 group (33.3%). Previous studies and reviews have reported variable coinfection rates, ranging from 3.5 to 14%, focusing on patients from general wards with bacterial infections (7, 8, 11, 12, 22–24). Only a few studies have reported coinfection data from COVID-19 cohort in the ICU (26.9–28%) (11, 25, 26). The coinfection rate in our ICU was higher than that in others. In addition, the main pathogens were gram-negative bacilli and *Aspergillus spp.*, which was not in accordance with previous studies that reported that *S. aureus* and other common community-acquired bacteria were prevalent in coinfections (8, 11, 26). In our influenza cohort, the prevalence of coinfections was similar to most of previous study (4, 27), but higher than others (28, 29).

Meanwhile, unlike other studies, which reported *S. aureus*, *S. pneumoniae* and *Hemophilus influenza* were the most commonly isolated co-infectious agents (4, 5, 27, 29), we found typical pathogens of nosocomial infections such as *A. baumannii* and *P. aeruginosa*. Some factors may explain this phenomenon. First, in our study, the time from onset to ICU admission was longer than in other studies (9 vs. 3–5.6 days). Only, 17.2% of the patients in our study were admitted directly from the emergency or outpatient department. The rest were hospitalized in general wards and other ICUs before being transferred to the ICU in our study. Second, we supposed that it was due to the inclusion bias in different studies but not the actual situation. The diagnostic criteria for coinfection were not standardized, which confounded pathogenicity and colonization. Most of studies that assumed coinfection as the pathogen were detected. In addition, the time of diagnosis of coinfection was not uniform. Many studies included secondary infection or mixed coinfection and secondary infection. Obviously, the proportion of coinfection with *Aspergillus* spp. was quite high in our study. Awareness of the possibility of fungal coinfection in COVID-19 is essential to initiate empirical antifungal therapy and fungal infection test as early as possible, which assisted in preventing severe illness and death from coinfection.

In the COVID-19 cohort, patients aged 50–70 years had the highest prevalence of coinfection (OR: 2.680), which was in agreement with studies that reported that older adult patients tended to have coinfection (30, 31). Hughes et al. (8) indicated that the age group of 55–81 years were predisposed to coinfection, which concorded with our study (50–70 years). APACHE II ≥18 and CD8+ T cell ≤90/μL were also independent risk factors for coinfection. These findings might imply that critical conditions due to disease and declined immune ability due to aging are the causes of coinfection in the older adult (32). In addition, BMI ≤23.5 kg/m^2 and WBC ≥10 × 10^9/L were predictive factors of influenza coinfection. A higher WBC count was a manifestation of coinfection. A large prospective study conducted by Langouche et al. reported that critical illness evokes adipose tissue accumulation of alternatively active M2 macrophages, which have local anti-inflammatory functions (33). Patients with a lower BMI may have weak anti-inflammatory abilities. Our study showed that patients with high-risk factors could be treated empirically with antibiotics after ICU admission. Antibiotics should cover both gram-positive and gram-negative bacteria. It should also be noted that patients with COVID-19 may have coinfection with fungi, and these patients should be promptly treated with antifungal therapy.

The pathogenesis of influenza coinfection has been elaborated. Influenza virus contributes to respiratory epithelial cell damage, bacterial mucociliary clearance dysfunction, and immune response dampening, enabling increased bacterial adherence and invasion (34, 35). As for COVID-19, the mechanism of the pathogenesis of coinfection remains indistinct, and we lack evidence to support the bacteria-virus association (7).

The incidence of ICU-acquired superinfections was similar in the COVID-19 and influenza cohort (43.9% vs. 53.4%). In both cohorts, gram-negative bacilli were responsible for most ICU-acquired superinfections. *A. baumannii*, *K. pneumoniae,* and *P. aeruginosa* were the most commonly identified bacteria. However, the COVID-19

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TABLE 3 Treatment and outcomes of COVID-19 and influenza patients with coinfection.

	COVID-19 N = 123			Influenza *N* = 145		
	None *N* = 82	Coinfection *N* = 41	*p*	None *N* = 94	Coinfection *N* = 51	*p*
Treatment, *n*%				–	–	–
Paxlovid	42 (54.5)	25 (62.5)	0.409	–	–	–
Corticosteroids	66 (85.7)	37 (92.5)	0.376	–	–	–
Anticoagulation	71 (92.2)	40 (100)	0.093	–	–	–
Bacterial antibiotic	71 (92.2)	40 (100)	0.093	–	–	–
Antifungal antibiotic	24 (31.6)	32 (80)	<0.001	–	–	–
Barotrauma	7 (11.5)	6 (16.2)	0.548	4 (4.3)	4 (7.8)	0.452
Acute kidney injury, n%	15 (20.3)	18 (51.4)	0.001	38 (40.4)	24 (47.1)	0.441
Cardiovascular failure, n%	27 (36.5)	15 (42.9)	0.523	21 (22.3)	14 (27.5)	0.492
Acute liver injury, n%	10 (13.5)	3 (8.6)	0.543	14 (14.9)	5 (9.8)	0.386
Hospital acquired pneumonia, n%	31 (37.8)	23 (56.1)	0.054	48 (51.1)	28 (54.9)	0.659
Urinary infection, n%	4 (5.3)	0	0.299	1 (1.1)	1 (2)	1
Abdominal infection, n%	2 (2.7)	1 (2.8)	1	0	2 (3.9)	0.122
Bloodstream infection, n%	3 (4)	4 (11)	0.211	2 (2.1)	2 (3.9)	0.613
Deep venous thrombosis, n%	19 (25.7)	9 (25.7)	0.997	–	–	–
Pulmonary embolism, n%	1 (1.4)	1 (2.8)	0.549	–	–	–
Gastrointestinal bleeding, n%	15 (20.3)	11 (31.4)	0.202	–	–	–
The need of CRRT, n%	18 (26.1)	13 (34.2)	0.375	30 (31.9)	20 (39.2)	0.377
The need of tracheal intubation, n%	36 (44.4)	33 (80.5)	<0.001	66 (71)	39 (76.5)	0.477
The need of tracheotomy, n%	19 (23.8)	23 (56.1)	<0.001	–	–	–
The need of ECMO, n%	3 (4.1)	8 (20.5)	0.008	25 (26.6)	11 (21.6)	0.503
The need of prone position, n%	19 (25.7)	24 (61.5)	<0.001	–	–	–
The need of recruitment, n%	4 (5.6)	3 (7.9)	0.691	–	–	–
The length of IPPV, days	0 (0–7.5)	6 (0.25–17)	0.003	11 (6–23.75)	7 (4–16.25)	0.126
ICU length of stay, days	8 (3–14)	9 (6–20)	0.121	11 (6–21)	9 (6–19.75)	0.497
Hospital length of stay, days	12 (7–18)	16 (7–25)	0.287	20 (10–59)	17 (10–28)	0.877
Hospital survival, n%	37 (45.1)	12 (9.8)	0.152	54 (57.4)	54 (45.1)	0.325

CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; IPPV, intensive positive–pressure ventilation; ICU, intensive care unit.

group had significantly higher rates of *C. striatum* and lower rates of *P. aeruginosa*, *S. maltophilia*, *B. cenocepacia,* and *CMV* than the influenza cohort. Possibly due to different periods, the distribution of nosocomial bacteria in the ICU was different. *CMV* infection is generally asymptomatic and usually presents as a latent infection in healthy individuals. Immune abnormalities caused by influenza may promote *CMV* expression (36).

Further, the incidence of MDR bacteria was similar between COVID-19 and influenza. In the COVID-19 group, 2 patients had ICU-acquired superinfections with *Aspergillus* spp. and *Rhizopus spp*. They all experienced kidney transplantation and took immunosuppressants for long periods, which accounted for the immune disorder. In a multicenter cohort study conducted by Rouze et al. (37), the incidence of ventilator-associated lower respiratory tract infection was significantly higher in the COVID-19 group (50.5%) than in the influenza group (30.3%). However, we did not reach this conclusion. The shorter duration of IPPV for COVID-19 in our study may have contributed to this difference. Like VAP in other diseases (38), ICU-acquired superinfection was associated with longer IPPV time, ICU stay and hospital stay. Another key finding from our study was that ICU-acquired superinfection was associated with a reduced survival rate in patients with COVID-19. This results agrees with those of other studies that have shown a negative association between secondary infection and an increased risk of death (39, 40).

This study identified many predictive factors for ICU-acquired superinfection of COVID-19. The decrease in CD8+ T cells and the increase of IL-8 levels indicated the cytokine storm activation and subsequent immunosuppression (41). Immune response dysfunction may be associated with a higher risk of ICU-acquired superinfection. Moreover, high blood glucose levels, hypertension, and smoking have all reported to be related to secondary infection (42–44). Based on the above results, early identification of high-risk patients for ICU-acquired superinfection and active examination for pathogens will facilitate timely and appropriate antibiotics, which is beneficial to prognosis.

TABLE 4 Treatment and outcomes of COVID-19 and influenza patients with ICU-acquired superinfections.

	COVID-19 *N* = 123			Influenza *N* = 145		
	None *N* = 69	ICU-acquired superinfection *N* = 54	*p*	None *N* = 69	ICU-acquired superinfection *N* = 76	*p*
Treatment, *n*%						
Paxlovid	32 (49.2)	35 (67.3)	0.05	–	–	–
Corticosteroids	56 (86.2)	47 (90.4)	0.484	–	–	–
Anticoagulation	62 (95.4)	49 (94.2)	1	–	–	–
Bacterial antibiotic	59 (90.8)	52 (100)	0.033	–	–	–
Antifungal antibiotic	19 (29.7)	37 (71.2)	<0.001	–	–	–
Barotrauma	5 (9.4)	8 (17.8)	0.225	2 (2.9)	6 (7.9)	0.280
Acute kidney injury, n%	9 (14.8)	24 (50)	<0.001	24 (34.8)	38 (50)	0.064
Cardiovascular failure, n%	17 (27.9)	25 (52.1)	0.010	16 (23.2)	23 (30.3)	0.337
Acute liver injury, n%	7 (11.5)	6 (12.5)	0.870	6 (8.7)	13 (17.1)	0.148
Hospital acquired pneumonia, n%	0	54 (100)	<0.001	0	76 (100)	<0.001
Urinary infection, n%	2 (3.2)	2 (3.9)	0.614	0	2 (2.6)	0.498
Abdominal infection, n%	1 (1.6)	2 (4.1)	0.582	0	2 (2.6)	0.498
Bloodstream infection, n%	2 (3.2)	5 (10.2)	0.237	1 (1.4)	3 (3.9)	0.622
Deep venous thrombosis, n%	18 (29)	10 (21.3)	0.359	–	–	–
Pulmonary embolism, n%	2 (3.2)	0	0.504	–	–	–
Gastrointestinal bleeding, n%	10 (16.1)	16 (34)	0.030	–	–	–
The need of CRRT, n%	9 (15.5)	22 (44.9)	0.001	19 (27.5)	31 (40.8)	0.094
The need of tracheal intubation, n%	20 (29.4)	49 (90.7)	<0.001	37 (54.4)	68 (89.5)	<0.001
The need of tracheotomy, n%	6 (9)	36 (66.7)	<0.001	–	–	–
The need of ECMO, n%	3 (4.9)	8 (15.4)	0.061	10 (14.5)	26 (34.2)	0.006
The need of prone position, n%	12 (19)	31 (62)	<0.001	–	–	–
The need of recruitment, n%	1 (1.7)	6 (11.8)	0.048	–	–	–
The length of IPPV, days	0 (0–1)	10.5 (6–15)	<0.001	5 (3–9.5)	14 (7–32)	<0.001
ICU length of stay, days	5.5 (3–10)	14 (8–20.8)	<0.001	7 (4–10.5)	18 (10–35.5)	<0.001
Hospital length of stay, days	11 (6.5–19.5)	15 (8–22)	0.104	11.5 (5.8–16.3)	28.5 (15–57.5)	<0.001
Hospital survival, n%	39 (56.5)	10 (18.5)	<0.001	36 (55.2)	41 (53.9)	0.455

CRRT, continuous renal replacement therapy; ECMO, extracorporeal membrane oxygenation; IPPV, intensive positive–pressure ventilation; ICU, intensive care unit.

To our knowledge, this is the first study to focus on the coinfections/ICU-acquired superinfections of COVID-19 versus influenza ICU patients in Asia. We clearly defined the time of sample positivity time from the date of ICU admission (<48 h vs. ≥ 48 h). However, this study had several limitations. First, this was a single-center retrospective study, and the study population was relatively small; therefore, possible selection and report biases exist, and it is difficult to generalize the results to other centers. Second, ICU management, ICU isolation measures, sampling methods, and infection diagnostic techniques differed between the COVID-19 and influenza pandemics. In addition, we distinguished infection and colonization using clinical judgment rather than bacterial count, which may have affected the detection rate. Most importantly, some patients were exposed to antibiotics and stayed in general wards or other ICUs before our ICU admission. This could have impacted pathogenic microorganism detection and potentially underestimated or overestimated the real coinfection rate.

Conclusion

Coinfections and ICU-acquired superinfections were frequent not only in COVID-19 patients but also in influenza patients admitted to the ICU. The represent agents of coinfections in ICU patients were different from those in the general ward. Our study provides evidence supporting close monitoring and empirical choice of antibiotics according to the pathogen for COVID-19 and influenza cases at risk of coinfections/ICU-acquired superinfections in the ICU. Apart from the limited study population, ICU management, ICU isolation measures, sampling methods, and infection diagnostic techniques may have impact on

our conclusion. A large-scale and well-designed RCT is needed in the future.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving humans were approved by China-Japan Friendship Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin due to the study's retrospective nature.

Author contributions

LH and QZ took full responsibility for the integrity of the submission and publication and were involved in the study design. QZ, LH, and ZC participated in the design of the study and coordination. ZC involved in data collection, had full access to all of the data in the study, took responsibility for the integrity of the data, and were responsible for data verification. ZC and LH took the responsibility for statistical analysis and drafted the manuscript. QZ provided crucial revision for important intellectual content. All authors made substantial contributions to the conception and design of the study or to the data acquisition, analysis or interpretation, reviewed and approved the final manuscript, and significantly contributed to this study.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1195048/full#supplementary-material

References

1. Greco M, de Corte T, Ercole A, Antonelli M, Azoulay E, Citerio G, et al. Clinical and organizational factors associated with mortality during the peak of first COVID-19 wave: the global UNITE-COVID study. *Intensive Care Med*. (2022) 48:690–5. doi: 10.1007/s00134-022-06705-1

2. Faust JS, du C, Liang C, Mayes KD, Renton B, Panthagani K, et al. Excess mortality in Massachusetts during the Delta and omicron waves of COVID-19. *JAMA*. (2022) 328:74–6. doi: 10.1001/jama.2022.8045

3. Sullivan SG, Cowling BJ. Reconciling estimates of the global influenza burden. *Lancet Respir Med*. (2019) 7:8–9. doi: 10.1016/S2213-2600(18)30511-3

4. Rice TW, Rubinson L, Uyeki TM, Vaughn FL, John BB, Miller R, et al. Critical illness from 2009 pandemic influenza a virus and bacterial coinfection in the United States. *Crit Care Med*. (2012) 40:1487–98. doi: 10.1097/CCM.0b013e3182416f23

5. Klein EY, Monteforte B, Gupta A, Jiang W, May L, Hsieh YH, et al. The frequency of influenza and bacterial coinfection: a systematic review and meta-analysis. *Influenza Other Respir Viruses*. (2016) 10:394–3. doi: 10.1111/irv.12398

6. McCullers JA. The co-pathogenesis of influenza viruses with bacteria in the lung. *Nat Rev Microbiol*. (2014) 12:252–2. doi: 10.1038/nrmicro3231

7. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis*. (2020) 71:2459–68. doi: 10.1093/cid/ciaa530

8. Hughes S, Troise O, Donaldson H, Mughal N, Moore LSP. Bacterial and fungal coinfection among hospitalized patients with COVID-19: a retrospective cohort study in a UK secondary-care setting. *Clin Microbiol Infect*. (2020) 26:1395–9. doi: 10.1016/j.cmi.2020.06.025

9. Moreno-García E, Puerta-Alcalde P, Letona L, Meira F, Dueñas G, Chumbita M, et al. Bacterial co-infection at hospital admission in patients with COVID-19. *Int J Infect Dis*. (2022) 118:197–2. doi: 10.1016/j.ijid.2022.03.003

10. Giannella M, Rinaldi M, Tesini G, Gallo M, Cipriani V, Vatamanu O, et al. Predictive model for bacterial co-infection in patients hospitalized for COVID-19: a multicenter observational cohort study. *Infection*. (2022) 50:1243–53. doi: 10.1007/s15010-022-01801-2

11. Lansbury L, Lim B, Baskaran V, Lim WS. Co-infections in people with COVID-19: a systematic review and meta-analysis. *J Infect*. (2020) 81:266–5. doi: 10.1016/j.jinf.2020.05.046

12. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR, et al. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. *Clin Microbiol Infect*. (2020) 26:1622–9. doi: 10.1016/j.cmi.2020.07.016

13. van der Sluijs KF, van der Poll T, Lutter R, Juffermans NP, Schultz MJ. Bench-to-bedside review: bacterial pneumonia with influenza - pathogenesis and clinical implications. *Crit Care*. (2010) 14:219. doi: 10.1186/cc8893

14. Gao R, Cao B, Hu Y, Feng Z, Wang D, Hu W, et al. Human infection with a novel avian-origin influenza a (H7N9) virus. *N Engl J Med*. (2013) 368:1888–97. doi: 10.1056/NEJMoa1304459

15. Huang L, Zhai T, Hua L, Zhan Q. Early identification of patients with severe influenza-associated aspergillosis (IAA) in the intensive care unit--an IAA prediction score system (asper-PreSS). *J Infect*. (2020) 81:639–6. doi: 10.1016/j.jinf.2020.07.036

16. 中华医学会呼吸病学分会. 中国成人社区获得性肺炎诊断和治疗指南(2016年版). 中华结核和呼吸杂志. (2016) 39:253–9.

17. 中华人民共和国国家卫生健康委员会. 新型冠状病毒感染诊疗方案(试行第十版). 中华临床感染病杂志. (2023) 16:1–9.

18. Nseir S, di Pompeo C, Pronnier P, Beague S, Onimus T, Saulnier F, et al. Nosocomial tracheobronchitis in mechanically ventilated patients: incidence, aetiology and outcome. *Eur Respir J*. (2002) 20:1483–9. doi: 10.1183/09031936.02.00012902

19. Kalil AC, Metersky M, Klompas M, Muscedere J, Sweeney DA, Palmer LB, et al. Management of Adults with Hospital-acquired and Ventilator-associated Pneumonia: 2016 clinical practice guidelines by the Infectious Diseases Society of America and the American Thoracic Society. *Clin Infect Dis*. (2016) 63:e61–e111. doi: 10.1093/cid/ciw353

20. Verweij PE, Brüggemann RJM, Azoulay E, Bassetti M, Blot S, Buil JB, et al. Taskforce report on the diagnosis and clinical management of COVID-19 associated pulmonary aspergillosis. *Intensive Care Med*. (2021) 47:819–4. doi: 10.1007/s00134-021-06449-4

21. Pappas PG, Kauffman CA, Andes DR, Clancy CJ, Marr KA, Ostrosky-Zeichner L, et al. Clinical practice guideline for the Management of Candidiasis: 2016 update by the Infectious Diseases Society of America. *Clin Infect Dis*. (2016) 62:e1–e50. doi: 10.1093/cid/civ933

22. Verroken A, Scohy A, Gérard L, Wittebole X, Collienne C, Laterre PF. Co-infections in COVID-19 critically ill and antibiotic management: a prospective cohort analysis. *Crit Care*. (2020) 24:410. doi: 10.1186/s13054-020-03135-7

23. Wang L, Amin AK, Khanna P, Aali A, McGregor A, Bassett P, et al. An observational cohort study of bacterial co-infection and implications for empirical antibiotic therapy in patients presenting with COVID-19 to hospitals in north West London. *J Antimicrob Chemother*. (2021) 76:796–3. doi: 10.1093/jac/dkaa475

24. Nori P, Cowman K, Chen V, Bartash R, Szymczak W, Madaline T, et al. Bacterial and fungal coinfections in COVID-19 patients hospitalized during the new York City pandemic surge. *Infect Control Hosp Epidemiol*. (2021) 42:84–8. doi: 10.1017/ice.2020.368

25. Pandey M, May A, Tan L, Hughes H, Jones JP, Harrison W, et al. Comparative incidence of early and late bloodstream and respiratory tract co-infection in patients admitted to ICU with COVID-19 pneumonia versus influenza a or B pneumonia versus no viral pneumonia: wales multicentre ICU cohort study. *Crit Care*. (2022) 26:158. doi: 10.1186/s13054-022-04026-9

26. Contou D, Claudinon A, Pajot O, Micaëlo M, Longuet Flandre P, Dubert M, et al. Bacterial and viral co-infections in patients with severe SARS-CoV-2 pneumonia admitted to a French ICU. *Ann Intensive Care*. (2020) 10:119. doi: 10.1186/s13613-020-00736-x

27. Dhanoa A, Fang NC, Hassan SS, Kaniappan P, Rajasekaram G. Epidemiology and clinical characteristics of hospitalized patients with pandemic influenza a (H1N1) 2009 infections: the effects of bacterial coinfection. *Virol J*. (2011) 8:501. doi: 10.1186/1743-422X-8-501

28. Cuquemelle E, et al. Can procalcitonin help identify associated bacterial infection in patients with severe influenza pneumonia? *Multicentre Study Intens Care Med*. (2011) 37:796–0. doi: 10.1007/s00134-011-2189-1

29. Martín-Loeches I, Sanchez-Corral A, Diaz E, Granada RM, Zaragoza R, Villavicencio C, et al. Community-acquired respiratory coinfection in critically ill patients with pandemic 2009 influenza a(H1N1) virus. *Chest*. (2011) 139:555–2. doi: 10.1378/chest.10-1396

30. Senok A, Alfaresi M, Khansaheb H, Nassar R, Hachim M, al Suwaidi H, et al. Coinfections in patients hospitalized with COVID-19: a descriptive study from the United Arab Emirates. *Infect Drug Resist*. (2021) 14:2289–96. doi: 10.2147/IDR.S314029

31. Seyedjavadi SS, Bagheri P, Nasiri MJ, Razzaghi-Abyaneh M, Goudarzi M. Fungal infection in co-infected patients with COVID-19: an overview of case reports/case series and systematic review. *Front Microbiol*. (2022) 13:888452. doi: 10.3389/fmicb.2022.888452

32. Rydyznski Moderbacher C, Ramirez SI, Dan JM, Grifoni A, Hastie KM, Weiskopf D, et al. Antigen-specific adaptive immunity to SARS-CoV-2 in acute COVID-19 and associations with age and disease severity. *Cells*. (2020) 183:996–1012.e19. doi: 10.1016/j.cell.2020.09.038

33. Langouche L, Marques MB, Ingels C, Gunst J, Derde S, Vander Perre S, et al. ritical illness induces alternative activation of M2 macrophages in adipose tissue. *Crit Care*. (2011) 15:R245. doi: 10.1186/cc10503

34. Chertow DS, Memoli MJ. Bacterial coinfection in influenza: a grand rounds review. *JAMA*. (2013) 309:275–2. doi: 10.1001/jama.2012.194139

35. Pittet LA, Hall-Stoodley L, Rutkowski MR, Harmsen AG. Influenza virus infection decreases tracheal mucociliary velocity and clearance of *Streptococcus pneumoniae*. *Am J Respir Cell Mol Biol*. (2010) 42:450–0. doi: 10.1165/rcmb.2007-0417OC

36. Merani S, Pawelec G, Kuchel GA, McElhaney JE. Impact of aging and cytomegalovirus on immunological response to influenza vaccination and infection. *Front Immunol*. (2017) 8:784. doi: 10.3389/fimmu.2017.00784

37. Rouzé A, Martin-Loeches I, Povoa P, Makris D, Artigas A, Bouchereau M, et al. Relationship between SARS-CoV-2 infection and the incidence of ventilator-associated lower respiratory tract infections: a European multicenter cohort study. *Intensive Care Med*. (2021) 47:188–8. doi: 10.1007/s00134-020-06323-9

38. Kalanuria AA, Ziai W, Mirski M. Ventilator-associated pneumonia in the ICU. *Crit Care*. (2014) 18:208. doi: 10.1186/cc13775

39. Musuuza JS, Watson L, Parmasad V, Putman-Buehler N, Christensen L, Safdar N. Prevalence and outcomes of co-infection and superinfection with SARS-CoV-2 and other pathogens: a systematic review and meta-analysis. *PLoS One*. (2021) 16:e0251170. doi: 10.1371/journal.pone.0251170

40. Rawson TM, Wilson RC, Holmes A. Understanding the role of bacterial and fungal infection in COVID-19. *Clin Microbiol Infect*. (2021) 27:9–11. doi: 10.1016/j.cmi.2020.09.025

41. Manna S, Baindara P, Mandal SM. Molecular pathogenesis of secondary bacterial infection associated to viral infections including SARS-CoV-2. *J Infect Public Health*. (2020) 13:1397–04. doi: 10.1016/j.jiph.2020.07.003

42. Hu SQ, Hu JN, Chen RD, Yu JS. A predictive model using risk factor categories for hospital-acquired pneumonia in patients with aneurysmal subarachnoid hemorrhage. *Front Neurol*. (2022) 13:1034313. doi: 10.3389/fneur.2022.1034313

43. Alfares M, Almrzouqi A, Alghamdi R, Alsharif R, Kurdi L, Kamfar S, et al. Risk factors of hospital-acquired pneumonia among hospitalized patients with cardiac diseases. *Cureus*. (2023) 15:e34253. doi: 10.7759/cureus.34253

44. Cancella de Abreu M, Rousseau A, Ehrminger S, Rufat P, Hausfater P. Predictive factors for secondary intensive care unit admission within 48 hours after hospitalization in a medical ward from the emergency room. *Eur J Emerg Med*. (2020) 27:186–2. doi: 10.1097/MEJ.0000000000000628

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Serum HBV RNA: a promising biomarker for blood product safety screening and enhanced diagnostic efficiency in chronic hepatitis B virus infection

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KEYWORDS

blood safety, HBV RNA, biomarker, safety screening, diagnosis

1. Introduction

The prevalence of Chronic Hepatitis B Virus (HBV) infection remains a considerable challenge to global health, impacting around 30% of the global populace (1). Although antiviral therapies have made significant progress, the complete elimination of the virus, specifically the covalently closed circular DNA (cccDNA), continues to be a challenging objective. Conventional diagnostic techniques, including HBV DNA quantification and serological markers, possess certain limitations in precisely reflecting viral activity and treatment response (2). Liu et al. reported the identification of serum HBV RNA as a biomarker has demonstrated encouraging prospects in enhancing diagnostic efficacy and screening for blood product safety in individuals with chronic HBV infection (3). The objective of this commentary is to examine the importance of detecting serum HBV RNA, recent advancements in enhancing its diagnostic precision, its potential application as a biomarker for screening blood products for safety, and the consequences for medical practice.

2. The significance of serum HBV RNA detection

Blood product safety screening biomarkers refer to distinct molecules or indicators employed to evaluate the quality and safety of blood products that are designated for transfusion or other therapeutic applications. Biomarkers are essential in the identification and mitigation of infectious diseases, including viral infections, bacterial contamination, and other hazardous agents that could be present in donated blood (4). The principal aim of screening blood products for safety is to detect and eradicate any plausible hazards linked to transfusions, thereby ensuring the protection of the recipients against unfavorable responses or transmission of contagious illnesses. The biomarkers utilized in this procedure are commonly assessed via laboratory examinations and function as indicators of diverse infectious agents or other anomalies that could potentially jeopardize the safety of blood products (5).

Serological tests are commonly employed by blood donation centers to screen donated blood for the existence of HBV. The tests are designed to identify particular antibodies against HBV antigens, namely hepatitis B surface antigen (HBsAg) and hepatitis B core antigen (anti-HBc). Blood units that have been donated and test positive for these markers

are typically deemed unsuitable for transfusion in order to avoid the transmission of HBV to the recipients (6). Moreover, the term "window period" pertains to the duration between the acquisition of HBV infection and the emergence of discernible antibodies. During this temporal phase, an individual has the potential to contract HBV, however, they may exhibit negative results for HBsAg and anti-HBc upon testing. In order to mitigate the potential for transmission of Hepatitis B Virus (HBV), blood centers frequently employ supplementary measures, including nucleic acid testing (NAT). This method is capable of identifying the genetic material (HBV DNA) of the virus during the window period (7). In some cases, individuals with chronic HBV infection may have very low levels of HBV DNA in their blood, referred to as occult HBV infection. This poses a challenge in detecting HBV using standard serological tests alone. Therefore, in certain situations, blood centers may employ molecular techniques, like polymerase chain reaction (PCR), to quantify HBV DNA levels and assess the risk of transmission more accurately (8). The diagnostic significance of HBV RNA in comparison to HBsAg, anti-HBc, or HBV DNA is not fully established, however, since HBV RNA is still in the clinical research stage and has not yet gained widespread acceptance in clinical practice.

Serum HBV RNA, specifically non- or partially reverse-transcribed pregenomic RNA (pgRNA) present in HBV virion-like particles, has emerged as a valuable surrogate marker for assessing viral activity and treatment response (9). Unlike HBV DNA, which can be lost during antiviral therapy, serum HBV pgRNA persists and provides valuable insights into the transcriptional activity of cccDNA (Figure 1). Monitoring serum HBV RNA levels has demonstrated its potential to predict treatment efficacy, evaluate the risk of viral rebound after drug withdrawal, and guide clinical decision-making (3, 10, 11).

Recent studies also identified the characteristics of chronic hepatitis B (CHB) participants who are positive HBV RNA. A study by Janssen et al. found that factors associated with serum HBV RNA level were HBsAg status, serum ALT, HBV genotype, and the presence of basal core promoter mutations (12). Ghany et al. reported HBV RNA was also quantifiable in 99% of positive HBsAg donors and 58% of negative HBsAg donors, and HBV RNA level was closely associated with CHB phages, but because of its close relationship with HBV DNA, HBV RNA as a marker also has significant limitations in additional information related to clinical disease indicators, necessitating further verification in large-sample clinical trials (13).

3. Optimizing serum HBV RNA detection

According to a study by Yu et al., HBV pgRNA undergoes dynamic changes in splicing variation and truncation at its 3′ terminal in several cohorts of people with chronic HBV infection (14). In this study, reverse transcription-quantitative polymerase chain reactions (RT-qPCRs) were used to target several areas of the HBV genome, and the amount of HBV RNA found in serum was assessed in relation to these RNA species. According to the findings, there is a correlation between which region of the HBV genome is amplified and how precise and sensitive the detection of HBV RNA in serum. The detection levels at the preC/C-RNA region consistently exceeded those at the SF-RNA section and the XR-DNA region. As a result, detection at the preC/C-RNA region avoids interruptions of main pgRNA splicing variants and truncated 3′ terminals. To accurately detect blood HBV RNA, the amplification site needs to be selected carefully. Research also showed that treatment-naive individuals had high proportions of spliced pgRNAs and 3′ terminal-truncated pgRNAs, despite an absence of influence of HBeAg status. In early stages of treatment, these proportions declined in patients receiving nucleoside analogs (NAs), but climbed once again after 2 years of treatment. Based on these findings, it appears that long-term treatment with NAs exacerbates the interference induced by splicing variations and truncated 3′ terminals of pgRNA. This highlights the necessity of appropriate amplification region selection, particularly in scenarios involving long-term treatment.

4. Implications for blood product safety screening

Serum HBV RNA detection holds great potential as a biomarker for blood product safety screening. The presence of HBV RNA in blood units indicates active viral replication and the potential risk of transmission. By implementing sensitive and standardized assays for serum HBV RNA detection, blood banks and transfusion services can enhance their screening protocols, ensuring the exclusion of HBV-infected units and minimizing the risk of transmission to recipients (15).

Currently, the detection of HBV DNA is the primary method utilized for checking the safety of blood products. Nevertheless, the shortcomings of HBV DNA measurement, such as the disappearance of detectable DNA after antiviral treatment, call for the investigation of other biomarkers. The permanence of serum HBV RNA and the fact that it directly reflects the activity of the virus make it a desirable supplement to the screening procedures that are already in use. According to Kramvis's work, the incorporation of serum HBV RNA detection into blood product screening processes has the potential to considerably increase the accuracy and efficiency of identifying HBV-infected blood units, thereby protecting the health of recipients and ensuring the safety of the transfusion process (16).

5. Future directions and conclusion

In chronic HBV infection, the identification and validation of serum HBV RNA as a biomarker have opened up new pathways for improved diagnostic efficiency and blood product safety screening. To fully investigate its clinical value and establish standardized techniques for its detection, additional research and validation studies are required. To validate the findings and evaluate the generalizability of the optimized tests, further studies should be conducted on CHB patients comprising larger patient cohorts. In addition, research should be conducted into the underlying processes of lower reverse transcription efficiency of pgRNA splicing variants in order to achieve a full understanding of the effect that these variants have on the amount of HBV RNA found

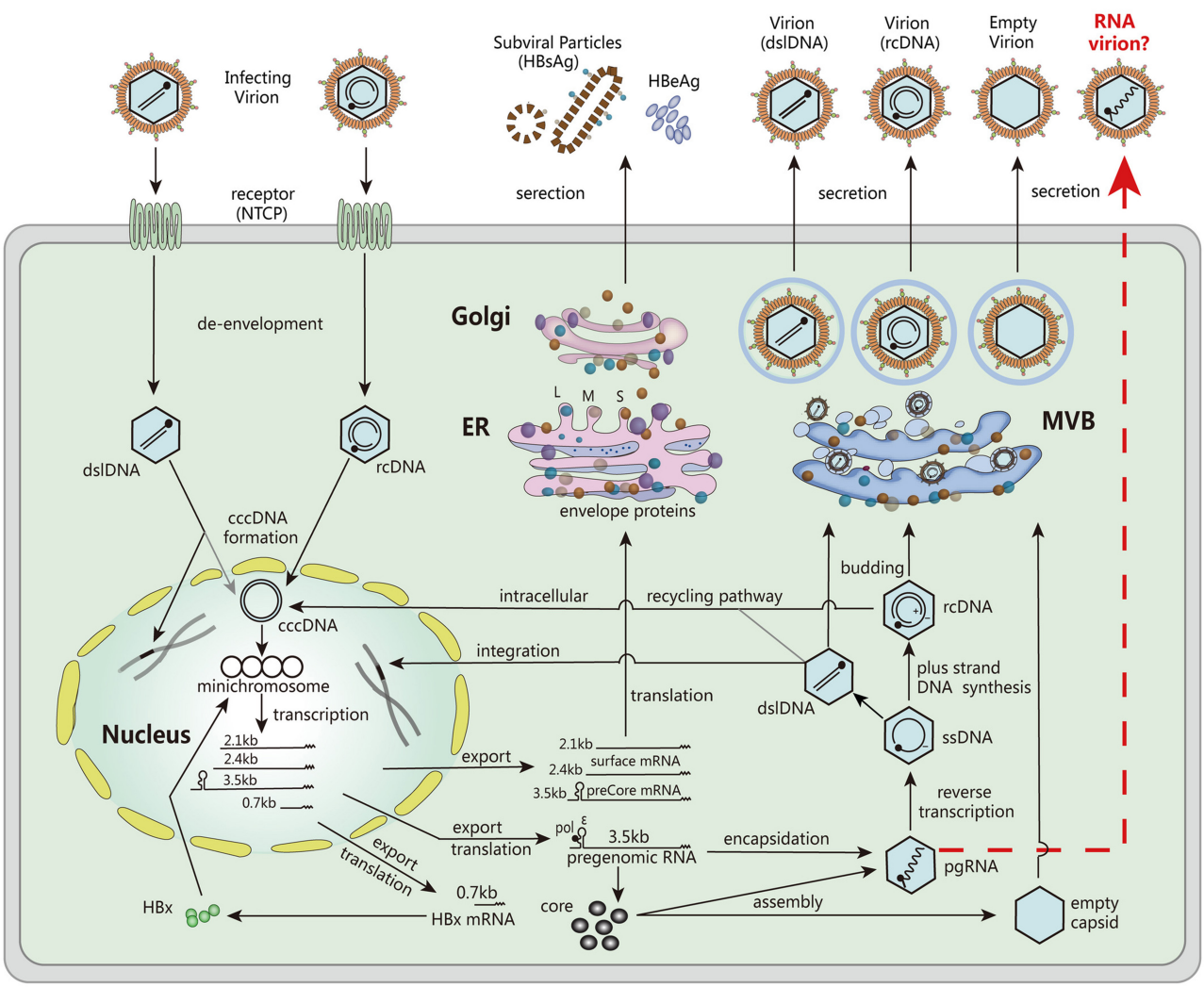

FIGURE 1
The major steps involved in the HBV life cycle. These steps include viral entry into host cells, de-envelopment of the viral particle, formation of cccDNA, transcription of mRNA, translation of viral proteins, encapsulation of pgRNA, DNA replication, assembly of viral particles, and secretion. Reproduced with permission (3). Copyright 2019, Wiley-VCH.

in serum. The interpretation of serum HBV RNA levels could be improved further by gaining a better understanding of the trans-packaging mechanism of pgRNA and the implications this has on the efficiency of reverse transcription.

In conclusion, the detection of HBV RNA in serum represents a substantial development in the effectiveness of the diagnostic process for chronic HBV infection. Recent research has shown that splicing variations and 3′ terminal truncations can have an effect on the amount of HBV RNA that can be measured in serum. These findings highlight how important it is to optimize assays and choose optimal amplification locations. The accuracy and sensitivity of HBV RNA quantification can be enhanced by healthcare providers by using standardized and sensitive assays. This enables improved monitoring of viral activity, assessment of treatment response, and informed decision-making regarding treatment discontinuation. In addition, the detection of HBV RNA in serum demonstrates excellent potential as a biomarker for the evaluation of the safety of blood products. Its incorporation into screening processes has the potential to assist in the detection of HBV-infected blood units, hence maximizing the efficacy of blood transfusions and lowering the probability of disease transmission. To further investigate the clinical value of serum HBV RNA detection and its application in bigger cohorts of CHB patients and blood product screening programs, continued research and validation studies are necessary. These studies must be carried out. In the end, the incorporation of serum HBV RNA detection into clinical practice and the implementation of blood safety precautions helps to a more effective management of chronic HBV infection and moves the aim of HBV eradication closer to realization.

Author contributions

SY and YG conducted the literature analysis and drafted the original manuscript. CZ revised and edited

the manuscript. All authors approved the final version of manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Clements CJ, Coghlan B, Creati M, Locarnini S, Tedder RS, Torresi J. Global control of hepatitis B virus: does treatment-induced antigenic change affect immunization? *Bulletin of the World Health Organization.* (2010) 88:66–73. doi: 10.2471/BLT.08.065722

2. Shen C, Feng X, Mao T, Yang D, Zou J, Zao X, et al. Yin-Yang 1 and HBx protein activate HBV transcription by mediating the spatial interaction of cccDNA minichromosome with cellular chromosome 19p13. *11 Emerging Microbes & Infections.* (2020) 9:2455–64. doi: 10.1080/22221751.2020.1840311

3. Liu S, Zhou B, Valdes JD, Sun J, Guo H. Serum hepatitis B virus RNA: a new potential biomarker for chronic hepatitis B virus infection. *Hepatology.* (2019) 69:1816–27. doi: 10.1002/hep.30325

4. Roberts N, James S, Delaney M, Fitzmaurice C. The global need and availability of blood products: a modelling study. *Lancet Haematol.* (2019) 6:e606–15. doi: 10.1016/S2352-3026(19)30200-5

5. Chang LE, Zhao L, Gong H, Wang L, Wang L. Severe acute respiratory syndrome coronavirus 2 RNA detected in blood donations. *Emerg Infect Dis.* (2020) 26:1631. doi: 10.3201/eid2607.200839

6. Nishida T, Matsubara T, Yakushijin T, Inada M. Prediction and clinical implications of HBV reactivation in lymphoma patients with resolved HBV infection: focus on anti-HBs and anti-HBc antibody titers. *Hepatol Int.* (2019) 13:407–15. doi: 10.1007/s12072-019-09966-z

7. Kusumoto S, Arcaini L, Hong X, Jin J, Kim WS, Kwong YL, et al. Risk of HBV reactivation in patients with B-cell lymphomas receiving obinutuzumab or rituximab immunochemotherapy. *J Am Soc Hematol.* (2019) 133:137–46. doi: 10.1182/blood-2018-04-848044

8. Wu D, Wang X, Feng F, Wang D, Hu Y, Yu Y, et al. Characteristic of HBV nucleic acid amplification testing yields from blood donors in China. *BMC Infect Dis.* (2021) 21:1–2. doi: 10.1186/s12879-021-06468-y

9. Mak LY, Huang Q, Wong DK, Stamm L, Cheung KS, Ko KL, et al. Residual HBV DNA and pgRNA viraemia is associated with hepatocellular carcinoma in chronic hepatitis B patients on antiviral therapy. *J Gastroenterol.* (2021) 56:479–88. doi: 10.1007/s00535-021-01780-5

10. Cloherty G, Butler E, Kuhns M. Serum hepatitis B virus RNA as a potential diagnostic biomarker during chronic hepatitis B virus infection. *Clin Liver Dis.* (2019) 13:90. doi: 10.1002/cld.774

11. Lin N, Ye A, Lin J, Liu C, Huang J, Fu Y, et al. Diagnostic value of detection of pregenomic RNA in sera of hepatitis B virus-infected patients with different clinical outcomes. *J Clin Microbiol.* (2020) 58:10–128. doi: 10.1128/JCM.01275-19

12. Van Campenhout MJH, Van Bömmel F, Pfefferkorn M, Fischer J, Deichsel D, Boonstra A, et al. Host and viral factors associated with serum hepatitis B virus RNA levels among patients in need for treatment. *Hepatology.* (2018) 68:839–47. doi: 10.1002/hep.29872

13. Ghany MG, King WC, Lisker-Melman M, Lok ASF, Terrault N, Janssen HLA, et al. Comparison of HBV RNA and Hepatitis B core related antigen with conventional HBV markers among untreated adults with chronic hepatitis B in North America. *Hepatology.* (2021) 74:2395–409. doi: 10.1002/hep.32018

14. Yu G, Chen R, Zheng S, Liu Y, Zou J, Gu Z, et al. A standardized assay for the quantitative detection of serum HBV RNA in chronic hepatitis B patients. *Emerg Microbes Infect.* (2022) 11:775–85. doi: 10.1080/22221751.2022.2045874

15. Ghany MG, Buti M, Lampertico P, Lee HM, Berg T, Brunetto MR, et al. Guidance on treatment endpoints and study design for clinical trials aiming to achieve cure in, chronic hepatitis B and D-Report from the 2022 AASLD-EASL HBV/HDV Treatment Endpoints Conference. *J Hepatol.* (2023) 15:431. doi: 10.1097/HEP.0000000000000431

16. Kramvis A, Chang KM, Dandri M, Farci P, Glebe D, Hu J, et al. A roadmap for serum biomarkers for hepatitis B virus: current status and future outlook. *Nat Rev Gastroenterol Hepatol.* (2022) 19:727–45. doi: 10.1038/s41575-022-00649-z

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Epidemiological and clinical characteristics of COVID-19 reinfection during the epidemic period in Yangzhou city, Jiangsu province

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Background: With the continuous progress of the epidemic of coronavirus disease 2019 (COVID-19) infection and the constant mutation of the virus strain, reinfection occurred in previously infected individuals and caused waves of the epidemic in many countries. Therefore, we aimed to explore the characteristics of COVID-19 reinfection during the epidemic period in Yangzhou and provide a scientific basis for assessing the COVID-19 situation and optimizing the allocation of medical resources.

Methods: We chose previously infected individuals of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) reported locally in Yangzhou city from January 2020 to November 30, 2022. A telephone follow-up of cases was conducted from February to March 2023 to collect the COVID-19 reinfection information. We conducted a face-to-face survey on that who met the definition of reinfection to collect information on clinical symptoms, vaccination status of COVID-19, and so on. Data were analyzed using SPSS 19.0.

Results: Among the 999 eligible respondents (92.24% of all the participants), consisting of 42.28% males and 57.72% females, the reinfection incidence of females was significantly higher than that of male cases ($\chi^2 = 5.197$, $P < 0.05$); the ages of the respondents ranged from 1 to 91 years, with the mean age of 42.28 (standard deviation 22.73) years; the most of the sufferers were infected initially with Delta variant (56.88%), followed by the Omicron subvariants BA.1/BA.2 (39.52%). Among all the eligible respondents, 126 (12.61%) reported COVID-19 reinfection appearing during the epidemic period, and the intervals between infections were from 73 to 1,082 days. The earlier the initial infection occurred, the higher the reinfection incidence and the reinfection incidence was significantly increased when the interval was beyond 1 year ($P < 0.01$) .119 reinfection cases (94.4%) were symptomatic when the most common symptoms included fever (65.54%) and cough (61.34%); compared with the initial infection cases, the proportion of clinical symptoms in the reinfected cases was significantly higher ($P < 0.01$). The reinfection incidence of COVID-19 vaccination groups with different doses was statistically significant ($P < 0.01$). Fewer reinfections were observed among the respondents with three doses of COVID-19 vaccination compared to the respondents with two doses (χ^2 =14.595, $P < 0.001$) or without COVID-19 vaccination (χ^2 =4.263, $P = 0.039$).

Conclusion: After the epidemic period of COVID-19, the reinfection incidence varied with different types of SARS-CoV-2 strains. The reinfection incidence was influenced by various factors such as virus characteristics, vaccination, epidemic prevention policies, and individual variations. As the SARS-CoV-2 continues to mutate, vaccination and appropriate personal protection have practical significance in reducing the risk of reinfection.

KEYWORDS

SARS-CoV-2, reinfection, epidemiological characteristics, clinical characteristics, epidemic

1. Introduction

Reinfections might occur with many respiratory viruses, including human coronaviruses, mainly due to weak or waning initial immune response, reinfection with another genotype/species or the high variability of the viruses (1). Reinfection was defined as recovery followed by a new infection due to the same variant previously infected or a new variant of the agent (2). In August 2020, To et al. (3) first described an asymptomatic patient from Hong Kong with a positive SARS-CoV-2 PCR test from a sample collected 142 days after a first symptomatic COVID-19 episode. Since then, the reinfection of COVID-19 in previously infected individuals has been reported in many parts of the world (4, 5), with the reinfection incidence of different variants varying considerably in different periods and regions. Due to the influence of epidemic prevention policies and the characteristics of the SARS-CoV-2, the reinfection incidence of COVID-19 in previously infected individuals was low before the emergence of the Omicron variant. Up to March 2021, a meta-analysis indicated the overall prevalence of reinfection among COVID-19 patients was 3‰ (95% confidence interval [CI]:0.8–5‰) (6). Since the worldwide outbreak of the Omicron variant in November 2021, the reinfection incidences reported in multiple regions have significantly increased. According to an analysis of a survey conducted in the UK, the highest reinfection incidence among individuals previously infected with different strains from February 2020 to November 2022 could reach 16.6% (7). The SARS-CoV-2 Omicron variant spread rapidly nationwide since November 2022, seriously affecting the health of residents. In previous studies, the factors associated with the infection rate of COVID-19 might include socio-demographic factors such as gender, age, and occupation, but the results were not entirely consistent (8, 9), whereas vaccination has presented a certain protective effect against COVID-19 (10, 11). And in the few studies of reinfection in China, the factors influencing reinfection rates were similar to those of initial infection (12, 13). This study was based on the survey of COVID-19-infected individuals in Yangzhou City during the epidemic period from January 2020 to November 30, 2022, to explore the characteristics of COVID-19-reinfection and the influencing factors, and provide a scientific basis for assessing the COVID-19 situation and optimizing the allocation of medical resources.

2. Participants and methods

2.1. Participants

Following the China Information System for Disease Control and Prevention (CISDCP), COVID-19 previously infected individuals reported locally in Yangzhou city from January 2020 to November 30, 2022, were chosen.

2.2. Methods

2.2.1. Investigation methods

Trained and assessed epidemiological investigators conducted the first round of telephone follow-up surveys on the cases or their guardians included in this study, followed by the second round of face-to-face surveys on the one that met the definition of reinfection.

2.2.2. Investigation content

The telephone survey mainly included demography basic information, reinfection status, SARS-CoV-2 nucleic acid or antigen test results, and information about close contacts, whether the contacts had positive antigen tests or COVID-19 symptoms.

Face-to-face surveys were conducted to collect information on the first infection of COVID-19, the diagnosis and treatment of the latest infection of COVID-19, and so on. The COVID-19 vaccination information was obtained from the records of the Jiangsu Province Vaccination Integrated Service Management Information System.

2.2.3. Definition of reinfection

(1) Confirmed case of SARS-CoV-2 reinfection: Identified as those who tested positive for SARS-CoV-2 nucleic acid or antigen more than 60 days after the last positive SARS-CoV-2 nucleic acid or antigen irrespective of symptoms.

(2) Probable case of SARS-CoV-2 reinfection: Defined as those who met an acute onset or worsening of any following signs or symptoms (fever, fatigue, cough, sore throat, hyposmia/anosmia, nasal congestion, runny nose, conjunctivitis, myalgia, diarrhea, etc.), and had an

epidemiological history (close contact with the case of positive SARS-CoV-2 nucleic acid test/rapid antigen test for COVID-19 or of similar symptoms), and the interval from the last positive SARS-CoV-2 nucleic acid test exceeded more than 60 days.

2.2.4. Genome sequencing

During January 2020 and November 30, 2022, nasopharyngeal swab samples from COVID-19-infected individuals reported locally in Yangzhou city were collected by the CDCs along with designated hospitals for COVID-19 diagnosis and treatment. These samples were promptly dispatched to the Yangzhou CDC for further analysis, including nucleic acid testing and genome sequencing.

Genome sequencing was implemented on all SARS-CoV-2 nucleic acid test-positive cases with cycle threshold (Ct) values $\leq$32. Strains of the local case reported in 2020 were all original. The chain of transmission for local outbreaks of COVID-19 in both 2021 July-August and 2022 was explicit, and the strains were Delta variant and Omicron subvariants BA.5.2/BF.7, respectively.

2.2.5. Statistical analysis

Epidemiological investigators conducted telephone and face-to-face questionnaires. Data were entered and managed using EpiData 3.1 and analyzed using SPSS 19.0. Categorical variables were compared using the Chi-squared test or Fisher's exact test. Whereas, the Shapiro-Wilk test was used to assess the normality of continuous variables which were expressed by mean±standard ($\bar{x} \pm s$) deviation after conforming to normal distribution. Homogeneity of variance between the groups was checked by Levene's test and the data were analyzed by t-test when homogeneity of variance. Multivariable logistic regression was done and a p-value of $\leq$ 0.05 was used to declare the level of significance.

2.2.6. Quality control

The survey group consisted of an investigation-leading group, a technical guidance group, and a quality control group, and epidemiological investigators were trained and assessed. The survey was conducted strictly under unified investigation methods and questionnaires. The investigators checked and corrected the questionnaire to ensure that there were no missing items or logic errors after the everyday survey. 5–10% of the questionnaire were randomly selected for review.

3. Results

3.1. Basic characteristics of respondents

Between January 2020 and November 30, 2022, a total of 1,083 previously infected individuals were reported locally, of which 999 completed telephone surveys with a response rate of 92.24%. The main period for the initial positive SARS-CoV-2 nucleic acid test of all previously infected individuals was July 28 to August 26, 2021 (n = 570, 52.63%). 42.28% of the respondents were male and 57.72% were female, with a sex ratio (M/F) of 0.73:1. The ages of the respondents ranged from 1 to 91 years old, with a mean age of 42.28 (standard deviation 22.73) years. Most of the sufferers were infected initially with the Delta variant (56.88%), followed by the Omicron subvariants BA.1/BA.2 (39.52%) (Table 1).

TABLE 1 Types of SARS-CoV-2 strains of previously infected individuals during the epidemic period in Yangzhou, China.

Virus type	Number of infection cases (N = 1,083)	Number of respondents (N = 999)	Response rate (%)
Delta variant (%)	616 (56.88)	553 (55.36)	89.77
Omicron variant (%)	428 (39.52)	408 (40.84)	95.33
Original strain (%)	39 (3.60)	38 (3.80)	97.44

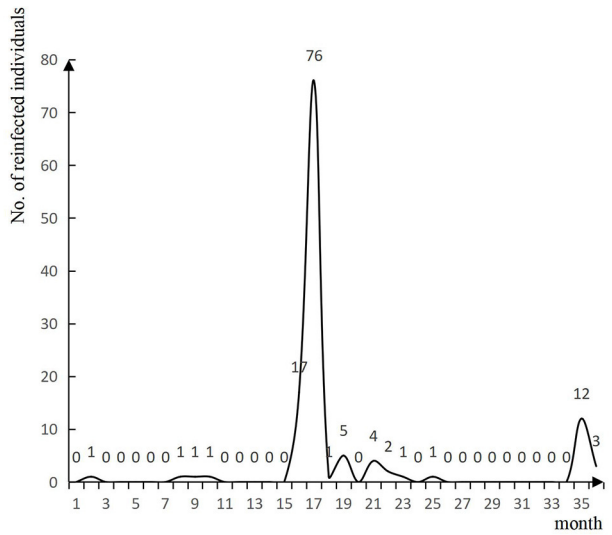

FIGURE 1
Intervals between infections in previously infected individuals.

3.2. Interval of reinfection

No individuals previously infected with COVID-19 were found to be reinfected before November 2022. Among all the 999 eligible respondents reported between November 2022 and January 2023, 126 met the definition of reinfection, with a reinfection incidence of 12.61%. Whereas, the intervals between infections ranged from 73 to 1,082 days, and the median interval (P25–P75) was 508 days (498–530) (Figure 1).

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TABLE 2 Reinfection incidences by gender, age, occupation, interval from initial infection to investigation and type of SARS-CoV-2 strains.

Variables	Reinfection status		Reinfection incidence (%)	χ^2/F/t	P-value
	Reinfected ($N = 126$)	Non reinfected ($N = 873$)			
Gender, *n* (%)				5.197	0.023
Male	53 (42.06)	462 (52.92)	10.30		
Female	73 (57.94)	411 (47.08)	15.10		
Mean age ± SD, yr	42.65 ± 20.39	46.44 ± 19.73	13.01	−2.031	0.043
Occupation, *n* (%)				0.509	0.775
Student and preschooler	15 (11.90)	96 (11.00)	13.50		
Medical staff	3 (2.38)	14 (1.60)	17.60		
Others	108 (85.71)	763 (87.40)	12.40		
Interval from initial infection to investigation, *n* (%)				84.950	<0.001
< 6 months	1 (0.79)	346 (39.63)	0.30		
6 months to 1 year	3 (2.38)	56 (6.41)	5.10		
>1 year	122 (96.83)	471 (53.95)	20.60		
Type of SARS-CoV-2 strains, *n* (%)				66.489	<0.001
Omicron variant	4 (3.17)	404 (46.28)	1.00		
Delta variant	106 (84.13)	447 (51.20)	19.17		
original strain	16 (12.70)	22 (2.52)	42.11		

3.3. Epidemiological characteristics of reinfected individuals

There was no statistically significant difference in reinfection incidences among different occupations, however, the statistical difference was observed in the following factors between the two groups, gender, age, interval from initial infection to the investigation, and type of SARS-CoV-2 strains. The results showed that the reinfection incidence of females was significantly higher than that of male cases (χ^2 =5.197, $P < 0.05$). The difference in the average age between reinfected and non-reinfected groups was statistically significant ($P < 0.05$). The earlier the initial infection occurred, the higher the reinfection incidence and the reinfection incidence was significantly increased when the interval was beyond 1 year ($P < 0.01$). Considering the impact of observation time on the reinfection incidence of different strains, the reinfection status of different SARS-CoV-2 strains was analyzed using incidence density. The incidence densities of the original strain, Delta variant, and Omicron variant were 168.42, 127.56, and 19.70 per 1,000 person-years, respectively (Tables 2, 3).

TABLE 3 The relative risk and results of the logistic regression model among previously infected individuals.

Variables	OR (95%CI)	P-value
Gender		
Male	1	
Female	1.578 (1.061–2.259)	0.023
Age, yr	0.994 (0.985–1.003)	0.203
Interval from initial infection to investigation		
< 6 months	1	
6 months to 1 year	89.813 (12.489–645.870)	<0.001
>1 year	18.211 (1.862–178.118)	0.013
Type of SARS-CoV-2 strains		
original strain	1	
Omicron variant	0.325 (0.165–0.641)	0.001
Delta variant	0.014 (0.004–0.044)	<0.001

3.4. Clinical characteristics of reinfected individuals

Among the 126 reinfected individuals, 119 (94.4%) were symptomatic. The most common symptoms included fever (n = 78,65.54%), cough (n = 73,61.34%), sore throat (n = 22,18.49%), feebleness (n = 19,15.97%), headache (n = 13,10.92%), hyposmia/anosmia (n = 6,5.04%) and symptoms of gastrointestinal (n = 3,2.52%). Additionally, 121 (96.03%) took medication on their own, and only the remaining 5 (3.97%) sought medical attention. There were no critical or hospitalized cases. Compared to the initial

TABLE 4 Clinical characteristics of reinfected individuals.

Variables	Infection ($N = 126$)	Reinfection ($N = 126$)	χ^2	P-value
Whether symptomatic			27.232	<0.001
Yes	87 (69.05)	119 (94.44)		
No	39 (30.95)	7 (5.56)		
Symptom				
Fever	66 (75.86)	78 (65.54)	2.542	0.111
Cough	48 (55.17)	73 (61.34)	0.790	0.374
Sore throat	17 (19.54)	22 (18.49)	0.036	0.849
Fatigue	9 (10.34)	19 (15.97)	1.352	0.245
Fever*			4.640	0.098
Low-grade	14 (21.21)	9 (11.54)		
Moderate-grade	27 (40.91)	45 (57.69)		
High-grade	25 (37.88)	24 (30.77)		

*Low-grade:37.3°C–37.9°C; Moderate-grade: 38°C–38.9°C; High-grade:39°C and above.

infection, the proportion of clinical symptoms in the reinfected population was significantly higher ($P < 0.01$). The proportion of clinical symptoms such as fever, cough, sore throat, and fatigue among the symptomatic individuals was not statistically significant at the time of initial infection and reinfection (Table 4).

3.5. Vaccination status of reinfected individuals

17.32% of the reinfected individuals received three or more doses of COVID-19 vaccination, and 14.91% received two doses. The reinfection incidence of COVID-19 vaccination groups with different doses was statistically significant ($P < 0.01$), fewer reinfections were observed among the respondents with three doses of COVID-19 vaccination compared to the respondents with two doses (χ^2 =14.595, $P < 0.001$) or without COVID-19 vaccination (χ^2 =4.263, $P = 0.039$). However, there was no statistically significant difference in the reinfection incidence between those who received COVID-19 vaccination or not in the past six months or after the initial infection (Table 5).

4. Discussion

As the main variant of SARS-CoV-2, Omicron could significantly reduce the neutralizing efficacy of neutralizing antibodies with different epitopes. Over 85% of the tested neutralizing antibodies were escaped by Omicron (14). The Omicron variant escaped almost all clinically approved antibody therapeutics, significantly impaired humoral immunity elicited by natural infection and vaccination, and had higher transmission rates among household contacts than those of the Delta variant, attributing to a higher risk of yet another resurgence of the pandemic (15).

COVID-19 previously infected individuals reported locally in Yangzhou city in recent 3 years were chosen. According to the weekly report of the Chinese Center for Disease Control and Prevention (CDC), during the period from December 2022 to February 2023, when COVID-19 prevention and control measures were relaxed, an estimated 82.4% of the population in China was infected (16), while the reinfection incidence of previously infected individuals in this survey was only 12.61%, far lower than that of the general population. Multiple surveys of reinfection of previously infected individuals have been conducted in different countries and regions at different periods. For example, France conducted three reinfection surveys at different periods, with reinfection incidences of 0.08% (June 2020 to February 2021), 0.4% (March 2020 to August 2021), and 3.1% (March 2021 to February 2022), respectively (17–19). 1.8% of Peruvian healthcare workers might have been reinfected with SARS-CoV-2 between March 2020 and August 2021 (20). The overall SARS-CoV-2 reinfection incidence was found to be 28.3% (95% CI: 23.7%–33.2%) in Guangdong Province between December 2022 and January 2023 (12). Chengdu reported 8.71% COVID-19 reinfection incidence during February-December, 2022 (13). The conclusions differed among countries and regions, mainly due to the different periods of the investigations and the different types of virus strains which were initially infected and reinfected. A stratified survey of individuals previously infected with different strains of the virus was conducted in the Yangzhou region. The Omicron variant reinfection incidences among previously infected individuals were 42.11% for the original strain, 19.17% for the Delta variant, and 1.00% for the Omicron variant, respectively. There were significant differences in the reinfection incidence of previously infected individuals with different strains of SARS-CoV-2. The reinfection incidence of infected individuals with the initial infection of the Omicron variant is significantly lower than that of those with the Delta variant, mainly because the immune system responded more strongly to the same type of SARS-CoV-2 strains that have been exposed again, meanwhile, produced a high level of neutralizing antibody; However, the reaction to the other newly exposed strains was weak, which can not produce neutralizing antibodies or can only produce low levels of neutralizing antibodies. This phenomenon was called original antigenic sin (OAS), also known as immune imprinting (21). A survey was conducted in the UK between February 2020 and November 2022 to investigate the reinfection with different types of SARS-CoV-2 strains among previously infected individuals, with reinfection incidences ranging from 0.3 to 16.6% (7). The results showed that there was some cross-immune protection between the different strains and that the protection gradually weakened over time. The same types of SARS-CoV-2 strains with different branches had a stronger protective effect and a lower possibility of reinfection. Of course, the individuals infected with COVID-19 during the domestic outbreak might have a stronger awareness of protection, leading to changes in health habits with limited social activities, and the data on reinfection incidence may be underestimated compared with the general population.

Reinfection referred to the reinfection of the same or different types of SARS-CoV-2 strains after the removal of the initial infection with COVID-19, due to factors such as the immune system not producing enough neutralizing antibodies after the initial infection, or the mutation of the key site of

TABLE 5 Vaccination status of COVID-19 among reinfected individuals.

Vaccination status*	Reinfection status		reinfection incidence (%)	χ^2	P-value
	Reinfected ($N = 126$)	Non reinfected ($N = 873$)			
Vaccination doses				15.190	0.002
Unvaccinated	19	111	14.60		
1 dose	9	49	15.50		
2 doses	56	264	17.50		
≥3 doses	42	449	8.60		
Vaccinated within the past 6 months**				0.174	0.677
No	108	760	12.49		
Yes	18	113	13.70		
Vaccinated after the initial infection				0.194	0.660
No	56	261	17.70		
Yes	70	299	18.97		

*Based on the records of the Jiangsu Province Vaccination Integrated Service Management Information System; **Vaccinated with COVID-19 vaccine or not since June 1, 2022.

receptor-binding domain (RBD) of SARS-CoV-2 S protein and other factors. Reinfection was different from the recurrence of positive (re-positive) nucleic acid detection, which was the result of persistent/fluctuant viral shedding or sample detection problems leading to a re-positive SARS-CoV-2 nucleic acid test within a short time after the initial infection. The interval for "re-positive" was usually short, while the reinfection was long. The interval of reinfection reported among countries and regions varied greatly. The relatively long intervals of reinfection reported in this study were mainly related to the strict prevention and control policies adopted by Yangzhou City. However, the shortest intervals for reinfection reported in different regions were inconsistent either, and it was 73 days in this survey. The intervals for "re-positive" and reinfection were not entirely consistent in reports and criteria of judgment around the world. An analysis of post-discharge re-positive in Guangdong Province (22) found that up to 85.27% of re-positive cases were confirmed within 14 d after discharge. Many other regions (23–25) had also reported "re-positive" within 14d−30d, but among the re-positives, there was no fever or other symptoms and almost no secondary cases. The definition of reinfection also varied among different countries. Following the criteria established by the US CDC, SARS-CoV-2 reinfection was defined as an infection occurring more than 90 days after the collection of the first positive specimen (26); and 60 days for the French Ministry of Health (19); The UK Office for National Statistics had set multiple standards for reinfection, one of which was met to qualify as reinfection, including an interval of at least 60 days (7). Therefore, it was difficult to distinguish between reinfection and "re-positive" by the SARS-CoV-2 nucleic acid test alone. A comprehensive assessment of factors such as intervals, clinical symptoms, and epidemiologic history was required.

Additionally, our survey revealed the incidence of COVID-19 reinfection varied with different population characteristics. The risk of reinfection was significantly higher among females and younger cases, which is consistent with some other research findings (27, 28). The higher screening rate for females and younger people, and the higher exposure to occupational and social activities, might be the reasons. Medical staff and long-term care facility residents had a slightly higher reinfection incidence and were persistently at a higher risk of exposure when compared to the general population (29, 30). However, in this survey, there was no statistically significant difference in reinfection incidences among different occupations, indicating that occupational exposure in Yangzhou did not affect the reinfection incidence. Reinfection rates differed significantly among individuals initially infected with various SARS-CoV-2 strains. The reinfection incidence for those primarily exposed to the Omicron variant was 1.00%, significantly lower than that for those initially infected with the Delta variant and original strain. The timing of the emergence of dominant SARS-CoV-2 strains has been influenced by different prevention and control policies around the world, along with the definition of reinfection and various natural and social factors, all led to varying results of reinfection incidences among studies.

In this study, fewer reinfections were observed among the respondents with three doses of COVID-19 vaccination compared to the respondents with two doses or without COVID-19 vaccination, which was consistent with the conclusion of Altarawneh et al. (31). Hybrid immunity induced by a combination of natural infections and vaccinations had not been detected in providing excellent protection against SARS-CoV-2 reinfection in this survey, which may be related to factors such as the small sample size of the study population, vaccine type, and vaccination time. However, several researches demonstrated that hybrid immunity was more protective against symptomatic disease and progression to critical illness and was associated with a longer time to reinfection (32–34). Therefore, it is recommended that both first-time infected and reinfected populations should continue to strengthen vaccination efforts to reduce the incidence of reinfection or critical cases.

This study has several limitations that warrant consideration. First, there was a certain bias in the investigation process, as some of the previously infected individuals in this study were lost to

interviews, and the previously infected individuals in Yangzhou had more intervened behavioral habits before the adjustment of epidemic prevention and control policies.

Second, the SARS-CoV-2 strains that infected some of the study subjects were directly identified based on the genome sequencing results of the key cases in the chain of transmission during the local epidemic. Although there were close epidemiological associations, individual abnormal situations might exist.

Third, the type of COVID-19 vaccine was not specifically analyzed as the main immunization protocol was with an inactivated vaccine. While adenovirus vector vaccine and recombinant protein (CHO cell) vaccine had relatively low vaccination rates. Additionally, as the sample size of this study was small, large-scale studies with extended follow-up periods are warranted to confirm relatively accurate conclusions.

The results of this study showed that after the epidemic period of COVID-19, there were significant differences in the reinfection incidence of previously infected individuals with different strains of SARS-CoV-2, indicating there was some cross-immune protection against various strains of SARS-CoV-2, and that the cross-immunoprotection gradually weakened over time. The infection incidence is influenced by various factors such as viral characteristics, vaccination, epidemic prevention policies, and individual variations. As the SARS-CoV-2 continues to mutate, vaccination and appropriate personal protection have practical significance in reducing the risk of reinfection.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of Yangzhou Center for Disease Control and Prevention. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

YW: Investigation, Methodology, Software, Writing—original draft, and Writing—review and editing. JL: Formal analysis, Investigation, and Writing—review and editing. HY: Formal analysis, Investigation, Software, and Writing—review and editing. LZ: Software, Supervision, and Writing—review and editing. JH: Funding acquisition, Software, Supervision, and Writing—review and editing. LX: Data curation, Formal analysis, and Writing—review and editing. JZ: Investigation, Methodology, and Writing—review and editing. XZ: Conceptualization, Writing—review and editing, and Supervision. YH: Validation, Resources, and Writing—review and editing. YD: Investigation, Writing—review and editing, and Formal analysis. CW: Investigation, Writing—review and editing, and Formal analysis.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1256768/full#supplementary-material

References

1. Yahav D, Yelin D, Eckerle I, Eberhardt CS, Wang J, Cao B, et al. Definitions for coronavirus disease 2019 reinfection, relapse and PCR re-positivity. *Clin Microbiol Infect.* (2021) 27:315–8. doi: 10.1016/j.cmi.2020.11.028

2. Wang H, Wright T, Everhart K, Oyeniran SJ, Mejias A, Leber AL. SARS-CoV-2 Reinfection with different SARS-CoV-2 variants in children, Ohio, United States. *J Pediatric Infect Dis Soc.* (2023) 12:198–204. doi: 10.1093/jpids/piad017

3. To KK-W, Hung IF-N, Ip JD, Chu AW-H, Chan W-M, Tam AR, et al. Coronavirus disease 2019 (COVID-19) Re-infection by a phylogenetically distinct severe acute respiratory syndrome coronavirus 2 strain confirmed by whole genome sequencing. *Clin Infect Dis.* (2021) 73:e2946–51. doi: 10.1093/cid/ciaa1275

4. Ren X, Zhou J, Guo J, Hao C, Zheng M, Zhang R, et al. Reinfection in patients with COVID-19: a systematic review. *Glob Health Res Policy.* (2022) 7:12. doi: 10.1186/s41256-022-00245-3

5. El-Shabasy RM, Nayel MA, Taher MM, Abdelmonem R, Shoueir KR, Kenawy ER. Three waves changes, new variant strains, and vaccination effect against COVID-19 pandemic. *Int J Biol Macromolecules.* (2022) 204: 161–168. doi: 10.1016/j.ijbiomac.2022.01.118

6. Ghorbani SS, Taherpour N, Bayat S, Ghajari H, Mohseni P, Nazari SSH. Epidemiologic characteristics of cases with reinfection, recurrence, and hospital readmission due to COVID-19: a systematic review and meta-analysis. *J Med Virol.* (2022) 94:44–53. doi: 10.1002/jmv.27281

7. Statics OFN. *Coronavirus (COVID-19) Infection Survey, characteristics of people testing positive for COVID-19, UK*. (2022). Available online at: https://www.ons.gov.uk/peoplepopulationandcommunity/healthandsocialcare/conditionsanddiseases/datasets/coronaviruscovid19infectionsinthecommunityinengland (accessed December 14, 2022).

8. Ma Y, Xu S, Luo Y, Qin Y, Li J, Lei L, et al. Epidemiological characteristics and transmission dynamics of the COVID-19 outbreak in Hohhot, China: a time-varying SQEIAHR model analysis. *Front Public Health.* (2023) 11:1175869. doi: 10.3389/fpubh.2023.1175869

9. Li ZX, Fu GT, Li XW. Epidemiological characteristics of COVID-19 patients in Shaanxi Province. *Shanghai J Prev Med.* (2022) 34:973–6. doi: 10.19428/j.cnki.sjpm.2022.2206

10. Wang J, Ma T, Ding S, Xu K, Zhang M, Zhang Z, et al. Dynamic characteristics of a COVID-19 outbreak in Nanjing, Jiangsu province, China. *Front Public Health.* (2022) 10:933075. doi: 10.3389/fpubh.2022.933075

11. Zhang GP, Su C, Yang J, Yan FZ, Wu WS, Bai JY, et al. Transmission characteristics and risk factors of household COVID-19 clusters caused by 2019-nCoV Omicron variant in Tianjin. *Zhonghua Liu Xing Bing Xue Za Zhi.* (2022) 43:1370–5. doi: 10.3760/cma.j.cn112338-20220425-00340

12. Cai C, Li Y, Hu T, Liang R, Wang K, Guo C, et al. The associated factors of SARS-CoV-2 reinfection by omicron variant - Guangdong Province, China, December 2022 to January 2023. *China CDC Wkly.* (2023) 5:391–6. doi: 10.46234/ccdcw2023.075

13. Dai YX, Wang Y, Du XB, Yan K, Zhang S, Liu H, et al. Characteristics of Omicron variant-induced SARS-CoV-2 reinfections in Chengdu, China. *Chin J Public Health.* (2023) 39:442–7. doi: 10.11847/zgggws1141316

14. Cao Y, Wang J, Jian F, Xiao T, Song W, Yisimayi A, et al. Omicron escapes the majority of existing SARS-CoV-2 neutralizing antibodies. *Nature.* (2022) 602:657–63. doi: 10.1038/s41586-021-04385-3

15. Cui Z, Liu P, Wang N, Wang L, Fan K. Structural and functional characterizations of infectivity and immune evasion of SARS-CoV-2 Omicron. *Cell.* (2022) 185:860–71. doi: 10.1016/j.cell.2022.01.019

16. Fu D, He G, Li H, Tan H, Ji X, Lin Z, et al. Effectiveness of COVID-19 vaccination against SARS-CoV-2 omicron variant infection and symptoms - China, December 2022-February 2023. *China CDC Wkly.* (2023) 5:369–73. doi: 10.46234/ccdcw2023.070

17. Gautret P, Houhamdi L, Nguyen NN, et al. Does SARS-CoV-2 reinfection depend on virus variant? *Clin Microbiol Inf.* (2021) 27:1374–5. doi: 10.1016/j.cmi.2021.06.029

18. Nguyen NN, Houhamdi L, Hoang VT, et al. SARS-CoV-2 reinfection and COVID-19 severity. *Emerging Microb Inf.* (2022) 11:894–901. doi: 10.1080/22221751.2022.2052358

19. Bastard J, Taisne B, Figoni J, Mailles A, Durand J, Fayad M, et al. Impact of the omicron variant on SARS-CoV-2 reinfections in France, March 2021 to February 2022. *EuroSurveillance.* (2022) 27:2200247. doi: 10.2807/1560-7917.ES.2022.27.13.2200247

20. Ramos W, Guerrero N, Napanga-Saldana EO, et al. Hospitalization, death, and probable reinfection in Peruvian healthcare workers infected with SARS-CoV-2: a national retrospective cohort study. *Hum Resour Health.* (2022) 20:86. doi: 10.1186/s12960-022-00787-0

21. Yu YL. Immune imprinting caused by infection/vaccination of severe acute respiratory syndrome coronavirus 2. *Prog Microbiol Immunol.* (2023) 51:1–5. doi: 10.13309/j.cnki.pmi.2023.03.001

22. Tan XH, Kang M, Deng AP, Li BS, Luo M, Yi Y, et al. Analysis on characteristics and influencing factors of COVID-19 confirmed cases with viral nucleic acid re-positive after discharge in Guangdong Province. *Chin J Prev Med.* (2022) 56:49–55. doi: 10.3760/cma.j.cn112150-20211108-01034

23. Hu M, Yue Y, Feng J, Tu ZH, Meng JT, Mao Y, et al. Prevalence and infectious characteristics of retest positive cases infected with SARS-CoV-2 in Chengdu city. *Chin J Public Health.* (2022) 38:758–61. doi: 10.11847/zgggws1137219

24. Zhang JR, Min Y, Zhang JP, Feng YJ, Bao ZW, Wu XX, et al. Characteristics of 637 patients infected with novel coronavirus Omicron variant retested positive in Suzhou. *J Clin Pulm Med.* (2023) 28:340–4. doi: 10.3969/j.issn.1009-6663.2023.03.004

25. Li ZL, Li Y, Chen QL, Yang XK, Zhao HT, Jiang XL, et al. Distribution and infectious characteristics of repositive cases infected with SARS-CoV-2. *Chin J Epidemiol.* (2021) 42:1750–6. doi: 10.3760/cma.j.cn112338-20210506-00367

26. Akinbami LJ, Biggerstaff BJ, Chan PA, McGibbon E, Pathela P, Petersen LR. Reinfection with severe acute respiratory syndrome coronavirus 2 among previously infected healthcare personnel and first responders. *Clin Infect Dis.* (2022) 75:e201–7. doi: 10.1093/cid/ciab952

27. Dhillon RA, Qamar MA, Gilani JA, Irfan O, Waqar U, Sajid MI, et al. The mystery of COVID-19 reinfections: a global systematic review and meta-analysis. *Ann Med Surg.* (2021) 72:103130. doi: 10.1016/j.amsu.2021.103130

28. Flacco ME, Soldato G, Martellucci CA, Di Martino G, Carota R, Caponetti A, et al. Risk of SARS-CoV-2 reinfection 18 months after primary infection: population-level observational study. *Front Public Health.* (2022) 10:884121. doi: 10.3389/fpubh.2022.884121

29. Malhotra S, Mani K, Lodha R, Bakhshi S, Mathur VP, Gupta P, et al. COVID-19 infection, and reinfection, and vaccine effectiveness against symptomatic infection among health care workers in the setting of omicron variant transmission in New Delhi, India. *Lancet Reg Health Southeast Asia.* (2022) 3:100023. doi: 10.1016/j.lansea.2022.100023

30. Jang EJ, Choe YJ, Yun G-W, Wang S, Cho UJ, Yi S, et al. Reinfection with SARS-CoV-2 in general population, South Korea; nationwide retrospective cohort study. *J Med Virol.* (2022) 94:5589–92. doi: 10.1002/jmv.28026

31. Altarawneh HN, Chemaitelly H, Ayoub HH, Tang P, Hasan MR, Yassine HM, et al. Effects of previous infection and vaccination on symptomatic omicron infections. *N Engl Med.* (2022) 387:21–34. doi: 10.1056/NEJMoa2203965

32. Hui DS. Hybrid immunity and strategies for COVID-19 vaccination. *Lancet Infect Dis.* (2023) 23:2–3. doi: 10.1016/S1473-3099(22)00640-5

33. Al-Otaiby M, Krissaane I, Al Seraihi A, Alshenaifi J, Qahtani MH, Aljeri T, et al. SARS-CoV-2 reinfection incidence and outcomes in Saudi Arabia: a national retrospective study. *Int J Infect Dis.* (2022) 122:758–66. doi: 10.1016/j.ijid.2022.07.025

34. Bobrovitz N, Ware H, Ma X, Li Z, Hosseini R, Cao C, et al. Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the omicron variant and severe disease: a systematic review and meta-regression. *Lancet Infect Dis.* (2023) 23:556–67. doi: 10.1016/S1473-3099(22)00801-5

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Impact of vaccination and non-pharmacological interventions on COVID-19: a review of simulation modeling studies in Asia

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Introduction: Epidemiological modeling is widely used to offer insights into the COVID-19 pandemic situation in Asia. We reviewed published computational (mathematical/simulation) models conducted in Asia that assessed impacts of pharmacological and non-pharmacological interventions against COVID-19 and their implications for vaccination strategy.

Methods: A search of the PubMed database for peer-reviewed, published, and accessible articles in English was performed up to November 2022 to capture studies in Asian populations based on computational modeling of outcomes in the COVID-19 pandemic. Extracted data included model type (mechanistic compartmental/agent-based, statistical, both), intervention type (pharmacological, non-pharmacological), and procedures for parameterizing age. Findings are summarized with descriptive statistics and discussed in terms of the evolving COVID-19 situation.

Results: The literature search identified 378 results, of which 59 met criteria for data extraction. China, Japan, and South Korea accounted for approximately half of studies, with fewer from South and South-East Asia. Mechanistic models were most common, either compartmental (61.0%), agent-based (1.7%), or combination (18.6%) models. Statistical modeling was applied less frequently (11.9%). Pharmacological interventions were examined in 59.3% of studies, and most considered vaccination, except one study of an antiviral treatment. Non-pharmacological interventions were also considered in 84.7% of studies. Infection, hospitalization, and mortality were outcomes in 91.5%, 30.5%, and 30.5% of studies, respectively. Approximately a third of studies accounted for age, including 10 that also examined mortality. Four of these studies emphasized benefits in terms of mortality from prioritizing older adults for vaccination under conditions of a limited supply; however, one study noted potential benefits to infection rates from early vaccination of younger adults. Few studies (5.1%) considered the impact of vaccination among children.

Conclusion: Early in the COVID-19 pandemic, non-pharmacological interventions helped to mitigate the health burden of COVID-19; however, modeling indicates

that high population coverage of effective vaccines will complement and reduce reliance on such interventions. Thus, increasing and maintaining immunity levels in populations through regular booster shots, particularly among at-risk and vulnerable groups, including older adults, might help to protect public health. Future modeling efforts should consider new vaccines and alternative therapies alongside an evolving virus in populations with varied vaccination histories.

KEYWORDS

COVID-19, vaccination, epidemiological modeling, Asia, intervention

1. Introduction

The SARS-CoV-2, which causes COVID-19, rapidly spread from the first reported symptoms in Wuhan, China, in December 2019 through to the declaration of an International Public Health Emergency by the World Health Organization in January 2020 (1, 2). The nature of the virus has since changed through its evolution into new variants of concern, with differing potentials for infection, severe disease course, and negation of immunity from vaccination or prior infection (3, 4). The human response to the pandemic has also developed from the implementation of non-pharmacological countermeasures through to pharmacological and vaccination strategies, which have mitigated the impact of the pandemic with varying degrees of effectiveness (5, 6).

In the early pandemic, non-pharmacological interventions were implemented across many regions to ease the immediate disease burden of COVID-19 and prevent the overwhelming of public health systems. Although lockdowns and school/workplace closures were effective in reducing transmission and patient healthcare costs, the economic disruption, declining mental health, and social discontent caused by these interventions raised concerns (7–9). The development and wider rollout of effective vaccines has further changed the dynamics of the situation (10–13). In the context of limited vaccine supply, the WHO recommended administration strategies that aimed to reduce mortality from COVID-19 infection (14). Individuals at risk of infection and/or severe disease were targeted, namely, front-line healthcare workers and older adults, the latter group being at particular risk of morbidity and mortality from COVID-19, compared with younger age groups (6, 15, 16). As supplies have stabilized, campaigns to vaccinate the wider public have faced challenges in the form of hesitancy among certain populations to receive initial doses of the vaccine and/or subsequent boosters (17, 18). Additionally, from 2022, the progression from a prevalent Delta to Omicron variant has shifted the burden of disease to younger people, including pediatric patients in Asia (19–21).

Ongoing high infection rates, an evolving virus, vaccine fatigue, and the relaxation of pandemic countermeasures suggest that COVID-19 will pose ongoing challenges to public health with potential differential effects across age groups (22, 23). Owing to large and diverse populations with different cultural practices and healthcare systems, the development of the COVID-19 situation in Asian settings is likely to pose unique questions for healthcare professionals and policymakers in the region. The dynamics of vaccination against SARS-CoV-2 are complicated by age-dependent factors, type of vaccines used and population vaccine coverage, changing levels of infection and the prevailing strains of concern, and the relaxation of non-pharmaceutical interventions, hence, necessitating the use of mathematical modeling studies in Asia.

Throughout the crisis, epidemiological models have been widely developed and used by policymakers to estimate the impact of interventions on projected disease burden and demands on public healthcare systems (24, 25). Mitigation strategies, including the mandating of mask-wearing, social distancing, restrictions on movement and gatherings, through to pharmaceutical and vaccination strategies, have been implemented to control the spread of virus with varying degrees of effectiveness (5, 6). Although models cannot exactly predict key factors such as the basic reproduction number (R_0), these tools have been applied both globally and in Asia to guide potential care needs in terms of stratifying risk, directing limited resources, and planning for future outbreaks (26, 27). Models of increasing complexity have also been developed to account for the dynamics of transmission among different age groups in the context of different vaccination statuses and waning vaccine effectiveness over time or against emerging variants (28).

Mechanistic models are formulated to mimic the nature of spreading diseases, allowing the simulation of the complex dynamics and nonlinear feedback of COVID-19 transmission within a population (29). An example is the Susceptible-Infected-Exposed-Removed (SEIR) compartmental model, which considers interactions among cohorts of a population, categorized according to disease status. Individuals within a compartment are not differentiated from one another but can flow from one compartment to another at rates defined by parameter inputs. This approach can be effective for examining disease dynamics at a macro level. As a type of mechanistic model, individual-level models treat members of a population as unique agents; this may better capture phenomena such as super-spreader events. Statistically derived models (for example, distribution fitting and regression-type analysis) can also be applied to accurately characterize data from a small number of parameters and are suitable for short-term forecasts that are accurate, repeatable, and sensitive to momentary changes in a system (30). These tools include regression using least-squares or Bayesian estimations. These features make statistical models valuable for producing up-to-date estimates of COVID-19's underlying nature as a disease (e.g., the basic reproduction rate) and the demographic patterns of the population (e.g., how long people self-isolate). Although statistical models can be highly accurate, they treat the situation as a black box and do not mimic the underlying nature of the disease or its consequences. Thus, these models are not well suited for long-term projections or for exploring hypothetical scenarios.

This review aims to examine published COVID-19 epidemiological modeling studies conducted in Asian settings and assess the models used and their findings regarding the impact of vaccination and non-pharmacological measures on COVID-19 infections, hospitalization, mortality, and policies. We discuss the implications of modeling studies in Asia in the context of the latest data on COVID-19 vaccines, the emergence of SARS-CoV-2 variants, and regional vaccination policies.

2. Methods

2.1. Literature search

For the purposes of this study, eligible reports examined the infection, mortality, hospitalization, vaccination, and/or policy outcomes from computational models for COVID-19 set in Asia. We followed the PRISMA guidelines in conducting systematic elements of this review. A literature search in the PubMed database for peer-reviewed, published, and accessible articles in English, available from the start of the COVID-19 pandemic, was conducted on 9 November 2022. Search terms focused on keywords identified from PICO (populations, intervention, comparison, outcome) elements and included terms common among modeling studies (e.g., Susceptible-Infected-Recovered, simulation, agent-based), types of outcomes (e.g., hospitalization, infection, and mortality) and the target interventions (e.g., vaccination) (See Supplementary Table S1 for full search terms). The search was filtered for articles that had available full text, and the results were exported to Microsoft Excel for initial screening based on the titles and abstracts. Articles that did not include an epidemiological model (e.g., laboratory and clinical studies) and/or were not concerned with COVID-19 (e.g., other diseases examined within a COVID-19 setting) were excluded.

Screened articles were further categorized based on the abstract and display items to identify a smaller set of articles that fulfilled any of the following conditions: (i) the article mentions collaborations with, or recommendations to, policy-makers; or (ii) the article considers the effects size of pharmacological and/or non-pharmacological interventions on any of the outcomes of interest (infection, mortality, hospitalization, and/or vaccination).

2.2. Data extraction

One research assistant performed data extraction for model classification, location, time, and scale, interventions applied, consideration of age groups, and impacts of interventions on rates of infection, hospitalization/severe disease, mortality, and/or policy. Another research assistant confirmed the extracted data.

Non-pharmacological interventions were categorized as "reducing contact" (measures that reduce transmission from infected individuals, such as lockdowns, school/workplace closure, and social distancing), "border control" (preventing infected individuals from entering the population; for instance, airport quarantine and border closure), "contact tracing" (testing/quarantining of people having close contact with infected individuals), "hygiene" (e.g., hand washing, ventilation), "PPE" (e.g., wearing of masks, gloves), and "other." In categorizing models employed in reports, we differentiated between "mechanistic" (i.e., any model that applied *a priori* mathematical equations describing physical systems, with or without stochastic elements) and "statistical models" (e.g., models based on regression or Bayesian analysis, or machine learning of data sets), and classified combinations as "both." We further categorized mechanistic models as compartmental (cohort), agent-based (individual) or combination models (combining elements from both types).

3. Results

3.1. Study settings

The literature search returned 378 results, which, after exclusions for irrelevance ($n = 127$) and ineligibility ($n = 192$), resulted in a set of 59 reports selected for data extraction (Figure 1; Table 1). Most reports were from East Asia (China, 21/59 [3 of which were set in Hong Kong], 35.6%; Japan, 7/59, 11.9%; South Korea 6/59, 10.2%), followed by South Asia (India, 5/59, 8.5%; Bangladesh, 3/59, 5.1%; Sri Lanka, 1/59, 1.7%) and South-East Asia (Malaysia, 3/59, 5.1%; the Philippines, 2/59, 3.4%; Thailand, 1/59, 1.7%; Vietnam; 1/59, 1.7%). Multiple countries were considered in 6.8% (4/59) of reports, and 5.1% (3/59) presented modeling results that examined specific Asian regions as part of a global setting. The majority of reports (59.3%, 35/59) applied a modeling start date in 2020, with fewer studies starting in 2021 (33.9%, 20/59) and 2022 (3.4%, 2/59).

3.2. Modeling approaches

Mechanistic models dominated the reports (79.7.0%, 47/59), with fewer statistical (11.9%, 7/59), and combinations of both approaches (8.5%, 5/59) (Table 1). Among the mechanistic models, the majority were based on a compartmental model (61.0%, 36/59). For example, Jung et al. (34) used a susceptible-infected-recovered (SIR) model with time-dependent parameters determined by machine learning. Only one study (1.7%) focused on an agent-based model constructed from statistical data on buildings and the local population to model individual interactions (45); however, a number of studies integrated combination analysis models (18.6%, 11/59), including a study by Shen et al. which compared results from separate SEIR and agent-based models (74).

These proportions were generally similar across the geographic regions (Figure 2A). More than a third of reports accounted for the effects of age in their modeling (37.3%, 22/59), either as an analytical parameter or by separation of specific age groups. For example, Sunohara et al. (89) applied a SEIR model, stratifying the population into three age groups: young (15–49 years), middle (50–64 years), and old (>64 years), ignoring children (0–14 years). A large proportion of studies were prospective (44.1%, 26/59) or included a component of prospective analysis (28.8%, 17/59); completely retrospective studies were less common (27.1%, 16/59).

3.3. Interventions

Pharmacological and non-pharmacological interventions were considered in 59.3% (35/59) and 84.7% (50/59) of reports, respectively

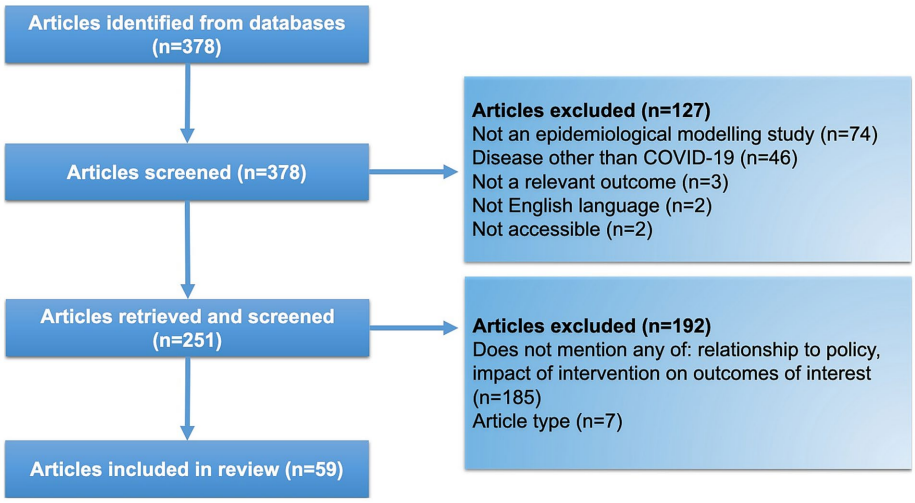

FIGURE 1
Literature search flow diagram.

(not mutually exclusive). Vaccination was the most common pharmacological intervention, considered in 57.6% (34/59) of studies. Notably, vaccination was a factor considered in more than half of the reports from East Asian settings (China, 13/21, 61.9%; Japan, 6/7, 85.7%; South Korea, 4/6, 66.7%), as well as India (3/5, 60.0%) and Thailand (1/1, 100%) (Figure 2B). However, models that incorporated vaccination were either absent or less common than models that incorporated non-pharmacological interventions in many regions of South Asia (Bangladesh, 0/3, 0%; Sri Lanka, 0/1, 0%) and South-East Asia (Malaysia, 0/3, 0%; the Philippines, 1/2, 50.0%; Vietnam; 0/1, 0%). Among studies concerning vaccination, 25.4% (15/59) also considered the influence of age in their models; however, vaccination was explicitly extended to pediatric patients (≥3 years old) in only three studies (32, 38, 55) and adolescents in one study (≥15 years old) (89). In other studies, vaccines were considered to be administered only to the adult population (typically ≥20 years old).

The type of vaccine was generally unspecified or maintained as a 'hypothetical' vaccine within the model with effectiveness as a variable. Where vaccine effectiveness was an input parameter in models, a wide range of values for effectiveness in reducing infection were typically used. Several models also parameterized the effectiveness of vaccination for reducing infectiousness and risk of mortality, as well as vaccination type, number of doses, and time from vaccination. Two studies from China specified the use of vaccines based on the inactivated virus, and two studies from Japan specified the use of mRNA vaccines. A study from South Korea compared a variety of different mRNA vaccines and a viral vector vaccine. Traditional Chinese medicine was also considered in one report and antiviral medication in two reports.

A large proportion of the reports that considered non-pharmacological interventions examined the effects of reducing contact (76.3%, 45/59). These kinds of interventions ranged from strict lockdowns on individual movement in regions that report infections (32) to studies that parameterized changes in individual mobility at public transport facilities and spaces (62). Contact tracing (20.3%, 12/59); border control (16.9%, 10/59); and the use of PPE (13.6%, 8/59), i.e., masks and face shields, were also relatively common targets. The effects of hygiene (3.4%, 2/59) and education (3.4%, 2/59) were less commonly measured. For several lower-income regions (i.e., Vietnam, Malaysia, Sri Lanka, Bangladesh), modeling targeted only non-pharmacological interventions (Figure 2B).

3.4. Outcomes

3.4.1. Infection

Symptomatic infection was a target outcome of almost all models (91.5%, 54/59) (Table 1; Figure 3). Among the 57.6% (34/59) of reports that considered the influence of vaccination, almost all (97.1%, 33/34) examined infection as an outcome. Of the 85.2% (46/54) of reports concerning non-pharmacological impacts on infection (Figure 3), those categorized as reducing contact were most common (93.5%, 43/46), followed by contact tracing (23.9%, 11/46), border control (21.7%, 10/46), and PPE (15.2%, 7/46). Fewer reports considered the effects of hygiene (4.3%, 2/46) or education (4.3%, 2/46) on infection.

Among models that considered infection, 35.2% (19/54) of reports accounted for the influence of age as a factor in their models, including 14 reports of vaccinated populations. With respect to recommendations for prioritizing vaccines to different age groups during the acute stage of the pandemic, only one study in Japan noted potential benefits to infection rates by vaccinating younger people first (89), and two South Korean reports specifically recommended prioritizing older adults first (49, 50). Other reports from China proposed targets for overall population coverage necessary to flatten case numbers. Two studies highlighted the importance of expanding vaccination programs to include young people (32, 55).

3.4.2. Hospitalization and severe disease

Incidences of severe cases of COVID-19 that required medical intervention and/or hospitalization were considered in 30.5% (18/59) of studies (Table 1; Figure 3). Vaccination was considered in 61.1% (11/18) of these studies. A third of reports (33.3%, 6/18) stratified this outcome by age, in which two studies noted that targeted vaccination

of older adults or children would decrease hospitalization (32, 38). Additionally, 77.8% (14/18) of reports considered non-pharmacological countermeasures, including reducing contact (66.7%, 12/18), contact tracing (22.2%, 4/18), and border control (11.1%, 2/18).

3.4.3. Mortality

Among the included reports, 30.5% (18/59) considered mortality as a modeling outcome (Table 1; Figure 3); of these, 72.2% (13/18) examined vaccination. Age was a factor in 55.6% (10/18) of reports, and 22.2% (4/18) explicitly noted that vaccination schedules prioritizing older adults during the acute phase of the pandemic would likely reduce overall mortality most effectively (46, 49, 50, 55). One Japanese report suggested that a vaccination strategy that prioritized younger generations might offer benefits in terms of overall mortality (89). However, the stringency of and compliance with non-pharmacological countermeasures was acknowledged to modulate the effectiveness of age-based strategies. Models that incorporated non-pharmacological countermeasures were a focus of 77.8% (14/18) of reports; these countermeasures included contact reduction (72.2%, 13/18), contact tracing (11.1%, 2/18), and border control (11.1%, 2/18).

4. Discussion

We examined modeling studies applied to the COVID-19 pandemic in Asia to identify patterns that might guide decision-making on appropriate and timely countermeasures for the ongoing endemic situation and management of future potential SARS-CoV-2 variants of interest. Although, global studies have raised concerns that modeling studies may not give accurate long-term forecasts of numbers of COVID-19 patients, hospitalizations, and deaths, the scenarios generated by such models are still useful for guiding decision-making and resource prioritization (90–92). Across many regions, modeling studies have also played roles in messaging to the public in order to explain and justify otherwise undesirable interventions (93). Our review showed that data-driven modeling studies play a critical role in the understanding of disease and various preventive policies in Asia.

We identified more reports from East Asian nations, particularly dominated by reports from China. However, the strong tendency to use mechanistic modeling was generally reflected across all regions. Despite the considerable differences in public health infrastructure and substantial variations in population densities, income levels, and sociocultural aspects (82), the common use of mechanistic cohort models across regions suggests that this approach is universally attractive for understanding general disease dynamics and the effectiveness of different interventions (94). This choice may also reflect the intuitive nature of this type of model and the ability to easily develop tools suitable for use by policymakers. Other key advantages of mechanistic models include the insights into the disease that might be gained, particularly from validation of the modeling against real-world evidence. Hence, mechanistic disease-spread models are often used as a basis for cost-effectiveness analyses (95). Additionally, the broad range of symptoms that manifest in COVID-19 introduces considerable uncertainty into national reporting of disease statistics; thus, more sophisticated models (i.e., agent-based models) may not offer particular benefits in accuracy based on available input data.

Particularly early in the pandemic, prior to the wider availability of vaccines, models examined the influence of non-pharmacological interventions on disease-related outcomes. Models set in South and South-East Asian regions were particularly focused on non-pharmacological interventions rather than vaccination, which may reflect the timing of this literature search and the slower vaccine approval, acquisition, and administration in these regions. The majority of models examined here looked at reducing contact through restrictions of movement and gatherings. This focus may reflect the economic disruption caused by these interventions. Although such measures are acknowledged to be effective in reducing disease transmission, their impact is difficult to assess in terms of financial burden and sustainability and compliance; thus, it remains challenging to gauge the accuracy of modeling applied to these measures (96). Few studies here included explicit cost-effectiveness assessments for managing COVID-19 with non-pharmacological interventions or vaccination strategies (47, 88, 89). Whereas vaccination is considered to be cost-effective in terms of treatment costs, strictly enforced countermeasures can pose considerable socio-economic and mental health burdens on populations, which are not fully considered in any of the models identified here (97, 98).

Following the introduction of vaccines from late 2020, reports more often incorporated the effects of vaccination into models. Although information on the specific vaccines used was often limited, actual administration patterns may be inferred from local approval schedules and government procurement policies. Whereas China has almost exclusively used inactivated vaccines (e.g., CoronaVac, Sinovac Biotech), Japan and South Korea granted early approval for mRNA vaccines (e.g., Comirnaty, Pfizer–BioNTech, Pfizer Inc.; Spikevax, Moderna Inc), which have been widely administered. In India, a viral vector vaccine (Vaxzevria, Oxford-AstraZeneca) was used extensively; and across other South and South-East Asian countries, a variety of vaccine types have been deployed due to varying availability and access.

Early recommendations on vaccination strategies were broadly similar, regardless of the vaccination type and local situation, emphasizing the need for rapid distribution and high overall population coverage. Models examining vaccination identified here underline the importance of rapidly achieving 67–83% population coverage with effective vaccines to reduce infection, hospitalization, and/or mortality rates (99). Direct comparisons of modeling results with actual real-world data are complicated by various factors. The methods of diagnosing infection have developed from polymerase chain reaction (PCR) testing initially, through to antigen rapid testing later in the pandemic. Additionally, the implementation of home-based recovery programs in some regions may have underestimated the actual number of infections and hospital admissions (100). Nevertheless, predictions of vaccination outcomes from Asian models have been widely confirmed by lower rates of infection and hospitalization among vaccinated individuals and higher excess mortality among unvaccinated adults across these regions (101, 102).

Reports diverged in terms of specific recommendations for prioritizing vaccination of different age groups. In China and South Korea, where strict social measures were implemented alongside widespread use of inactivated virus or mRNA vaccines, respectively, the majority of models recommended priority vaccination of older

TABLE 1 Key characteristics of included reports (*N* = 59 studies).

Author Year (citation)	Locality	Intervention			Model type		Age groups	Outcomes		
		Nonpharmacological	Pharmacological	Vaccination efficacy parameters[a]				Infection	Hospitalization and severe disease	Mortality
Baniasad 2021 (31)	Iran, Turkey, Saudi Arabia, United Arab Emirates, India, Russia, Philippines, South Korea	Contact tracing, reducing contact	Vaccination (unspecified)	Unspecified	Both	Combination	N/A	X	X	X
Cai 2022 (32)	China	Reducing contact	Vaccination (Sinovac/ CoronaVac)	51.8%, as a variable	Mechanistic	Compartmental	14 age groups	X	X	X
Ferguson 2022 (33)	Bangladesh	Reducing contact, PPE	N/A	N/A	Mechanistic	Compartmental	N/A	X	X	X
Jung 2020 (34)	North Korea	Reducing contact	Vaccination (unspecified)	50–80% in reducing susceptibility 80–95% reducing hospitalization	Mechanistic	Compartmental	N/A	X	X	
Leung 2021 (35)	Global, Hong Kong, China	Border control	Vaccination (unspecified)	Weighted average of available vaccines	Mechanistic	Compartmental	Age as variable	X	X	X
Qian 2022 (36)	China	Contact tracing, reducing contact	Vaccination (unspecified)	Unspecified	Mechanistic	Compartmental	N/A	X	X	X
Suphanchaimat 2021 (37)	Thailand	N/A	Vaccination (unspecified)	50% against infection	Mechanistic	Compartmental	N/A	X	X	X
Cai 2022 (38)	China	N/A	Vaccination (unspecified)	Variable	Mechanistic	Compartmental	16 age groups	X	X	
Ediriweera 2020 (39)	Sri Lanka	Reducing contact	N/A	Weighted average of available vaccines	Mechanistic	Compartmental	N/A	X	X	
Kong 2022 (40)	Japan, China	Contact tracing, border control, reducing contact	Vaccination (unspecified)	Unspecified	Mechanistic	Compartmental	N/A	X	X	
Rajput 2021 (41)	India	Contact tracing, reducing contact	Vaccination (unspecified)	80%, variable	Mechanistic	Compartmental	N/A	X	X	
Shah 2022 (42)	India	Reducing contact	N/A	N/A	Mechanistic	Combination	N/A	X	X	

(Continued)

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TABLE 1 (Continued)

Author Year (citation)	Locality	Intervention			Model type		Age groups	Outcomes		
		Nonpharmacological	Pharmacological	Vaccination efficacy parameters[a]				Infection	Hospitalization and severe disease	Mortality
Shankaranarayanan 2022 (43)	India	Reducing contact	Vaccination (unspecified)		Statistical	N/A	N/A	X	X	
De Lara-Tuprio 2022 (44)	The Philippines	Contact tracing, reducing contact, hygiene, PPE	N/A	N/A	Mechanistic	Compartmental	N/A	X		
Dong 2022 (45)	China	Reducing contact	N/A	N/A	Mechanistic	Agent-based	Age as variable	X		
Foy 2021 (46)	India	Reducing contact	Vaccination (unspecified)	0–100%	Mechanistic	Compartmental	7 age groups	X		X
Fu 2022 (47)	China	N/A	Vaccination (CoronaVac/BBIBP-CorV)	Unspecified	Statistical	N/A	3 age groups	X	X	
Gaudou 2020 (48)	Vietnam	Reducing contact, PPE	N/A	N/A	Mechanistic	Combination	Age as variable	X		X
Ko 2021 (49)	South Korea	Border control, reducing contact	Vaccination (unspecified)	Variable	Mechanistic	Compartmental	4 age groups	X		X
Ko 2021 (50)	South Korea	Reducing contact	Vaccination (unspecified)	84%	Both	Compartmental	5 age groups	X		X
Lin 2021 (51)	China	N/A	Antivirals	N/A	Mechanistic	Compartmental	4 age groups	X		X
Omae 2021 (52)	Japan	N/A	BNT162b2	Decreased infection by 60 and 92% for 1st and 2nd doses	Mechanistic	Compartmental	N/A	X		X
Yufeng 2022 (53)	China	Reducing contact	N/A	N/A	Mechanistic	Compartmental	N/A	X		X
Zhang 2022 (54)	Pakistan, Bangladesh	Reducing contact	Vaccination (unspecified)	Unspecified	Mechanistic	Compartmental	N/A	X		X
Zhao 2021 (55)	China	N/A	Vaccination (unspecified)	Unspecified	Mechanistic	Compartmental	4 age groups	X		X
Akamatsu 2021 (56)	Japan	Reducing contact	N/A	N/A	Mechanistic	Compartmental	Age as variable		X	X
Alsayed (57)	Malaysia	Reducing contact	N/A	N/A	Mechanistic	Combination	N/A	X		
Chen 2021 (58)	China, Singapore	Border control	N/A	N/A	Mechanistic	Compartmental	N/A	X		

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TABLE 1 (Continued)

Author Year (citation)	Locality	Intervention			Model type		Age groups	Outcomes		
		Nonpharmacological	Pharmacological	Vaccination efficacy parameters[a]				Infection	Hospitalization and severe disease	Mortality
Estadilla 2021 (59)	The Philippines	Contact tracing, reducing contact	Vaccination (unspecified)	Weighted average of available vaccines	Mechanistic	Compartmental	N/A	X		
Hassan 2020 (60)	Bangladesh	Reducing contact	N/A	N/A	Mechanistic	Combination	N/A	X		
Herng 2022 (61)	Malaysia	Reducing contact	N/A	N/A	Both	Combination		X		
Hirata 2022 (62)	Japan	Reducing contact	Vaccination (Pfizer/ BioNTech)		Statistical	N/A	N/A	X		
Islam 2021 (63)	Bangladesh	Border control, reducing contact	N/A	N/A	Statistical	N/A	N/A	X		
Kobayashi 2022 (64)	Japan	Reducing contact	Vaccination (unspecified)	Estimated at population level	Mechanistic	Compartmental	Age as variable	X		
Kong 2021 (65)	Global	Border control, reducing contact	N/A	N/A	Mechanistic	Compartmental	N/A	X		
Libotte 2020 (66)	China	N/A	Vaccination (unspecified)	Variable	Mechanistic	Compartmental	N/A	X		
Liu 2022 (67)	China	Contact tracing, border control, reducing contact, hygiene, PPE	N/A	N/A	Both	Compartmental	N/A	X		
Liu 2022 (68)	China	Reducing contact, PPE	Vaccination (unspecified)	Parameterized for susceptibility and infectiousness	Mechanistic	Compartmental	N/A	X		
Lym 2022 (69)	South Korea	Reducing contact	N/A	N/A	Statistical	N/A	2 age groups	X		
Mandal 2020 (70)	India	Border control, reducing contact	N/A	N/A	Mechanistic	Compartmental	N/A	X		
Min 2021 (71)	South Korea	Reducing contact	AstraZeneca, Moderna, Janssen, Pfizer, COVAX facility	52–94%	Mechanistic	Compartmental	3 age groups	X		
Salman 2021 (72)	Malaysia	Reducing contact, education	N/A	N/A	Mechanistic	Combination	N/A	X		
Seok 2022 (73)	South Korea	Contact tracing, reducing contact, Other	Vaccination (unspecified)	48.1–96.1%	Mechanistic	Compartmental	9 age groups	X		
Shen 2022 (74)	China	Reducing contact	Vaccination (unspecified)	Unspecified	Mechanistic	Combination	3 age groups	X		

(Continued)

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TABLE 1 (Continued)

Author Year (citation)	Locality	Intervention			Model type		Age groups	Outcomes		
		Nonpharmacological	Pharmacological	Vaccination efficacy parameters[a]				Infection	Hospitalization and severe disease	Mortality
Wu 2022 (75)	China	Reducing contact	Vaccination (unspecified)	30%	Mechanistic	Statistical	N/A	X		
Xing 2021 (76)	Global	Reducing contact	N/A	N/A	Statistical	N/A	N/A	X		
Yasuda 2022 (77)	Japan	N/A	Vaccination (unspecified)		Mechanistic	Compartmental	2 age groups	X		
Yin 2021 (78)	China	Contact tracing, PPE	N/A	N/A	Mechanistic	Combination	Age as a variable	X		
Yu 2021 (79)	China, Hong Kong	Contact tracing, reducing contact	Vaccination (unspecified)	30, 50, 70%	Mechanistic	Compartmental	N/A	X		
Zhang 2021 (80)	China, Hong Kong	Border control, contact tracing, reducing contact	Vaccination (unspecified)	0–80%	Mechanistic	Combination	4 age groups	X		
Zhao 2021 (81)	China	Education	Vaccination (unspecified)	100%	Mechanistic	Compartmental	N/A	X		
Zhao 2021 (82)	South East Asia	Contact tracing, reducing contact, PPE	Vaccination (unspecified)	70%	Mechanistic	Compartmental	N/A	X		
Zhou 2020 (83)	China	Reducing contact	N/A	N/A	Mechanistic	Compartmental	N/A	X		
Zhu 2021 (84)	Japan	Border control, reducing contact	Vaccination (unspecified)	78.1%	Both	Compartmental	N/A	X		
Zou 2022 (85)	China	Reducing contact	Vaccination (unspecified)	50–90%	Mechanistic	Compartmental	N/A	X		
Hou 2021 (86)	China	Other	N/A	N/A	Mechanistic	Combination	N/A		X	
Jung 2022 (87)	South Korea	Reducing contact, PPE	N/A	N/A	Mechanistic	Compartmental	N/A		X	X
Li 2020 (88)	China	N/A	N/A	N/A	Statistical	N/A	Age as variable		X	
Sunohara 2021 (89)	Japan	Reducing contact	Vaccination (unspecified)	100% against transmission	Mechanistic	Compartmental	3 age groups			X

[a]Referring to infection unless otherwise specified.

10.3389/fpubh.2023.1252719

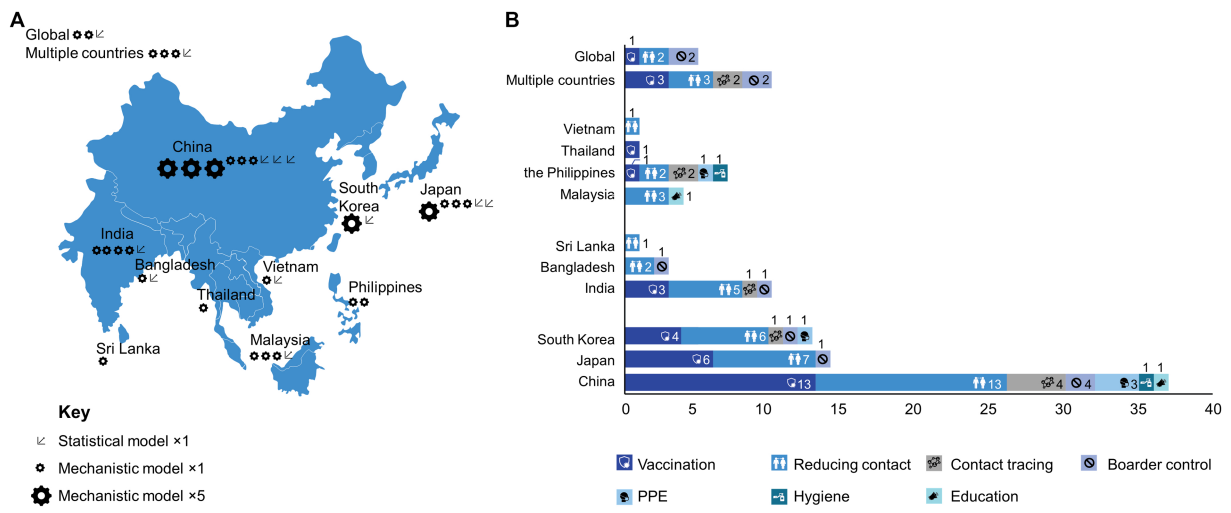

FIGURE 2
Geographical distribution of reports by **(A)** use of statistical and mechanistic model; **(B)** intervention category.

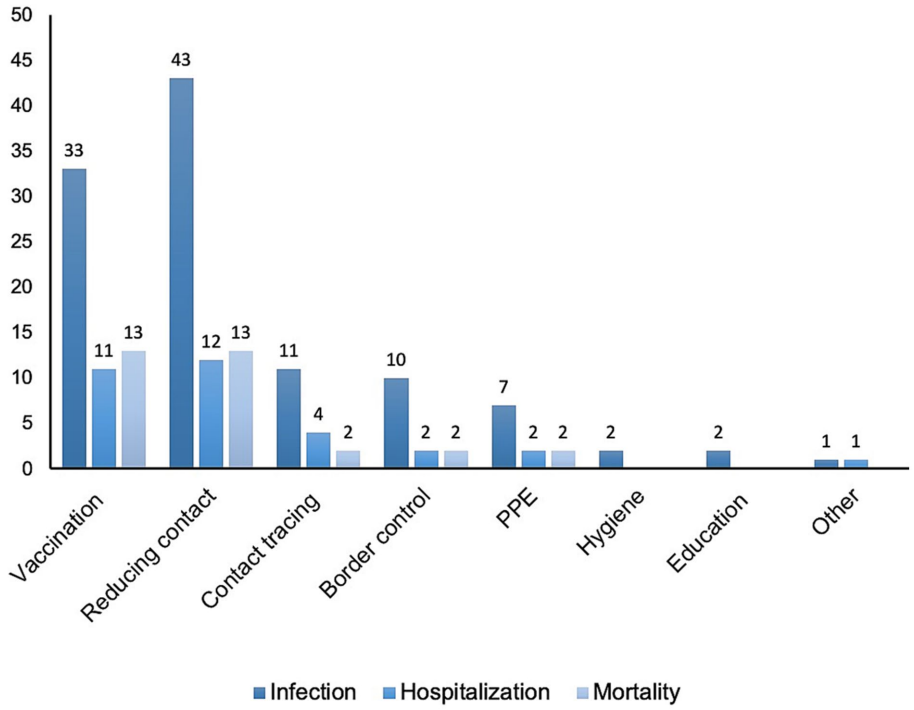

FIGURE 3
Outcome categories explored in models targeting specific interventions.

adults, a groups that has being prioritized for vaccination since the beginning of the pandemic (103). This is consistent with results from early global modeling studies that also prioritized older adults (90, 104, 105).

In Japan, which implemented voluntary social distancing and predominantly administered mRNA vaccines, one study noted a potential for benefits in terms of indirectly reducing mortality by targeting younger age groups to reduce overall transmission, depending on the strength of the lockdown (89). The actual vaccination strategy implemented in Japan, however, prioritized older adults, which may have helped reduce the hospital burden but could have been suboptimal in reducing transmission, particularly in the context of weakly enforced social measures. However, attitudes towards COVID-19 suggested remarkable concern among the Japanese public, which contributed to good compliance with government advice to wear face masks, social distance, and accept vaccination/boosters (106). Globally, the attitudes of populations towards the disease, trust in policy makers, and enforcement of

interventions are factors that may need to be accounted for in modeling studies (107).

The evolution of new viral strains may also influence vaccination strategies. The main variants of SARS-CoV-2 circulating in different regions have changed, from the original strain prevalent in 2020, to four main variants of concern – the Alpha, Beta, and Delta strains, common across Asia in 2021 – through to the Omicron variants from 2022 (3, 4, 108). Notably, the changes from the original variant to the Delta variant and then later to the Omicron variant were accompanied by increases in R_0 values, from 2.79 to 5.08 to 8.20, respectively (109, 110). The recently emerged Omicron subvariants, such as XBB and XBF, appear to be key drivers of infection waves, although they appear to pose lower risks of mortality (111). The emergence of new variants presents challenges for modeling in terms of shifting profiles for infectiousness, severe disease and mortality, and breakthrough infection against different vaccines and vaccination statuses (i.e., number/type of vaccination and time since last booster) (112). For example, individuals with hybrid immunity (developed through both infection and vaccination) might experience greater and more sustained protection, which might allow for longer intervals between boosters (113).

The outcomes of many models have anticipated that sufficiently high vaccination coverage in a population might 'break' transmission, allowing for the relaxation of non-pharmacological interventions (71, 114). In reality, vaccinated populations in Asia have continued to fall short of the high proportions predicted to be necessary to confer 'herd immunity' despite large numbers having received initial vaccinations and boosters (115). The waning effectiveness of mRNA vaccination/booster shots over time (after approximately 20 weeks) – particularly among older adults, who typically have weakened immune systems – may increase rates of infection (116). The potential impact of immune imprinting also motivates further studies to understand the effects and limitations of boosters (117). Reduced effectiveness of vaccines against Omicron strains might also shift initial targets for vaccination coverage in a population upwards, obstructing the relaxation of non-pharmacological interventions (118). Ongoing viral transmission from viral shedding from convalescent individuals, in addition to cessation of mask usage, may contribute to future surges of the disease.

There is also a need to deeply understand the importance of using vaccines that confer broad protection in the Omicron era, such as bivalent vaccines (119). Recent real-world evidence on bivalent vaccines points to additional protection, primarily against hospitalization, among older adults with monovalent vaccines; however, there may also be some additional benefits against symptomatic disease in other age groups during Omicron subvariant circulation (120). The high-priority populations are defined as older adults, adults with significant comorbidities, children or adults with immunocompromising conditions, pregnant women, and frontline health workers.

We noted that few studies (32, 38, 55) examined here modeled the vaccination of pediatric populations in Asia. Modeling of the impact of vaccinating children (5–11 years) in Europe has been proposed as a route to the relaxation of restrictions in schools (121). Modeling studies have also suggested that extending vaccination to children may reduce hospitalization and mortality across all age groups in developing countries (122). The latest update from the WHO's Strategic Advisory Group of Experts on Immunization (SAGE) on COVID-19 vaccination continues to prioritize the populations at greatest risk of mortality or severe disease to safeguard healthcare systems. As a low priority group, children are not routinely recommended for vaccination and boosters, but are instead determined by individual countries' evaluation of the burden, cost, and effectiveness (123). Although data on the impact of COVID-19 among children are lacking overall, there appears to be greater risk of mortality and hospitalization in low and middle-income countries (124). Thus, there is a need for further studies focused on the local burden of COVID-19 disease among younger populations in Asia, where future modeling studies might also guide decision-making on the impact of vaccinations (121, 122, 125).

Moving into a period where more vaccines are approved and made accessible, the hesitancy of uptake is likely to pose an ongoing barrier to booster strategies and sustainable protection (126). Among children and their caregivers, common reasons for fear and hesitancy to receive a vaccine include assumptions that the vaccine has side effects and may not be safe (18). Adults may also be complacent that the COVID-19 situation is no longer severe, and/or a negative perception of the vaccine, government, and/or pharmaceutical company may discourage them from receiving initial vaccination and/or boosters (17, 18). General fatigue over the topics of COVID-19 and vaccines and a wider circulation of misinformation on vaccines may contribute to these attitudes (127). Some reports examined here accounted for vaccine hesitancy, but this factor will likely be more important in future COVID-19 models, where the majority of a population have been vaccinated but coverage falls short of requirements for herd immunity. Modeling may also need to account for the impacts of educational interventions to address gaps in knowledge and attitudes among hesitant groups. Therefore, from a public health perspective, it is crucial to understand the drivers of vaccine uptake and vaccine hesitancy; this might help to identify groups that might have lower than average uptake and plan accordingly. Such pockets of immunity gaps and high susceptibility in the population could result in small-scale outbreaks that reduce the effect of population immunity. We predict that relaxation of control measures might be associated with new waves of infection and associated deaths; however, these outcomes will be reduced by increased levels of vaccine-derived immunity as well as hybrid immunity from infections in vaccinated individuals in the population.

The evolving nature of the COVID-19 situation especially motivates a dynamic vaccine-development pipeline to deliver more effective options against new strains of SARS-CoV-2 (128, 129). Furthermore, in an endemic scenario, non-vaccine pharmacological interventions are likely to become important for the treatment of severe disease. Here, two studies were identified that considered the impact of antiviral treatments, which may reflect the regional timeline for approval of such medicines. The introduction of monoclonal antibodies may help to manage cases of severe COVID-19 and improve these outcomes (10).

There are several limitations to this study. First, the scope of the literature search, particularly the focus on journal articles published in the English language, may have omitted more recent pre-print and local-language documents. The specific search terms may also have introduced bias into the types of modeling studies captured here. Furthermore, the large diversity of regional situations, model designs, interventions, and outcome targets limit the ability to systematically

extract relevant quantitative data from these reports. Additionally, few studies examined the influence of pharmacological interventions other than vaccines. As common limitations of all modeling studies, there is considerable uncertainty in many parameters such as population immunity, infectious rate, and contact and the level of health-related behaviors in study populations (130). Thus, modeling studies are subject to unreliable predictions on the impact on outcomes of interest.

5. Conclusion

Computational modeling is an important tool to address a limited understanding of SARS-CoV-2 epidemiology, and the quantitative estimates of the duration of protection from infection and vaccinations. Whereas non-pharmacological interventions have had an early role in managing health outcomes of the COVID-19 pandemic, modeling studies underline the importance of vaccines. High population coverage and vaccine effectiveness are key to mitigating the outcomes of the disease and supporting the relaxation of disruptive restrictions on movement. Compartmental mechanistic modeling offers an adequate approach to projecting disease outcomes across large populations; however, future models may need to account for complications from evolving variants and vaccination statuses/inclinations within populations, age group stratification, especially including pediatric populations. Deeper consideration of the socioeconomic burdens associated with strict non-pharmacological interventions may also be useful for policymakers and further underscore the cost-effectiveness and social benefits of vaccination programs. In the near future, increasing vaccine coverage, particularly among at-risk populations and through outreach to vaccine-adverse groups, may help to ease infection and severe disease. The rapidly evolving nature of the virus also motivates the development of new vaccines and alternative therapies. Future administration strategies may also be nuanced by competing needs to reduce overall transmissibility or severe disease burden in high-risk groups.

Author contributions

KT, JS, EO, JY, and MK: conceptualization and methodology. KT, JS, JY, MK, and CM: formal analysis and data curation. KT, JS, JY, MK, EO, CM, and HO: validation, writing, reviewing, and editing of manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

MK, CM, EO, JS, KT, and JY were employed by Pfizer Inc.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1252719/full#supplementary-material

References

1. Habibzadeh P, Stoneman EK. The novel coronavirus: a Bird's eye view. *Int J Occup Environ Med*. (2020) 11:65–71. doi: 10.15171/ijoem.2020.1921

2. Cheng ZJ, Shan J. 2019 novel coronavirus: where we are and what we know. *Infection*. (2020) 48:155–63. doi: 10.1007/s15010-020-01401-y

3. Chinta S, Rodriguez-Guerra M, Shaban M, Pandey N, Jaquez-Duran M, Vittorio TJ. COVID-19 therapy and vaccination: a clinical narrative review. *Drugs Context*. (2023):12. doi: 10.7573/dic.2022-7-2

4. Wolf JM, Wolf LM, Bello GL, Maccari JG, Nasi LA. Molecular evolution of SARS-CoV-2 from December 2019 to august 2022. *J Med Virol*. (2023) 95:e28366. doi: 10.1002/jmv.28366

5. Flaxman S, Mishra S, Gandy A, Unwin HJT, Mellan TA, Coupland H, et al. Estimating the effects of non-pharmaceutical interventions on COVID-19 in Europe. *Nature*. (2020) 584:257–61. doi: 10.1038/s41586-020-2405-7

6. CDC. Assessing risk factors for severe COVID-19 illness. (2022). Available at: https://www.cdc.gov/coronavirus/2019-ncov/covid-data/investigations-discovery/assessing-risk-factors.html

7. Segarra-Blasco A, Teruel M, Cattaruzzo S. The economic reaction to non-pharmaceutical interventions during Covid-19. *Econ Anal Policy*. (2021) 72:592–608. doi: 10.1016/j.eap.2021.10.006

8. Toffolutti V, Plach S, Maksimovic T, Piccitto G, Mascherini M, Mencarini L, et al. The association between COVID-19 policy responses and mental well-being: evidence from 28 European countries. *Soc Sci Med*. (2022) 301:114906. doi: 10.1016/j.socscimed.2022.114906

9. Zhou Y, Rahman MM, Khanam R, Taylor BR. Individual preferences, government policy, and COVID-19: a game-theoretic epidemiological analysis. *Appl Math Model*. (2023) 122:401–16. doi: 10.1016/j.apm.2023.06.014

10. Chary M, Barbuto AF, Izadmehr S, Tarsillo M, Fleischer E, Burns MM. COVID-19 therapeutics: use, mechanism of action, and toxicity (vaccines, monoclonal antibodies, and immunotherapeutics). *J Med Toxicol*. (2023) 19:205–18. doi: 10.1007/s13181-023-00931-9

11. Hillary VE, Ceasar SA. An update on COVID-19: SARS-CoV-2 variants, antiviral drugs, and vaccines. *Heliyon*. (2023) 9:e13952. doi: 10.1016/j.heliyon.2023.e13952

12. Oyebanji OA, Mylonakis E, Canaday DH. Vaccines for the prevention of coronavirus disease 2019 in older adults. *Infect Dis Clin N Am*. (2023) 37:27–45. doi: 10.1016/j.idc.2022.11.002

13. WHO. *COVID-19 vaccine tracker*. (2021). Available at: https://covid19.trackvaccines.org/agency/who/

14. WHO. *WHO SAGE roadmap for prioritizing uses of COVID-19 vaccines in the context of limited supply: An approach to inform planning and subsequent recommendations based on epidemiological setting and vaccine supply scenarios*. Geneva: WHO (2021).

15. Felsenstein S, Hedrich CM. SARS-CoV-2 infections in children and young people. *Clin Immunol*. (2020) 220:108588. doi: 10.1016/j.clim.2020.108588

16. Liu Y, Mao B, Liang S, Yang JW, Lu HW, Chai YH, et al. Shanghai clinical treatment experts group for COVID-19. Association between age and clinical characteristics and outcomes of COVID-19. *Eur Respir J*. (2020) 55:2001112. doi: 10.1183/13993003.01112-2020

17. Hong Y, Xie F, An X, Lan X, Liu C, Yan L, et al. Evolution of public attitudes and opinions regarding COVID-19 vaccination during the vaccine campaign in China: year-long infodemiology study of Weibo posts. *J Med Internet Res*. (2023) 25:e42671. doi: 10.2196/42671

18. Zaufishan Z, Usman M, Fishan Mumtaz K, Bilal R, Arshad A, Khan HM. Safety, effectiveness and hesitancy of COVID-19 vaccination in children: a cross-sectional study in Pakistan. *Front Public Health*. (2022) 10:1084017. doi: 10.3389/fpubh.2022.1084017

19. Fergie J, Moran MM, Cane A, Pather S, Tureci Ö, Srivastava A. COVID-19 epidemiology, immunity, and vaccine development in children: a review. *Vaccines (Basel)*. (2022) 10. doi: 10.3390/vaccines10122039

20. Shi DS, Whitaker M, Marks KJ, Anglin O, Milucky J, Patel K, et al. Hospitalizations of children aged 5-11 years with laboratory-confirmed COVID-19 - COVID-NET, 14 states, March 2020-February 2022. *MMWR Morb Mortal Wkly Rep*. (2022) 71:574–81. doi: 10.15585/mmwr.mm7116e1

21. Choi SH, Choi JH, Lee JK, Eun BW, Song SH, Ahn B, et al. Clinical characteristics and outcomes of children with SARS-CoV-2 infection during the Delta and omicron variant-dominant periods in Korea. *J Korean Med Sci*. (2023) 38:e65. doi: 10.3346/jkms.2023.38.e65

22. Graham F. Daily briefing: no room for COVID complacency in 2023. *Nature*. (2022). doi: 10.1038/d41586-022-04588-2 [E-pub ahead of print].

23. Sarfraz A, Hasan Siddiqui S, Iqbal J, Ali SA, Hasan Z, Sarfraz Z, et al. COVID-19 age-dependent immunology and clinical outcomes: implications for vaccines. *J Dev Orig Health Dis*. (2022) 13:277–83. doi: 10.1017/S2040174421000398

24. Jewell NP, Lewnard JA, Jewell BL. Predictive mathematical models of the COVID-19 pandemic: underlying principles and value of projections. *JAMA*. (2020) 323:1893–4. doi: 10.1001/jama.2020.6585

25. Afzal A, Saleel CA, Bhattacharyya S, Satish N, Samuel OD, Badruddin IA. Merits and limitations of mathematical Modeling and computational simulations in mitigation of COVID-19 pandemic: a comprehensive review. *Arch Comput Methods Eng*. (2022) 29:1311–37. doi: 10.1007/s11831-021-09634-2

26. Pandey A, Sah P, Moghadas SM, Mandal S, Banerjee S, Hotez PJ, et al. Challenges facing COVID-19 vaccination in India: lessons from the initial vaccine rollout. *J Glob Health*. (2021) 11:03083. doi: 10.7189/jogh.11.03083

27. Nojiri S, Irie Y, Kanamori R, Naito T, Nishizaki Y. Mortality prediction of COVID-19 in hospitalized patients using the 2020 diagnosis procedure combination administrative database of Japan. *Intern Med*. (2023) 62:201–13. doi: 10.2169/internalmedicine.0086-22

28. Roy J, Heath SM, Wang S, Ramkrishna D. Modeling COVID-19 transmission between age groups in the United States considering virus mutations, vaccinations, and reinfection. *Sci Rep*. (2022) 12:20098. doi: 10.1038/s41598-022-21559-9

29. Lessler J, Azman AS, Grabowski MK, Salje H, Rodriguez-Barraquer I. Trends in the mechanistic and dynamic modeling of infectious diseases. *Curr Epidemiol Rep*. (2016) 3:212–22. doi: 10.1007/s40471-016-0078-4

30. Sharif O, Hasan MZ, Rahman A. Determining an effective short term COVID-19 prediction model in ASEAN countries. *Sci Rep*. (2022) 12:5083. doi: 10.1038/s41598-022-08486-5

31. Baniasad M, Mofrad MG, Bahmanabadi B, Jamshidi S. COVID-19 in Asia: transmission factors, re-opening policies, and vaccination simulation. *Environ Res*. (2021) 202:111657. doi: 10.1016/j.envres.2021.111657

32. Cai J, Deng X, Yang J, Sun K, Liu H, Chen Z, et al. Modeling transmission of SARS-CoV-2 omicron in China. *Nat Med*. (2022) 28:1468–75. doi: 10.1038/s41591-022-01855-7

33. Ferguson EA, Brum E, Chowdhury A, Chowdhury S, Kundegorski M, Mahmud AS, et al. Modelling how face masks and symptoms-based quarantine synergistically and cost-effectively reduce SARS-CoV-2 transmission in Bangladesh. *Epidemics*. (2022) 40:100592. doi: 10.1016/j.epidem.2022.100592

34. Jung SY, Jo H, Son H, Hwang HJ. Real-world implications of a rapidly responsive COVID-19 spread model with time-dependent parameters via deep learning: model development and validation. *J Med Internet Res*. (2020) 22:e19907. doi: 10.2196/19907

35. Leung K, Wu JT, Leung GM. Effects of adjusting public health, travel, and social measures during the roll-out of COVID-19 vaccination: a modelling study. *Lancet Public Health*. (2021) 6:e674–82. doi: 10.1016/S2468-2667(21)00167-5

36. Qian Y, Cao S, Zhao L, Yan Y, Huang J. Policy choices for Shanghai responding to challenges of Omicron. *Front Public Health*. (2022) 10:927387. doi: 10.3389/fpubh.2022.927387

37. Suphanchaimat R, Tuangratananon T, Rajatanavin N, Phaiyarom M, Jaruwanno W, Uansri S. Prioritization of the target population for coronavirus disease 2019 (COVID-19) vaccination program in Thailand. *Int J Environ Res Public Health*. (2021) 18:10803. doi: 10.3390/ijerph182010803

38. Cai J, Yang J, Deng X, Peng C, Chen X, Wu Q, et al. Assessing the transition of COVID-19 burden towards the young population while vaccines are rolled out in China. *Emerg Microbes Infect*. (2022) 11:1205–14. doi: 10.1080/22221751.2022.2063073

39. Ediriweera DS, de Silva NR, Malavige GN, de Silva HJ. An epidemiological model to aid decision-making for COVID-19 control in Sri Lanka. *PLoS One*. (2020) 15:e0238340. doi: 10.1371/journal.pone.0238340

40. Kong L, Duan M, Shi J, Hong J, Zhou X, Yang X, et al. Optimization of COVID-19 prevention and control measures during the Beijing 2022 winter Olympics: a model-based study. *Infect Dis Poverty*. (2022) 11:95. doi: 10.1186/s40249-022-01019-2

41. Rajput A, Sajid M, Tanvi SC, Aggarwal R. Optimal control strategies on COVID-19 infection to bolster the efficacy of vaccination in India. *Sci Rep*. (2021) 11:20124. doi: 10.1038/s41598-021-99088-0

42. Shah PV. Prediction of the peak, effect of intervention, and total infected by COVID-19 in India. *Disaster Med Public Health Prep*. (2022) 16:40–50. doi: 10.1017/dmp.2020.321

43. Shankaranarayanan A, Wei HC. Mathematical modeling of SARS-nCoV-2 virus in Tamil Nadu, South India. *Math Biosci Eng*. (2022) 19:11324–44. doi: 10.3934/mbe.2022527

44. de Lara-Tuprio E, Estadilla CDS, Macalalag JMR, Teng TR, Uyheng J, Espina KE, et al. Policy-driven mathematical modeling for COVID-19 pandemic response in the Philippines. *Epidemics*. (2022) 40:100599. doi: 10.1016/j.epidem.2022.100599

45. Dong T, Dong W, Xu Q. Agent simulation model of COVID-19 epidemic agent-based on GIS: a case study of Huangpu District, Shanghai. *Int J Environ Res Public Health*. (2022) 19:10242. doi: 10.3390/ijerph191610242

46. Foy BH, Wahl B, Mehta K, Shet A, Menon GI, Britto C. Comparing COVID-19 vaccine allocation strategies in India: a mathematical modelling study. *Int J Infect Dis*. (2021) 103:431–8. doi: 10.1016/j.ijid.2020.12.075

47. Fu Y, Zhao J, Wei X, Han P, Yang L, Ren T, et al. Effectiveness and cost-effectiveness of inactivated vaccine to address COVID-19 pandemic in China: evidence from randomized control trials and real-world studies. *Front Public Health*. (2022) 10:917732. doi: 10.3389/fpubh.2022.917732

48. Gaudou B, Huynh NQ, Philippon D, Brugiere A, Chapuis K, Taillandier P, et al. COMOKIT: a modeling kit to understand, analyze, and compare the impacts of mitigation policies against the COVID-19 epidemic at the scale of a City. *Front Public Health*. (2020) 8:563247. doi: 10.3389/fpubh.2020.563247

49. Ko Y, Lee J, Kim Y, Kwon D, Jung E. COVID-19 vaccine priority strategy using a heterogenous transmission model based on maximum likelihood estimation in the Republic of Korea. *Int J Environ Res Public Health*. (2021) 18:6469. doi: 10.3390/ijerph18126469

50. Ko Y, Lee J, Seo Y, Jung E. Risk of COVID-19 transmission in heterogeneous age groups and effective vaccination strategy in Korea: a mathematical modeling study. *Epidemiol Health*. (2021) 43:e2021059. doi: 10.4178/epih.e2021059

51. Lin SN, Rui J, Chen QP, Zhao B, Yu SS, Li ZY, et al. Effectiveness of potential antiviral treatments in COVID-19 transmission control: a modelling study. *Infect Dis Poverty*. (2021) 10:53. doi: 10.1186/s40249-021-00835-2

52. Omae Y, Kakimoto Y, Sasaki M, Toyotani J, Hara K, Gon Y, et al. SIRVVD model-based verification of the effect of first and second doses of COVID-19/SARS-CoV-2 vaccination in Japan. *Math Biosci Eng*. (2022) 19:1026–40. doi: 10.3934/mbe.2022047

53. Yufeng Z, Huaxin P, Lanting L, Pei Z, Kaining W, Shengxing C, et al. Risk assessment and analysis of traditional Chinese medicine intervention in coronavirus disease. *J Tradit Chin Med*. (2022) 42:472–8. doi: 10.19852/j.cnki.jtcm.20220408.001

54. Zhang W, Huggins T, Zheng W, Liu S, Du Z, Zhu H, et al. Assessing the dynamic outcomes of containment strategies against COVID-19 under different public health governance structures: a comparison between Pakistan and Bangladesh. *Int J Environ Res Public Health*. (2022) 19:9239. doi: 10.3390/ijerph19159239

55. Zhao ZY, Niu Y, Luo L, Hu QQ, Yang TL, Chu MJ, et al. The optimal vaccination strategy to control COVID-19: a modeling study in Wuhan City, China. *Infect Dis Poverty*. (2021) 10:140. doi: 10.1186/s40249-021-00922-4

56. Akamatsu T, Nagae T, Osawa M, Satsukawa K, Sakai T, Mizutani D. Model-based analysis on social acceptability and feasibility of a focused protection strategy against the COVID-19 pandemic. *Sci Rep*. (2021) 11:2003. doi: 10.1038/s41598-021-81630-9

57. Alsayed A, Sadir H, Kamil R, Sari H. Prediction of epidemic peak and infected cases for COVID-19 disease in Malaysia, 2020. *Int J Environ Res Public Health*. (2020) 17:4076. doi: 10.3390/ijerph17114076

58. Chen T, Huang S, Li G, Zhang Y, Li Y, Zhu J, et al. An integrated framework for modelling quantitative effects of entry restrictions and travel quarantine on importation risk of COVID-19. *J Biomed Inform*. (2021) 118:103800. doi: 10.1016/j.jbi.2021.103800

59. Estadilla CDS, Uyheng J, de Lara-Tuprio EP, Teng TR, Macalalag JMR, Estuar M. Impact of vaccine supplies and delays on optimal control of the COVID-19 pandemic:

mapping interventions for the Philippines. *Infect Dis Poverty*. (2021) 10:107. doi: 10.1186/s40249-021-00886-5

60. Hassan R, Dosar AS, Mondol JK, Khan TH, Noman AA, Sayem MS, et al. Prediction of epidemics trend of COVID-19 in Bangladesh. *Front Public Health*. (2020) 8:559437. doi: 10.3389/fpubh.2020.559437

61. Herng LC, Singh S, Sundram BM, Zamri A, Vei TC, Aris T, et al. The effects of super spreading events and movement control measures on the COVID-19 pandemic in Malaysia. *Sci Rep*. (2022) 12:2197. doi: 10.1038/s41598-022-06341-1

62. Hirata A, Kodera S, Diao Y, Rashed EA. Did the Tokyo Olympic games enhance the transmission of COVID-19? An interpretation with machine learning. *Comput Biol Med*. (2022) 146:105548. doi: 10.1016/j.compbiomed.2022.105548

63. Islam A, Sayeed MA, Rahman MK, Zamil S, Abedin J, Saha O, et al. Assessment of basic reproduction number (R(0)), spatial and temporal epidemiological determinants, and genetic characterization of SARS-CoV-2 in Bangladesh. *Infect Genet Evol*. (2021) 92:104884. doi: 10.1016/j.meegid.2021.104884

64. Kobayashi T, Nishiura H. Prioritizing COVID-19 vaccination. Part 2: real-time comparison between single-dose and double-dose in Japan. *Math Biosci Eng*. (2022) 19:7410–24. doi: 10.3934/mbe.2022350

65. Kong L, Hu Y, Wang Q, Chen X, Yao T, Wang Y, et al. Could COVID-19 pandemic be stopped with joint efforts of travel restrictions and public health countermeasures? A modelling study. *BMJ Open*. (2021) 11:e046157. doi: 10.1136/bmjopen-2020-046157

66. Libotte GB, Lobato FS, Platt GM, Silva Neto AJ. Determination of an optimal control strategy for vaccine administration in COVID-19 pandemic treatment. *Comput Methods Prog Biomed*. (2020) 196:105664. doi: 10.1016/j.cmpb.2020.105664

67. Liu W, Guo Z, Abudunaibi B, Ouyang X, Wang D, Yang T, et al. Model-based evaluation of transmissibility and intervention measures for a COVID-19 outbreak in Xiamen City. *China Front Public Health*. (2022) 10:887146. doi: 10.3389/fpubh.2022.887146

68. Liu J, Gao B, Bao HX, Shi Z. Isolating the net effect of multiple government interventions with an extended susceptible-exposed-infectious-recovered (SEIR) framework: empirical evidence from the second wave of COVID-19 pandemic in China. *BMJ Open*. (2022) 12:e060996. doi: 10.1136/bmjopen-2022-060996

69. Lym Y, Lym H, Kim K, Kim KJ. Spatiotemporal associations between local safety level index and COVID-19 infection risks across capital regions in South Korea. *Int J Environ Res Public Health*. (2022) 19:824. doi: 10.3390/ijerph19020824

70. Mandal S, Bhatnagar T, Arinaminpathy N, Agarwal A, Chowdhury A, Murhekar M, et al. Prudent public health intervention strategies to control the coronavirus disease 2019 transmission in India: a mathematical model-based approach. *Indian J Med Res*. (2020) 151:190–9. doi: 10.4103/ijmr.IJMR_504_20

71. Min KD, Tak S. Dynamics of the COVID-19 epidemic in the post-vaccination period in Korea: a rapid assessment. *Epidemiol Health*. (2021) 43:e2021040. doi: 10.4178/epih.e2021040

72. Salman AM, Ahmed I, Mohd MH, Jamiluddin MS, Dheyab MA. Scenario analysis of COVID-19 transmission dynamics in Malaysia with the possibility of reinfection and limited medical resources scenarios. *Comput Biol Med*. (2021) 133:104372. doi: 10.1016/j.compbiomed.2021.104372

73. Seok J, Lee Y, Choi JY, Choi JP, Seo H, Lee S, et al. Evaluation of intervention policies for the COVID-19 epidemic in the Seoul/Gyeonggi region through a model simulation. *Yonsei Med J*. (2022) 63:707–16. doi: 10.3349/ymj.2022.63.8.707

74. Shen S, Li W, Wei H, Zhao L, Ye R, Ma K, et al. A chess and card room-induced COVID-19 outbreak and its agent-based simulation in Yangzhou, China. *Front Public Health*. (2022) 10:915716. doi: 10.3389/fpubh.2022.915716

75. Wu JW, Jiao XK, Du XH, Jiao ZT, Liang ZR, Pang MF, et al. Assessment of the benefits of targeted interventions for pandemic control in China based on machine learning method and web service for COVID-19 policy simulation. *Biomed Environ Sci*. (2022) 35:412–8. doi: 10.3967/bes2022.057

76. Xing X, Xiong Y, Yang R, Wang R, Wang W, Kan H, et al. Predicting the effect of confinement on the COVID-19 spread using machine learning enriched with satellite air pollution observations. *Proc Natl Acad Sci U S A*. (2021) 118:e2109098118. doi: 10.1073/pnas.2109098118

77. Yasuda H, Ito F, Hanaki KI, Suzuki K. COVID-19 pandemic vaccination strategies of early 2021 based on behavioral differences between residents of Tokyo and Osaka, Japan. *Arch Public Health*. (2022) 80:180. doi: 10.1186/s13690-022-00933-z

78. Yin L, Zhang H, Li Y, Liu K, Chen T, Luo W, et al. A data driven agent-based model that recommends non-pharmaceutical interventions to suppress coronavirus disease 2019 resurgence in megacities. *J R Soc Interface*. (2021) 18:20210112. doi: 10.1098/rsif.2021.0112

79. Yu Z, Zhu X, Liu X, Wei T, Yuan HY, Xu Y, et al. Reopening international boarders without quarantine: contact tracing integrated policy against COVID-19. *Int J Environ Res Public Health*. (2021) 18:7494. doi: 10.3390/ijerph18147494

80. Zhang N, Jack Chan PT, Jia W, Dung CH, Zhao P, Lei H, et al. Analysis of efficacy of intervention strategies for COVID-19 transmission: a case study of Hong Kong. *Environ Int*. (2021) 156:106723. doi: 10.1016/j.envint.2021.106723

81. Zhao X, Zhou Q, Wang A, Zhu F, Meng Z, Zuo C. The impact of awareness diffusion on the spread of COVID-19 based on a two-layer SEIR/V-UA epidemic model. *J Med Virol*. (2021) 93:4342–50. doi: 10.1002/jmv.26945

82. Zhao Z, Li X, Liu F, Jin R, Ma C, Huang B, et al. Stringent nonpharmaceutical interventions are crucial for curbing COVID-19 transmission in the course of vaccination: a case study of South and Southeast Asian countries. *Healthcare (Basel)*. (2021) 9:1292. doi: 10.3390/healthcare9101292

83. Zhou Y, Xu R, Hu D, Yue Y, Li Q, Xia J. Effects of human mobility restrictions on the spread of COVID-19 in Shenzhen, China: a modelling study using mobile phone data. *Lancet Digit Health*. (2020) 2:e417–24. doi: 10.1016/S2589-7500(20)30165-5

84. Zhu W, Feng J, Li C, Wang H, Zhong Y, Zhou L, et al. COVID-19 risk assessment for the Tokyo Olympic games. *Front Public Health*. (2021) 9:730611. doi: 10.3389/fpubh.2021.730611

85. Zou Y, Yang W, Lai J, Hou J, Lin W. Vaccination and quarantine effect on COVID-19 transmission dynamics incorporating Chinese-spring-festival travel rush: modeling and simulations. *Bull Math Biol*. (2022) 84:30. doi: 10.1007/s11538-021-00958-5

86. Hou C, Jiang H. Methodology of emergency medical logistics for multiple epidemic areas in public health emergency. *PLoS One*. (2021) 16:e0253978. doi: 10.1371/journal.pone.0253978

87. Jung SM, Jung J. The possible impact of Nationwide vaccination on outcomes of the COVID-19 epidemic in North Korea: a modelling study. *J Korean Med Sci*. (2022) 37:e300. doi: 10.3346/jkms.2022.37.e300

88. Li XZ, Jin F, Zhang JG, Deng YF, Shu W, Qin JM, et al. Treatment of coronavirus disease 2019 in Shandong, China: a cost and affordability analysis. *Infect Dis Poverty*. (2020) 9:78. doi: 10.1186/s40249-020-00689-0

89. Sunohara S, Asakura T, Kimura T, Ozawa S, Oshima S, Yamauchi D, et al. Effective vaccine allocation strategies, balancing economy with infection control against COVID-19 in Japan. *PLoS One*. (2021) 16:e0257107. doi: 10.1371/journal.pone.0257107

90. Gerlee P, Joud A, Spreco A, Timpka T. Computational models predicting the early development of the COVID-19 pandemic in Sweden: systematic review, data synthesis, and secondary validation of accuracy. *Sci Rep*. (2022) 12:13256. doi: 10.1038/s41598-022-16159-6

91. Cramer EY, Ray EL, Lopez VK, Bracher J, Brennen A, Castro Rivadeneira AJ, et al. Evaluation of individual and ensemble probabilistic forecasts of COVID-19 mortality in the United States. *Proc Natl Acad Sci U S A*. (2022) 119:e2113561119. doi: 10.1073/pnas.2113561119

92. Childs ML, Kain MP, Harris MJ, Kirk D, Couper L, Nova N, et al. The impact of long-term non-pharmaceutical interventions on COVID-19 epidemic dynamics and control: the value and limitations of early models. *Proc Biol Sci*. (1957) 2021:20210811. doi: 10.1098/rspb.2021.0811

93. Imai N, Gaythorpe KAM, Abbott S, Bhatia S, van Elsland S, Prem K, et al. Adoption and impact of non-pharmaceutical interventions for COVID-19. *Wellcome Open Res*. (2020) 5:59. doi: 10.12688/wellcomeopenres.15808.1

94. Ahmed DA, Ansari AR, Imran M, Dingle K, Bonsall MB. Mechanistic modelling of COVID-19 and the impact of lockdowns on a short-time scale. *PLoS One*. (2021) 16:e0258084. doi: 10.1371/journal.pone.0258084

95. Lessler J, Cummings DA. Mechanistic models of infectious disease and their impact on public health. *Am J Epidemiol*. (2016) 183:415–22. doi: 10.1093/aje/kww021

96. Brauner JM, Mindermann S, Sharma M, Johnston D, Salvatier J, Gavenciak T, et al. Inferring the effectiveness of government interventions against COVID-19. *Science*. (2021) 371. doi: 10.1126/science.abd9338

97. Reuge N, Jenkins R, Brossard M, Soobrayan B, Mizunoya S, Ackers J, et al. Education response to COVID 19 pandemic, a special issue proposed by UNICEF: editorial review. *Int J Educ Dev*. (2021) 87:102485. doi: 10.1016/j.ijedudev.2021.102485

98. Peng W, Berry EM. Coping with the challenges of COVID-19 using the Sociotype framework: a rehearsal for the next pandemic. *Rambam Maimonides Med J*. (2021) 12:e0005. doi: 10.5041/RMMJ.10425

99. Steele MK, Couture A, Reed C, Iuliano D, Whitaker M, Fast H, et al. Estimated number of COVID-19 infections, hospitalizations, and deaths prevented among vaccinated persons in the US, December 2020 to September 2021. *JAMA Netw Open*. (2022) 5:e2220385. doi: 10.1001/jamanetworkopen.2022.20385

100. WHO. Eight steps to help with safe home-based recovery for COVID-19. (2022). Available at: https://www.who.int/westernpacific/news-room/feature-stories/item/eight-steps-to-help-with-safe-home-based-recovery-for-covid-19

101. Smith DJ, Hakim AJ, Leung GM, Xu W, Schluter WW, Novak RT, et al. COVID-19 mortality and vaccine coverage - Hong Kong special administrative region, China, January 6, 2022-march 21, 2022. *MMWR Morb Mortal Wkly Rep*. (2022) 71:545–8. doi: 10.15585/mmwr.mm7115e1

102. Wichaidit M, Nopsopon T, Sunan K, Phutrakool P, Ruchikachorn P, Wanvarie D, et al. Breakthrough infections, hospital admissions, and mortality after major COVID-19 vaccination profiles: a prospective cohort study. *Lancet Reg Health Southeast Asia*. (2023) 8:100106. doi: 10.1016/j.lansea.2022.100106

103. WHO. *WHO SAGE roadmap on uses of COVID-19 vaccines in the context of OMICRON and substantial population immunity*. Geneva: WHO (2020).

104. Moore S, Hill EM, Dyson L, Tildesley MJ, Keeling MJ. Modelling optimal vaccination strategy for SARS-CoV-2 in the UK. *PLoS Comput Biol*. (2021) 17:e1008849. doi: 10.1371/journal.pcbi.1008849

105. Hogan AB, Winskill P, Watson OJ, Walker PGT, Whittaker C, Baguelin M, et al. Within-country age-based prioritisation, global allocation, and public health impact of a vaccine against SARS-CoV-2: a mathematical modelling analysis. *Vaccine*. (2021) 39:2995–3006. doi: 10.1016/j.vaccine.2021.04.002

106. Innovation IoGH. *COVID-19 global behaviours and attitudes*. Spokane, WA: Innovation IoGH (2022).

107. Chowdhury R, Heng K, Shawon MSR, Goh G, Okonofua D, Ochoa-Rosales C, et al. Global dynamic interventions strategies for COVID-19 collaborative group. Dynamic interventions to control COVID-19 pandemic: a multivariate prediction modelling study comparing 16 worldwide countries. *Eur J Epidemiol*. (2020) 35:389–99. doi: 10.1007/s10654-020-00649-w

108. CoVarients. *Overview of variants in countries*. Available at: https://covariants.org/per-country

109. Liu Y, Rocklöv J. The reproductive number of the Delta variant of SARS-CoV-2 is far higher compared to the ancestral SARS-CoV-2 virus. *J Travel Med*. (2021) 28:taab124. doi: 10.1093/jtm/taab124

110. Liu Y, Rocklöv J. The effective reproductive number of the Omicron variant of SARS-CoV-2 is several times relative to Delta. *J Travel Med*. (2022) 29. doi: 10.1093/jtm/taac037

111. Chatterjee S, Bhattacharya M, Nag S, Dhama K, Chakraborty C. A detailed overview of SARS-CoV-2 Omicron: its sub-variants, mutations and pathophysiology, clinical characteristics, immunological landscape, immune escape, and therapies. *Viruses*. (2023) 15:167. doi: 10.3390/v15010167

112. Li T, Xiao Y. Optimal strategies for coordinating infection control and socio-economic activities. *Math Comput Simul*. (2023) 207:533–55. doi: 10.1016/j.matcom.2023.01.017

113. Bobrovitz N, Ware H, Ma X, Li Z, Hosseini R, Cao C, et al. Protective effectiveness of previous SARS-CoV-2 infection and hybrid immunity against the Omicron variant and severe disease: a systematic review and meta-regression. *Lancet Infect Dis*. (2023) 23:556–67. doi: 10.1016/S1473-3099(22)00801-5

114. Pan J, Zhu W, Tian J, Liu Z, Xu A, Yao Y, et al. Vaccination as an alternative to non-drug interventions to prevent local resurgence of COVID-19. *Infect Dis Poverty*. (2022) 11:36. doi: 10.1186/s40249-022-00960-6

115. Zhang Y, Quigley A, Wang Q, MacIntyre CR. Non-pharmaceutical interventions during the roll out of covid-19 vaccines. *BMJ*. (2021) 375:n2314. doi: 10.1136/bmj.n2314

116. Andrews N, Tessier E, Stowe J, Gower C, Kirsebom F, Simmons R, et al. Duration of protection against mild and severe disease by Covid-19 vaccines. *N Engl J Med*. (2022) 386:340–50. doi: 10.1056/NEJMoa2115481

117. Chemaitelly H, Ayoub HH, Tang P, Coyle P, Yassine HM, Al Thani AA, et al. Long-term COVID-19 booster effectiveness by infection history and clinical vulnerability and immune imprinting: a retrospective population-based cohort study. *Lancet Infect Dis*. (2023). doi: 10.1016/S1473-3099(23)00058-0

118. Chen B, Zhao Y, Jin Z, He D, Li H. Twice evasions of omicron variants explain the temporal patterns in six Asian and Oceanic countries. *BMC Infect Dis*. (2023) 23:25. doi: 10.1186/s12879-023-07984-9

119. Lin DY, Xu Y, Gu Y, Zeng D, Wheeler B, Young H, et al. Effectiveness of bivalent boosters against severe Omicron infection. *N Engl J Med*. (2023) 388:764–6. doi: 10.1056/NEJMc2215471

120. Surie D, DeCuir J, Zhu Y, Gaglani M, Ginde AA, Douin DJ, et al. *Early estimates of bivalent mRNA vaccine effectiveness in preventing COVID-19–associated hospitalization among immunocompetent adults aged ≥65 years — IVY network, 18 states, September 8–November 30, 2022*. *MMWR Morb Mortal Wkly Rep*. (2022) 71:1625–30. doi: 10.15585/mmwr.mm715152e2

121. Colosi E, Bassignana G, Contreras DA, Poirier C, Boelle PY, Cauchemez S, et al. Screening and vaccination against COVID-19 to minimise school closure: a modelling study. *Lancet Infect Dis*. (2022) 22:977–89. doi: 10.1016/S1473-3099(22)00138-4

122. Muller GC, Ferreira LS, Mesias Campos FE, Borges ME, Berg de Almeida G, Poloni S, et al. Modeling the impact of child vaccination (5-11 y) on overall COVID-19 related hospitalizations and mortality in a context of Omicron variant predominance and different vaccination coverage paces in Brazil. *Lancet Reg Health Am*. (2023) 17:100396. doi: 10.1016/j.lana.2022.100396

123. WHO. *SAGE updates COVID-19 vaccination guidance*. Geneva: WHO (2023).

124. Kitano T, Kitano M, Krueger C, Jamal H, Al Rawahi H, Lee-Krueger R, et al. The differential impact of pediatric COVID-19 between high-income countries and low- and middle-income countries: a systematic review of fatality and ICU admission in children worldwide. *PLoS One*. (2021) 16:e0246326. doi: 10.1371/journal.pone.0246326

125. Link-Gelles R, Ciesla AA, Roper LE, Scobie HM, Ali AR, Miller JD, et al. Early estimates of bivalent mRNA booster dose vaccine effectiveness in preventing symptomatic SARS-CoV-2 infection attributable to Omicron BA.5- and XBB/XBB.1.5-related sublineages among immunocompetent adults - increasing community access to testing program, United States, December 2022-January 2023. *MMWR Morb Mortal Wkly Rep*. (2023) 72:119–24. doi: 10.15585/mmwr.mm7205e1

126. Andreas M, Iannizzi C, Bohndorf E, Monsef I, Piechotta V, Meerpohl JJ, et al. Interventions to increase COVID-19 vaccine uptake: a scoping review. *Cochrane Database Syst Rev*. (2022) 8:CD015270. doi: 10.1002/14651858.CD015270

127. Zimmerman T, Shiroma K, Fleischmann KR, Xie B, Jia C, Verma N, et al. Misinformation and COVID-19 vaccine hesitancy. *Vaccine*. (2023) 41:136–44. doi: 10.1016/j.vaccine.2022.11.014

128. Mirtaleb MS, Falak R, Heshmatnia J, Bakhshandeh B, Taheri RA, Soleimanjahi H, et al. An insight overview on COVID-19 mRNA vaccines: advantageous, pharmacology, mechanism of action, and prospective considerations. *Int Immunopharmacol*. (2023) 117:109934. doi: 10.1016/j.intimp.2023.109934

129. Khoshnood S, Ghanavati R, Shirani M, Ghahramanpour H, Sholeh M, Shariati A, et al. Viral vector and nucleic acid vaccines against COVID-19: a narrative review. *Front Microbiol*. (2022) 13:984536. doi: 10.3389/fmicb.2022.984536

130. Holmdahl I, Buckee C. Wrong but useful – what Covid-19 epidemiologic models can and cannot tell us. *N Engl J Med*. (2020) 383:303–5. doi: 10.1056/NEJMp2016822

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An integrated public health response to an outbreak of Murray Valley encephalitis virus infection during the 2022–2023 mosquito season in Victoria

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Introduction: Murray Valley encephalitis virus (MVEV) is a mosquito-borne flavivirus known to cause infrequent yet substantial human outbreaks around the Murray Valley region of south-eastern Australia, resulting in significant mortality.

Methods: The public health response to MVEV in Victoria in 2022–2023 included a climate informed pre-season risk assessment, and vector surveillance with mosquito trapping and laboratory testing for MVEV. Human cases were investigated to collect enhanced surveillance data, and human clinical samples were subject to serological and molecular testing algorithms to assess for co-circulating flaviviruses. Equine surveillance was carried out via enhanced investigation of cases of encephalitic illness. Integrated mosquito management and active health promotion were implemented throughout the season and in response to surveillance signals.

Findings: Mosquito surveillance included a total of 3,186 individual trapping events between 1 July 2022 and 20 June 2023. MVEV was detected in mosquitoes on 48 occasions. From 2 January 2023 to 23 April 2023, 580 samples (sera and CSF) were tested for flaviviruses. Human surveillance detected 6 confirmed cases of MVEV infection and 2 cases of "flavivirus-unspecified." From 1 September 2022 to 30 May 2023, 88 horses with clinical signs consistent with flavivirus infection were tested, finding one probable and no confirmed cases of MVE.

Discussion: The expanded, climate-informed vector surveillance system in Victoria detected MVEV in mosquitoes in advance of human cases, acting as an effective early warning system. This informed a one-health oriented public health response including enhanced human, vector and animal surveillance, integrated mosquito management, and health promotion.

KEYWORDS

Murray Valley encephalitis virus, vector-borne disease, mosquito-borne disease, mosquitoes, flavivirus, encephalitis, surveillance, outbreak

Introduction

In south-east Australia, the epidemiology of Murray Valley encephalitis virus (MVEV), a mosquito-borne flavivirus, is particularly unusual due to long periods of inactivity punctuated by substantial outbreaks resulting in significant mortality. The virus, which is genetically and antigenically related to Japanese encephalitis virus (JEV), was first isolated from the brain tissue of human cases of encephalitis in 1951 (1). These cases were part of an outbreak of encephalitis centred around the Murray Valley region of south-eastern Australia. The virus has also been implicated in similar outbreaks in 1917, 1919, and 1925 (2), and proven in subsequent outbreaks in 1974 and 2011 (2, 3). Further study identified the freshwater breeding mosquito, *Culex annulirostris*, as the primary disease vector, and water birds, particularly egrets and the Nankeen night heron, as important amplifying animal hosts (Figure 1) (4, 5). Humans, along with terrestrial vertebrates such as horses, are "dead-end" hosts and do not become significantly viraemic to facilitate onward transmission of virus (6).

Human infection with MVEV has an incubation period of 1–4 weeks followed by a variable prodrome of fever and headache (7). The progression to encephalitis is characterised by neurological signs and symptoms which may at first appear non-specific. Only one in every 150–1,000 people infected with MVEV suffer severe disease, with the vast majority of infections being asymptomatic (8, 9). Clinical illness in horses is similar, with a subset of infections progressing to encephalitis and death (10). The illness is difficult to clinically distinguish from infection with JEV and the Australian sublineage of West Nile virus, Kunjin virus (KUNV) and diagnosis relies on serology and molecular methods (7).

Epidemiological and genomic analyses have clarified the ecological patterns of MVEV activity in Australia (11). In the south-east the virus is epizootic, evidenced by infrequent large human outbreaks (12). In the north-west, there are enzootic foci of extant virus circulation evidenced by sporadic human cases and pockets of high seropositivity (9).

The largest outbreak on record in south-east Australia was in 1974, with 58 human cases (27 in Victoria) and a case fatality rate of 20% (13). The most recent epidemic occurred in 2011 with 17 cases across New South Wales (NSW), South Australia (SA), and Western Australia (3). Confirmed human cases were conspicuously absent in Victoria in 2011 despite sentinel chicken seroconversions and a significant equine outbreak (14). In keeping with such infrequent appearances of MVEV in the south-east, there is known to be relatively low seroprevalence amongst people born after 1974 (12).

The dominant explanatory theory of MVEV reintroduction posits that the movement of migratory waterbirds from an area of enzootic activity, under optimal conditions for waterbird and mosquito breeding, allows for amplification of the virus in wildlife and a spill-over phenomena resulting in human cases of infection (15, 16). Consequently, research and public health efforts in the south-eastern states of Australia have focussed on predicting the circumstances that increase the likelihood of outbreaks.

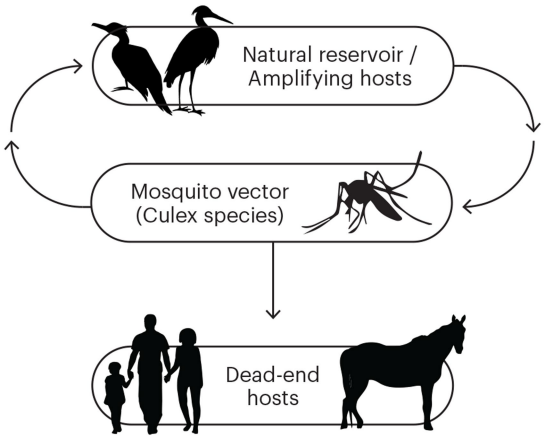

FIGURE 1
The transmission cycle for Murray Valley encephalitis virus (MVEV). The natural and amplifying hosts are waterbirds, and the primary vector is *Culex* species mosquitoes. Humans and horses are dead-end hosts.

Historically, three climate models, tested against historic outbreaks, have been used to assess the risk for MVEV activity in the Murray Valley region in any given year. Forbes' hypothesis, based on a historical analysis published in 1978, predicts an outbreak when rainfall averages in four major river basins are above the 7th decile value in the previous summer, and immediate spring/early summer period, preceding an enhanced mosquito season (17). The Nicholls hypothesis uses average mean sea level pressure (MSLP) in Darwin, a surrogate for the Southern Oscillation Index, and the Bennett hypothesis uses a negative Indian Ocean Dipole to predict MVEV activity (18, 19). Given the short historic time frame on which these hypotheses are based, and an imperfect track record of prediction, they are useful yet imprecise elements of a pre-season risk assessment.

Prior to 2021, MVEV and KUNV were the only two encephalitic flaviviruses known to cause locally transmitted disease in Victoria. In March 2021, a case of JEV infection on the Tiwi Islands in northern Australia was identified as a sentinel case (20) preceding an outbreak in the 2021–2022 mosquito season which resulted in 45 cases and seven deaths, centred around the south-east of Australia (21). This outbreak primed the public health system to prepare for further flavivirus outbreaks in the 2022–2023 mosquito season.

Here we describe the 2023 outbreak of MVEV in Victoria, Australia [population 6·7 million (22)] beginning with the pre-season risk assessment and preparedness activities and including the integrated public health response incorporating surveillance activities in humans and animals, laboratory diagnostics, vector control, risk communication and serosurveillance. The Victorian mosquito breeding season typically occurs from October to April each year (23) and collection of surveillance data occurs throughout the season with some programmes beginning before and finishing after the breeding season. Human surveillance data were collected as part of routine notifiable disease reporting and investigation, therefore human research ethics committee approval was not required.

Abbreviations: CDNA, Communicable Disease Network Australia; CSF, Cerebrospinal fluid; DEB, Defined Epitope Blocking ELISA; ELISA, Enzyme-linked immunosorbent assay; IFA, Immunofluorescence assay; IMT, Incident Management Team; JEV, Japanese encephalitis virus; KUNV, Kunjin virus; LGA, Local Government Area; MSLP , Mean sea level pressure; MVE, Murray Valley encephalitis; MVEV, Murray Valley encephalitis virus; NSW, New South Wales; PCR, Polymerase chain reaction; PRNT, Plaque reduction neutralisation test; SA, South Australia; VIDRL, Victorian Infectious Diseases Reference Laboratory.

Pre-season risk assessment

Pre-season mosquito risk assessments directly influence both surveillance and programme activities. The variability in mosquito-borne disease risk is routinely monitored through historic human epidemiology, vertebrate and mosquito surveillance, and predictive climatic factors. The unexpected JEV outbreak in south-east Australia in early 2022 resulted in expansion of mosquito-borne disease surveillance and preparedness for the 2022–2023 season.

The 2022–2023 mosquito season occurred on a background of three sequential years of La Niña weather patterns. By September 2022, water levels in the Murray River had reached the highest level since 1985 (24). October 2022 was the wettest month ever recorded in Victoria resulting in extensive flooding along the Murray, and associated rivers in northern Victoria (25). In addition, rainfall in the Darling, Northern Lake Eyre, and Gulf of Carpentaria basins satisfied Forbes' hypothesis predicting an outbreak of Murray Valley encephalitis (MVE) in the region of the Murray River (17, 26). The Nicholls hypothesis was also satisfied with MSLP readings in Darwin below the predictive threshold in autumn, winter, and spring of 2022 (18, 26). Further, a negative Indian Ocean Dipole event in 2022 likely contributed to rainfall over south-east Australia and satisfied Bennett's hypothesis (19). Based on these factors, the relative risk of MVEV activity in Victoria was predicted to be high, compared to previous years.

The geographic scope of predicted risk across Victoria was determined using historic signals of MVEV activity, namely human cases from the 1951 and 1974 outbreaks, equine cases, and sentinel chicken seroconversions. Assuming the potential for an overlapping ecological niche, JEV geospatial modelling (Shearer FM, unpublished) was incorporated into the risk assessment to produce a final composite map of at-risk Local Government Areas (LGAs) (Figure 2).

Public health response

The public health response to the 2022–2023 MVEV outbreak in Victoria, led by the Victorian Department of Health, was coordinated by an Incident Management Team (IMT) initiated on 28 October 2022.

Mosquito surveillance

The Victorian Arbovirus Disease Control Program which has operated in Victoria since 1974 funds strategically located local governments in inland and coastal areas considered high-risk for mosquito-borne diseases to undertake regular mosquito surveillance throughout the Victorian mosquito breeding season (27).

The state-wide surveillance system involves monitoring adult mosquito populations on a weekly basis at strategic sites across Victoria, allowing for long-term trend analysis. In inland Victoria, the most common mosquito trap in use is a form of Encephalitis Vector Surveillance trap baited with carbon dioxide and light to attract primarily night-time biting mosquitoes (28). Mosquito specimens are frozen to preserve virus RNA and transported to the Centre for AgriBioscience (Melbourne, Victoria, Australia) for mosquito counting, morphological species identification, and viral testing.

Targeted polymerase chain reaction (PCR) testing is conducted for the presence of three flaviviruses; JEV, MVEV, and KUNV, and two alphaviruses; Ross River virus and Barmah Forest virus (29, 30). In most instances, after mosquito counting and species identification is complete, all mosquitoes in a single trap are re-combined back into their original single, mixed-species pool, and then tested as a "whole trap grind" to accelerate the availability of virus testing results. Where mosquito abundance is high, pool size is limited to 1,000 mosquitoes and multiple pools from a single trap are tested.

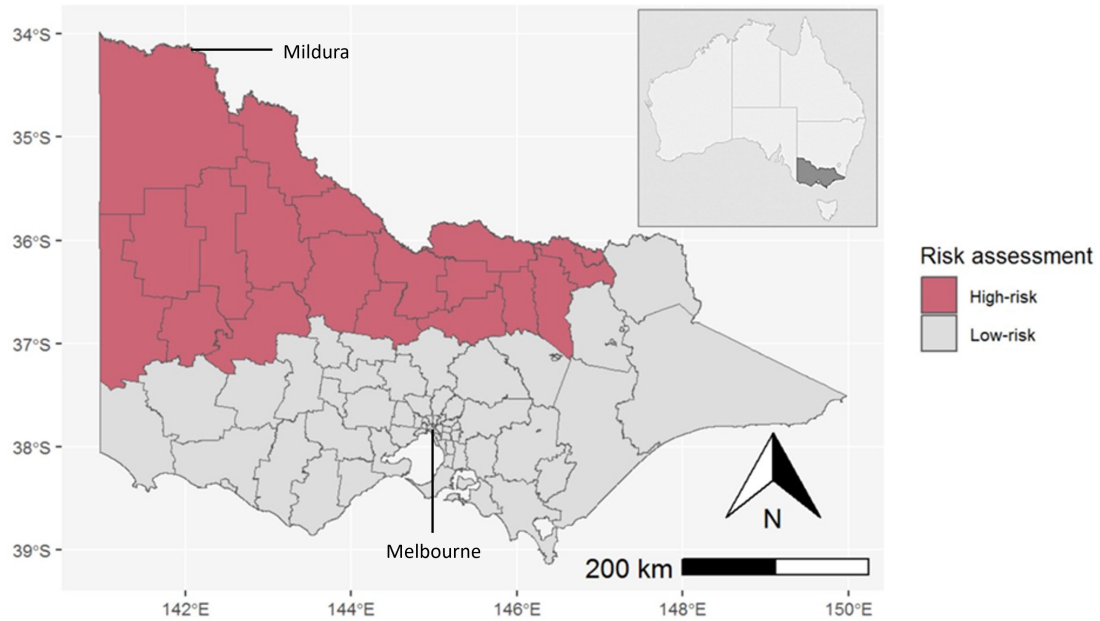

FIGURE 2
LGAs assessed as high-risk for MVEV/JEV activity in the state of Victoria, Australia (inset). The Murray River runs along the northern border of Victoria.

Mosquito surveillance results inform updates to local and state-wide risk assessments, identify locations of increased risk to human populations, and inform vector control activities. Programme expansion targeted areas identified as higher risk and without existing surveillance. Further traps were added throughout the season in response to evolving risk, for example into areas impacted by the October 2022 floods in northern Victoria.

Mosquito trapping for surveillance in the 2022–2023 season was expanded from 13 to 22 LGAs (including 315 unique trap sites) across Victoria, leading to a total of 3,186 individual trapping events and a total of 1,027,867 mosquitoes captured between 1 July 2022 and 20 June 2023. Excluding damaged traps and those which collected no mosquitoes, a total of 2,411 traps were analysed and 3,995 PCR tests were performed on whole trap grinds or mosquito species-specific pools (29).

Data demonstrated high to very high levels of mosquitoes from late October 2022 to early January 2023, particularly in LGAs in northern areas of Victoria adjacent or inland to the Murray River. Mosquito numbers declined to moderate and low levels from February 2023.

In high-risk LGAs, a trend was observed with *Culex australicus* [primarily a bird biting species (31, 32)] breeding in very high numbers immediately after flooding, followed by a transition to *Culex annulirostris* [a bird and human biting species (33)] in early December 2022. This provided a pre-warning for the potential of amplification of mosquito-borne viruses within bird populations that can spill over into humans.

MVEV was first detected in a trap in Mildura, the most north-west LGA in Victoria, on 4 January 2023. There were a total of 48 MVEV detections from traps across 11 LGAs between 4 January and 28 March 2023 (Table 1). The highest number of detections consistently occurred in the north-west of the state (Figure 3).

Mosquito surveillance was flexible and adaptive to the situation, whereby additional traps in areas of high risk or areas of confirmed human cases (including potential exposure sites) were quickly deployed to obtain additional information.

TABLE 1 MVEV detections in mosquito traps by LGA for the 2022–2023 season.

Local government area	Collection date of first detection	Collection date of last detection	Total number of detections
Mildura	4 January 2023	7 March 2023	23
Greater Bendigo	5 January 2023	–	1
Indigo	10 January 2023	15 February 2023	8
Loddon	10 January 2023	31 January 2023	5
Campaspe	17 January 2023	31 January 2023	3
Greater Shepparton	17 January 2023	7 February 2023	3
Horsham	24 January 2023	–	1
Wodonga	24 January 2023	–	1
Swan Hill	7 February 2023	–	1
Wangaratta	15 March 2023	–	1
Gannawarra	28 March 2023	–	1
Total detections (all LGAs)			48

Mosquito surveillance and virus screening can provide multiple pieces of intelligence. In addition to presence/absence of virus, testing of multiple pools in a large trap can provide semi-quantitative data about the infection rate of mosquitoes, and testing in species-specific pools can enhance our understanding of vector transmission. However, the latter requires morphological identification of entire traps, and post-flood conditions that lead to significantly increased mosquito numbers impact lab capacity and trap sub-sampling is implemented. This prevents species-specific testing in peak periods. Where resources allowed, some species-specific testing did occur and MVEV was detected in 11 of 19 *Culex annulirostris* specific pools and 1 of 4 *Culex australicus* pools. Species specific testing of *Aedes notoscriptus* (2 pools); *Culex quinquefasciatus* (3 pools); *Anopheles annulipes* (4 pools); *Aedes vittiger* (1 pool); *Aedes theobaldi* (1 pool), and *Culex molestus* (2 pools) all yielded negative results for MVEV.

Laboratory diagnostics

An enhanced diagnostic approach was implemented as part of the public health response. Due to lack of systematic assessment of serological and viral dynamics from historical outbreaks, the testing algorithm (Figure 4) was designed to both improve case-finding in high-risk areas to characterise the current outbreak and inform future diagnostics. All requests from medical practitioners for flavivirus serology were reflexively followed-up by serological testing for JEV, MVEV, and KUNV, in addition to proactively recommending PCR [blood, urine and cerebrospinal fluid (CSF)] and serology testing (CSF) for encephalitic cases. All flavivirus testing in Victoria took place at the Victorian Infectious Diseases Reference Laboratory (VIDRL) (Melbourne, Victoria, Australia).

MVEV can be detected by molecular techniques on whole blood, urine, CSF and brain tissue, however viraemia is typically brief and serology is often required for diagnosis (7). Enhanced testing on encephalitic cases with strong epidemiological evidence included performing MVEV IgM [Immunofluorescence Assay (IFA)] and MVEV total antibody [Defined Epitope Blocking (DEB) Enzyme Linked Immunosorbent Assay (ELISA)] in parallel and convalescent serology (at least 2–4 weeks apart). DEB is a blocking ELISA using MVEV specific monoclonal antibodies and has been used in Australia with comparable test characteristics to more labour-intensive neutralisation assays (34, 35). Recent MVEV infection was determined by either positivity of both IFA (IgM) and DEB or MVEV antibody seroconversion. Benchmarking of diagnostic assays (molecular, serology, viral culture, and genomics) was performed across multiple public health reference laboratories in Australia as part of the National JEV Diagnostic Project to further inform assay optimisation. Rapid sequencing/genotyping was performed at VIDRL using both Sanger sequencing and next-generation sequencing (Oxford Nanopore) to allow geographical linkage of human and mosquito detections (36).

From 2 January 2023 to 23 April 2023, 580 samples (sera and CSF) were tested for flavivirus antibodies (MVEV DEB, MVEV IFA, KUNV DEB, and JEV IFA; >2,300 tests performed) and flavivirus PCR (Pan-Flavivirus PCR and real-time PCR for JEV, MVEV, KUNV,

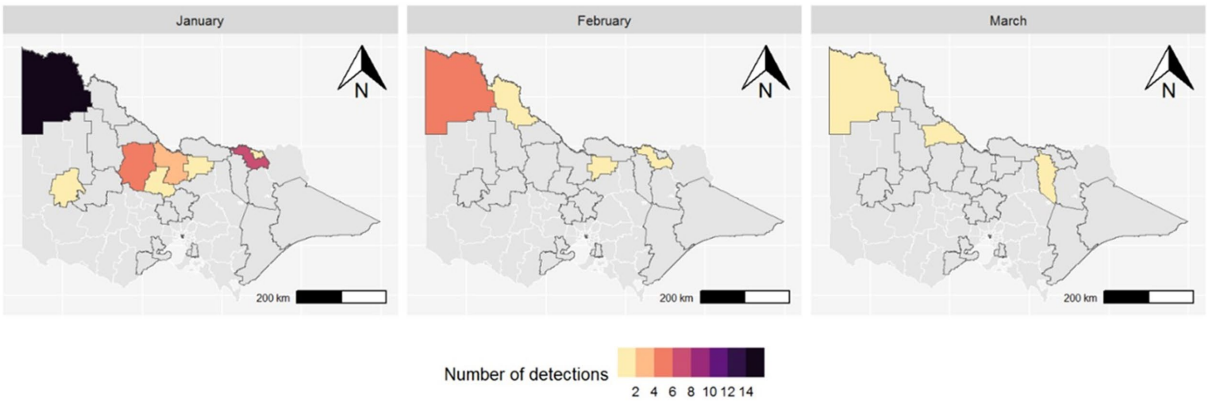

FIGURE 3
Map of Victorian LGAs with MVEV detected in mosquitoes by month (2023); LGAs outlined in black are those where mosquito surveillance occurred during the season.

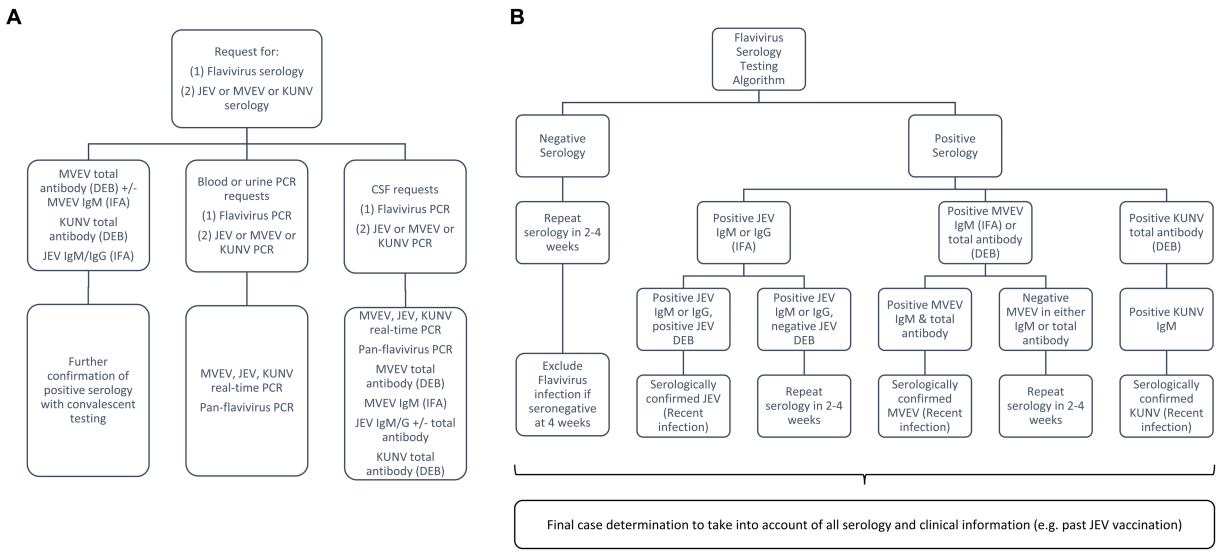

FIGURE 4
Laboratory testing algorithm for the flavivirus outbreak: **(A)** broad testing approach to capture symptomatic individuals from high-risk areas; **(B)** approach to serological testing and interpretation for those with encephalitis, taking into consideration local epidemiology and vaccination history. MVEV, Murray Valley encephalitis virus; JEV, Japanese encephalitis virus; KUNV, Kunjin virus; DEB, Defined Epitope Blocking Enzyme Linked Immunosorbent Assay (ELISA); IFA, Immunofluorescence Assay.

WNV, yellow fever virus, Zika virus, dengue virus; >2,400 tests performed).

Human surveillance

MVE is an "urgent" notifiable condition in Victoria under the Public Health and Wellbeing Regulations 2019 and must be notified immediately upon initial diagnosis (clinically suspected or laboratory confirmed) by medical practitioners and laboratories to the Department of Health (37).

The Department of Health urgently follows up all notifications of suspected or confirmed cases of MVE in accordance with national guidelines (38). Interviews with cases under investigation, next of kin and/or treating clinicians are conducted to establish the clinical presentation, potential mosquito exposures, and JEV vaccination status. Treating clinicians are provided with advice on testing, collection, and transport of samples.

Cases are assessed against the Communicable Diseases Network Australia (CDNA) case definitions for MVE and related flaviviruses, where a confirmed case of MVE requires clinical and laboratory (molecular or serological) evidence (39). A risk assessment is undertaken, considering exposure during acquisition period (5–28 days prior to symptom onset), local epidemiology and mosquito surveillance information.

Throughout the 2022–2023 season (1 October 2022–30 April 2023), the Department of Health received notification of 491 Victorian residents who underwent testing for MVEV. There were six confirmed

human MVE cases, of which five were fatal. Two additional recovered cases, with clinical and epidemiological findings consistent with MVE, were classified as "flavivirus infection - unspecified" because of cross-reactivity between MVEV and other flavivirus assays, excluding the ability to differentiate the causative virus, or the presence of co-infection. The median age of cases was 67 years (range 34–72 years) and two of eight cases were female. The range of symptom onset was from 16 January to 29 March 2023. Seven cases were reported to have encephalitis, whilst only one case had a non-encephalitic illness. Possible mosquito bite exposure during the acquisition period for these cases occurred across seven Victorian LGAs, all of which were designated high-risk by the pre-season risk assessment (Figure 5). There were five cases who had also spent time in MVEV risk areas in other Australian states during their acquisition period. Six cases were residents of a high-risk area. Travel to a risk area, fishing, camping, and river swimming were other reported risk factors for exposure. Only one case had a history of prior vaccination against JEV.

Five of eight cases (including both "flavivirus infection – unspecified" cases) were confirmed by serology. The remaining three cases had molecular diagnosis by PCR, one from CSF and two from post-mortem brain tissue, all of which were determined to belong to MVEV genotype 1a, consistent with samples collected from mosquitoes in Victoria.

Equine surveillance

Given the historic co-occurrence of human and equine MVE outbreaks, Agriculture Victoria collaborated on the state outbreak response.

MVEV is not a notifiable disease in animals in Victoria, however the Victorian Significant Disease Investigation (SDI) Program encourages flavivirus testing by private veterinarians for horses displaying significant or unusual symptoms (40). Initial testing with pan-flavivirus ELISA is performed at the Centre for AgriBioscience, whilst confirmatory testing via serum plaque reduction neutralisation test (PRNT) assay is performed at the Australian Centre for Disease Preparedness (Geelong, Victoria, Australia).

A confirmed case of MVE in a horse is defined as a clinical presentation consistent with flavivirus infection and laboratory results which reveal detection of MVEV antigen via culture, molecular or immunohistochemical methods. In contrast to human case definitions, equine case definitions included a "probable" category which is satisfied by serologic criteria. For probable or confirmed equine cases additional information is collected from the notifying private veterinarian and the owner, including clinical, exposure and travel history. Owners are provided with information and education on prevention and vector control for horses and humans, and encouraged to contact their local public health unit or doctor if they have any health concerns.

From 1 September 2022 to 30 May 2023, 88 horses with clinical signs consistent with flavivirus infection were tested. One probable and no confirmed cases of MVE were detected. The distribution of these horses was state-wide, with tested horses residing across 35 LGAs. At 28 June 2023, three suspected equine cases remained under investigation (awaiting PRNT results to differentiate the causal flavivirus).

Risk communication

Risk communication and dissemination of key public health messages coordinated by the Department of Health was a core component of the MVE public health response. Risk communication to members of the public impacted by flooding occurred from October

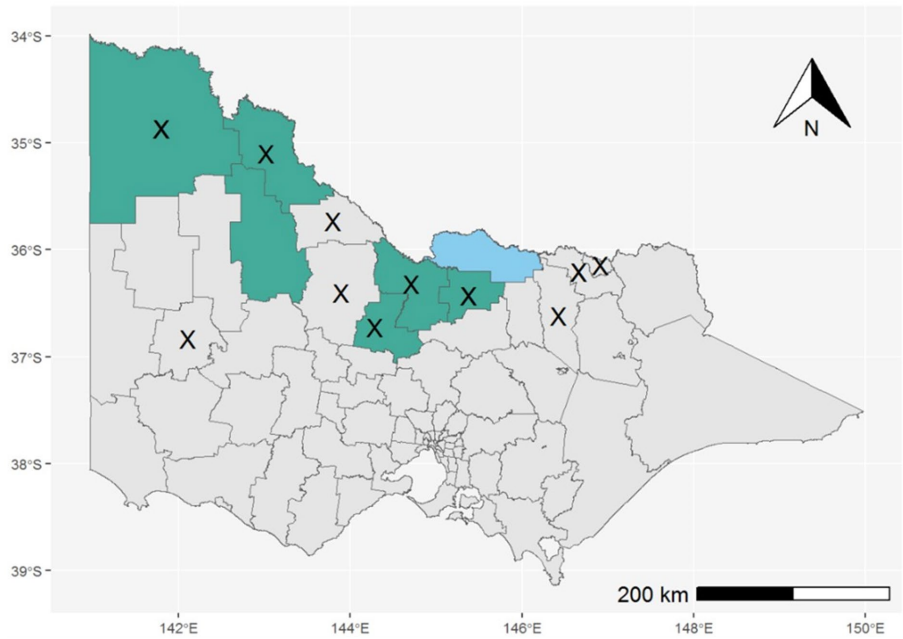

FIGURE 5
LGAs reported as primary exposure sites for confirmed cases of MVE (shaded green) or "flavivirus infection – unspecified" (shaded blue); LGAs with MVEV detections in mosquitoes are marked with an X.

2022 as mosquito numbers boomed across the north-west of the state. A new state-wide health promotion campaign titled "Do not wing it with mosquitoes" was launched in December 2022 featuring new imagery and messaging about mosquito bite prevention (41). A suite of digital, print and other media resources were deployed and translated into 23 languages to cater for culturally and linguistically diverse communities. This included a stakeholder pack containing social media resources for use by local governments and other stakeholders to promote mosquito avoidance. A mosquito-borne diseases webpage was developed as a central repository for all Victorian public information on mosquito-borne diseases, including weekly mosquito surveillance reports and human case surveillance data (42).

Three alerts/advisories were released in late 2022 highlighting the increased risk of mosquito-borne diseases due to flooding and at the beginning of the mosquito season. Further to this, seven reactive alerts and advisories targeting the Victorian community, tourists and health professionals were released in response to new cases and significant surveillance signals throughout the season (43). These provided information on risk, recommendations to reduce risk and detailed clinical and testing information for clinicians.

Targeted risk communication was undertaken through key stakeholders for select populations at higher risk of exposure to mosquitoes such as those with insecure housing, those residing in dwellings without insect screens, populations residing in flood-affected areas, populations displaced by flooding, and children attending early childhood education centres and schools. Clinicians and laboratories were further engaged through webinars to promote clinical awareness of MVE, indications for testing and notification requirements. Human health public awareness campaigns were disseminated to equine and agriculture industry bodies. Targeted communications were also undertaken by local public health units in affected areas.

Integrated vector control

Integrated Mosquito Management (44, 45) is utilised in Victoria each mosquito season to manage the human health risks associated with mosquito-borne diseases. It is informed by mosquito surveillance data (species and abundance), virus detection in mosquito populations, human case exposure information, environmental conditions, and an assessment of the risk of disease to localised populations. A range of control methods (physical and chemical) are utilised to manage larval and adult mosquito populations, targeted to where and when people are at greatest risk. Integrated mosquito management aims to limit the transmission of pathogens by reducing or eliminating vectors (in this case mosquitoes) from human contact (46).

In Victoria, landowners or land occupiers are responsible for mosquito management on their properties (37), and local governments as large landowners in regional areas play a significant role in local vector control. In the 2022–2023 season, the Department of Health worked collaboratively with local government to support, coordinate, and implement vector control activities in areas of heightened public health risk. This included investment in high-capacity vector control equipment, delivery of vector control training (e.g., in the safe handling and use of chemicals to ensure efficacy of treatments whilst minimising non-target impacts), coordination of resources and expertise across LGAs, and provision of expert advice to enable councils to effectively reduce the risk posed by mosquitoes in their local communities.

Chemical applications including larvicide and adulticide are used by local government to reduce vector numbers where they pose a risk to large human populations. Larviciding, including the use of S-Methoprene and *Bacillus thuringiensis israelensis* (*Bti*) were deployed in habitats where large numbers of larvae were observed. Fogging, involving the application of an adulticide using a natural or synthetic pyrethroid chemical in the form of a fine mist or aerosol, is utilised to target adult mosquitoes, particularly when abundance is elevated, or virus is detected in mosquitoes. Residual barrier treatments through the application of a synthetic pyrethroid to surfaces where adult mosquitoes may land were deployed in areas where people were found to congregate (outdoor school settings, campgrounds, toilet blocks, barbeque, or playground areas). These strategies were implemented proactively based on detailed local knowledge of mosquito breeding sites in proximity to human populations. Intelligence from field observations, mosquito trapping data, and human and animal surveillance data allowed the Department of Health to direct and redeploy appropriate vector control capacity in a pragmatic manner, associated with local risk throughout the season.

Discussion

The mosquito breeding season of 2022–2023 resulted in the first outbreak of human cases of MVE in the state of Victoria since 1974 and the first outbreak in south-eastern Australia since 2011. Crucially, the outbreak of human cases was preceded by early warning signals from mosquito surveillance, with the virus being detected in mosquitoes from 4 January 2023, 12 days before the first case's symptoms began, 8 days before sentinel chickens in the adjacent state of NSW first seroconverted (47) and 15 days before sentinel chickens in the adjacent state of SA first seroconverted (48). This demonstrates that vector surveillance, with virus detection, has the capacity to serve as a critical early warning system (49). In Victoria, routine vector surveillance expanded significantly during the 2022–2023 season, plausibly increasing the sensitivity of the system as an early warning tool. The representativeness of trapped mosquitoes is influenced by several factors. These include weather, sample size, and the condition of mosquitoes at the time of collection. Consequently, the methodology of mosquito surveillance must be adaptable throughout the season, such as when flooding renders trap sites inaccessible. Whilst these pragmatic adjustments may confound an isolated evaluation of the system, they represent important learnings in management of mosquito-borne diseases through surveillance in real-world settings.

Vector control activities, performed both pre-emptively and in response to surveillance signals, form an important part of the public health response. Adulticide fogging is the only means of killing adult mosquitoes that are known to be carrying disease. Fogging creates a protective buffer between mosquito populations and residential areas when the disease risk and/or vector abundance is high, and where the use of larvicides is not feasible due to the increased size and extent of breeding sites after major flooding events. However, fogging only temporarily reduces the number of adult mosquitoes. In contrast, barrier treatments applied to surfaces bind, and provide mosquito

control for up to 6–8 weeks (50, 51), but cannot be applied on a wide scale and are reserved for smaller, targeted areas.

The real-world nature of the integrated mosquito management programme in Victoria, whilst based on available evidence and best practice principles, is one of a suite of interventions, and therefore direct measurement of the success of vector control in isolation is limited. There is, however, a supportive body of observational evidence showing fogging has been demonstrated to kill 90% of adult dengue mosquitoes (52), and residual surface sprays have been demonstrated to reduce adult mosquito populations by 87–100% for 9 weeks post spraying (53). Further, larviciding has been shown to reduce the next cohort of emerging mosquitoes by approximately 95% (54). Given the high numbers of viral detections in adult mosquito surveillance samples, these evidence-based measures to reduce mosquito numbers present a logical intervention to prevent human disease where human populations are in close contact with mosquito populations.

The MVEV outbreak in 2022–2023 led to high mortality rates amongst confirmed cases of MVE, yielding a case fatality rate of 83%. High mortality is consistent with previous outbreaks and this case fatality rate is higher than in 1974, when 20% of cases succumbed to infection (13). A detailed clinical case series may highlight contributory factors behind case fatality however it is notable that median age for the outbreak was 67 years, compared to a median of 42 years in a case series from 1974 (13). The small number of cases and advances in diagnostics and case definitions further limits comparison between these two outbreaks, yet the findings underscore the population's ongoing susceptibility to severe disease, supported by a 2011 serosurvey showing a seroprevalence of only 2.2% in high-risk regions (12). The notified clinical cases likely underrepresent the actual burden of infection and repeat serosurveys will be crucial in determining the population's ongoing vulnerability.

Half a century on from the first isolation of MVEV, no directed therapy nor vaccine is available. The role of JEV vaccination in MVEV prevention, whilst particularly relevant for Australia after the 2021–2022 JEV outbreak (55), is uncertain, with insights from animal studies being mixed (56). Only one clinical case in this outbreak was vaccinated against JEV but had premorbid immune suppression making the significance of vaccination in this case unclear.

Serological interpretation to confirm MVEV infection is fundamentally complex due to cross-reactivity between viruses, persisting antibody response to prior infection or vaccine, and confounding anamnestic responses in the setting of acute infection. On a local level, regular public health and laboratory case conferences guided serological interpretation. Additional enhanced testing was also performed on encephalitic cases, in which MVEV IgM (IFA) and MVEV total antibody (DEB) were performed in parallel, alongside assays for the concurrently circulating flaviviruses JEV and KUNV. Despite post-mortem molecular diagnosis of two MVE cases being clinically complex and delayed, these samples allowed for sequencing, providing valuable genomic information that may improve our understanding of MVEV in Australia.

The propensity for flaviviruses to cause disease in agriculturally significant animals makes surveillance and control a clear one-health priority (14). The signs of MVE in horses can be clinically indistinguishable from other notifiable diseases, most notably Hendra virus. Consequently, on notification of a horse with encephalitis, testing for MVEV, in addition to measures related to suspected Hendra virus, is recommended. When carefully overseen, as described in Victoria, this system may act as a *de-facto* surveillance herd of horses across broad geographic areas evidenced by the testing of 88 horses across 35 LGAs. It is surprising that only one equine case of MVEV infection was diagnosed over the 2022–2023 mosquito season. This may speak to the limitations of testing in horses which, in addition to serological cross-reactivity similar to that in humans, is also limited by sample collection for molecular testing due to risks associated with lumbar puncture and autopsy. Evidence on the difference in host tropism of MVEV in different vertebrates is unavailable but may be another possible explanation for this apparent discrepancy. Horses, like humans are considered dead-end hosts for MVEV and do not contribute to onward amplification of the virus (6). Wild birds, however, are the natural reservoirs for MVEV and whilst not tested during this outbreak, a future serological survey of birds, as has been performed for other flaviviruses, may shed further light on the local epidemiology (5, 57).

Shifts in the distribution of vector-borne diseases due to climate change are the subject of global research and speculation. Direct attribution of any one outbreak to the effects of climate change is difficult, however ecological modelling can highlight areas of future risk (58). There are international examples of significant public health risk from emergent mosquito-borne disease, most famously the spread of West Nile virus throughout North America with ongoing seasonal outbreaks (59). For public health authorities concerned about emerging vector-borne diseases, our climate-informed surveillance programme paired with integrated vector control, targeted health promotion and a one-health focus can provide a preparedness framework for these emerging threats.

Conclusion

Although outbreaks of MVE are rare and infrequent, they are significant public health events when they do occur. Comprehensive vector surveillance, informed by climate predictions, serves as an essential warning tool to trigger a robust public health response. The potential widespread impact of MVEV and similar vector-borne diseases necessitates improvement-focussed analyses of surveillance activities within a one-health framework. As long as pharmacological interventions for MVE remain elusive, prevention and control of outbreaks will remain the mainstay of reducing the burden of these infections.

Author contributions

MB: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. HO'B: Conceptualization, Data curation, Formal analysis, Methodology, Writing – original draft. CL: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. RF: Data curation, Formal analysis, Methodology, Writing – review & editing. CB: Data curation, formal Analysis, Writing – original draft. PN: Data curation, formal Analysis, Investigation, Methodology, Writing – review & editing. CRB: Data curation, Formal analysis, Investigation, Writing – original draft. GB-S: Data curation, Formal analysis, Methodology, Writing – review & editing. M-HJ: Writing – original draft. AY: Data curation, Formal analysis, Investigation, Visualisation, Writing – review &

editing. NH: Writing – review & editing. NF: Conceptualization, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

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References

1. Burnet FM. Murray Valley encephalitis. *Am J Public Health Nat Health*. (1952) 42:1519–21. doi: 10.2105/AJPH.42.12.1519

2. Mackenzie JS, Broom AK. Australian X disease, Murray Valley encephalitis and the French connection. *Vet Microbiol*. (1995) 46:79–90. doi: 10.1016/0378-1135(95)00074-k

3. Selvey LA, Dailey L, Lindsay M, Armstrong P, Tobin S, Koehler AP, et al. The changing epidemiology of Murray Valley encephalitis in Australia: the 2011 outbreak and a review of the literature. *PLoS Negl Trop Dis*. (2014) 8:e2656. doi: 10.1371/journal.pntd.0002656

4. Doherty RL, Carley J, Mackerras MJ, Marks EN. Studies of arthropod-borne virus infections in Queensland: iii. Isolation and characterization of virus strains from wild-caught mosquitoes in North Queensland. Australian journal of experimental biology and medical. *Science*. (1963) 41:17–39. doi: 10.1038/icb.1963.2

5. Marshall ID, Brown BK, Keith K, Gard GP, Thibos E. Variation in arbovirus infection rates in species of birds sampled in a serological survey during an encephalitis epidemic in the Murray Valley of south-eastern Australia, February 1974. *Aust J Exp Biol Med Sci*. (1982) 60:471–8. doi: 10.1038/icb.1982.52

6. Hollidge BS, González-Scarano F, Soldan SS. Arboviral Encephalitides: transmission, emergence, and pathogenesis. *J Neuroimmune Pharmacol*. (2010) 5:428–42. doi: 10.1007/s11481-010-9234-7

7. Knox J, Cowan RU, Doyle JS, Ligtermoet MK, Archer JS, Burrow JN, et al. Murray Valley encephalitis: a review of clinical features. *Diagn Treatment Med J Aust*. (2012) 196:322–6. doi: 10.5694/mja11.11026

8. Anderson SG, Donnelley M, Stevenson WJ, Caldwell NJ, Eagle M. Murray-Valley encephalitis; surveys of human and animal sera. *Med J Aust*. (1952) 1:110–4. doi: 10.5694/j.1326-5377.1952.tb74988.x

9. Broom AK, Lindsay MD, Plant AJ, Wright AE, Condon RJ, Mackenzie JS. Epizootic activity of Murray Valley encephalitis virus in an aboriginal Community in the Southeast Kimberley Region of Western Australia: results of cross-sectional and longitudinal serologic studies. *Am J Trop Med Hyg*. (2002) 67:319–23. doi: 10.4269/ajtmh.2002.67.319

10. Kay B, Pollitt C, Fanning I, Hall R. The experimental infection of horses with Murray Valley encephalitis and Ross River viruses. *Aust Vet J*. (1987) 64:52–5. doi: 10.1111/j.1751-0813.1987.tb16129.x

11. Knope K, Whelan P, Smith D, Nicholson J, Moran R, Doggett S, et al. Arboviral diseases and malaria in Australia, 2010-11: annual report of the National Arbovirus and malaria advisory committee. *Commun Dis Intell Q Rep*. (2013) 37:E55–9.

12. Williams SA, Richards JS, Faddy HM, Leydon J, Moran R, Nicholson S, et al. Low Seroprevalence of Murray Valley encephalitis and Kunjin viruses in an opportunistic Serosurvey, Victoria 2011. *Aust N Z J Public Health*. (2013) 37:427–33. doi: 10.1111/1753-6405.12113

13. Bennett NM. Murray Valley encephalitis, 1974: clinical features. *Med J Aust*. (1976) 2:446–50. doi: 10.5694/j.1326-5377.1976.tb130324.x

14. Roche SE, Wicks R, Garner MG, East IJ, Paskin R, Moloney BJ, et al. Descriptive overview of the 2011 epidemic of Arboviral disease in horses in Australia. *Aust Vet J*. (2013) 91:5–13. Epub 20121219. doi: 10.1111/avj.12018

15. Mackenzie JS, Lindsay MD, Coelen RJ, Broom AK, Hall RA, Smith DW. Arboviruses causing human disease in the Australasian zoogeographic region. *Arch Virol*. (1994) 136:447–67. doi: 10.1007/BF01321074

16. Smith DW, Speers DJ, Mackenzie JS. The viruses of Australia and the risk to tourists. *Travel Med Infect Dis*. (2011) 9:113–25. doi: 10.1016/j.tmaid.2010.05.005

17. Forbes JA. Murray Valley encephalitis 1974, also, the epidemic variance since 1914 and predisposing rainfall patterns: Australasian medical publishing company. (1978).

18. Nicholls N. A method for predicting Murray Valley encephalitis in Southeast Australia using the southern oscillation. *Aust J Exp Biol Med Sci*. (1986) 64:587–94. doi: 10.1038/icb.1986.62

19. Bennett N, Moran R. Peculiarities of Murray Valley encephalitis (Mve) epidemics in South–Eastern Australia: the Indian Ocean dipole (Iod) as a predictor of epidemics. *Victorian Infect Dis Bull Dec*. (2009) 12:112–5.

20. Waller C, Tiemensma M, Currie BJ, Williams DT, Baird RW, Krause VL. Japanese encephalitis in Australia - a sentinel case. *N Engl J Med*. (2022) 387:661–2. doi: 10.1056/NEJMc2207004

21. Australian Government Department of Health and Aged Care. Health alert: Japanese encephalitis virus (Jev). (2023). Available at: https://www.health.gov.au/health-alerts/japanese-encephalitis-virus-jev/ (updated 16 June 2023; cited 2023 June 18).

22. Australian Bureau of Statistics. National, state and territory population. (2023).

23. Russell RC. Seasonal abundance of mosquitoes in a native Forest of the Murray Valley of Victoria, 1979–1985. *Aust J Entomol*. (1986) 25:235–40. doi: 10.1111/j.1440-6055.1986.tb01109.x

24. Murray Darling Basin Authority. River Murray weekly report: For the week ending Wednesday (2022) 7:2022.

25. Australian Government Bureau of Meteorology. Annual climate summary for Victoria. (2023).

26. Australian Government Bureau of Meteorology. Long range weather and climate reports. (2022).

27. Victorian Department of HealthMosquito Management in Victoria. (2023). Available at: https://www.betterhealth.vic.gov.au/health/healthyliving/mosquito-management-victoria (Accessed June 1, 2023).

28. Rohe DFR. A miniature battery powered Co2-baited trap for mosquito-borne encephalitis surveillance. *Bull Soc Vector Ecol*. (1979) 4:24–7.

29. Natsuda Puttharak MG, Ronke J, Chandel A, Brohier N, Wang X, O'Riley K, et al. *Mosquito and arbovirus surveillance report 2022–2023*. Melbourne: Agriculture Victoria Research (2023).

30. Batovska J, Mee PT, Lynch SE, Sawbridge TI, Rodoni BC. Sensitivity and specificity of Metatranscriptomics as an arbovirus surveillance tool. *Sci Rep*. (2019) 9:19398. doi: 10.1038/s41598-019-55741-3

31. Kay BH, Boyd AM, Ryan PA, Hall RA. Mosquito feeding patterns and natural infection of vertebrates with Ross River and Barmah Forest viruses in Brisbane, Australia. *Am J Trop Med Hyg*. (2007) 76:417–23. doi: 10.4269/ajtmh.2007.76.417

32. Jansen CC, Ritchie SA, Van den Hurk AF. The role of Australian mosquito species in the transmission of endemic and exotic West Nile virus strains. *Int J Environ Res Public Health*. (2013) 10:3735–52. doi: 10.3390/ijerph10083735

33. Webb C, Doggett S, Russell R. *A Guide to Mosquitoes of*. Australia: CSIRO Publishing (2016).

34. Hawkes RA, Roehrig JT, Boughton CR, Naim HM, Orwell R, Anderson-Stuart P. Defined epitope blocking with Murray Valley encephalitis virus and monoclonal antibodies: laboratory and field studies. *J Med Virol*. (1990) 32:31–8. doi: 10.1002/jmv.1890320106

35. Hall RA, Broom AK, Hartnett AC, Howard MJ, Mackenzie JS. Immunodominant epitopes on the Ns1 protein of Mve and kun viruses serve as targets for a blocking Elisa to detect virus-specific antibodies in sentinel animal serum. *J Virol Methods*. (1995) 51:201–10. doi: 10.1016/0166-0934(94)00105-p

36. Russell JS, Caly L, Kostecki R, McGuinness SL, Carter G, Bulach D, et al. The first isolation and whole genome sequencing of Murray Valley encephalitis virus from cerebrospinal fluid of a patient with encephalitis. *Viruses*. (2018) 10:319. doi: 10.3390/v10060319

37. Public Health and Wellbeing Regulations (2019). Public Health and Wellbeing Regulations 2019 (Vic). Sect. 7.

38. Communicable Diseases Network Australia. Murray Valley encephalitis – Series of National Guidelines – Guidelines for public health units. (2013).

39. Communicable Diseases Network Australia. Murray Valley encephalitis: Australian national notifiable diseases case definition. (2010).

40. Agriculture Victoria. Significant disease investigation (Sdi) program. (2023). Available at: https://agriculture.vic.gov.au/biosecurity/animal-diseases/significant-disease-investigation-sdi-program (Accessed June 1, 2023).

41. Victorian Department of Health. Better Health Channel: Protect yourself from mosquito-borne disease (2022). Available at: https://www.betterhealth.vic.gov.au/campaigns/protect-yourself-mosquito-borne-disease (Accessed June 1, 2023).

42. Victorian Department of Health. Mosquito-borne diseases: Vector-borne disease control. (2023). Available at: https://www.health.vic.gov.au/infectious-diseases/mosquito-borne-diseases (Accessed June 1, 2023).

43. Victorian Department of Health. Health alerts and advisories. (2023). Available at: https://www.health.vic.gov.au/news-and-events/healthalerts (Accessed June 1, 2023).

44. Rose RI. Pesticides and public health: integrated methods of mosquito management. *Emerg Infect Dis*. (2001) 7:17–23. doi: 10.3201/eid0701.010103

45. Fouet C, Kamdem C. Integrated mosquito management: is precision control a luxury or necessity? *Trends Parasitol*. (2019) 35:85–95. doi: 10.1016/j.pt.2018.10.004

46. Wilson AL, Courtenay O, Kelly-Hope LA, Scott TW, Takken W, Torr SJ, et al. The importance of vector control for the control and elimination of vector-borne diseases. *PLoS Negl Trop Dis*. (2020) 14:e0007831. doi: 10.1371/journal.pntd.0007831

47. New South Wales Health. *Nsw arbovirus surveillance and mosquito monitoring 2022–2023: Report number 15*. (2023) 28/1/2023. Report No.

48. South Australia Health. The south Australian arbovirus and mosquito monitoring report: January 2023. (2023).

49. Braddick M, Yuen A, Feldman R, Friedman ND. Early detection of Murray Valley encephalitis virus activity in Victoria using mosquito surveillance. *Med J Aust*. (2023) 219:40–1. doi: 10.5694/mja2.51987

50. Cilek J. Application of insecticides to vegetation as barriers against host-seeking mosquitoes. *J Am Mosq Control Assoc*. (2008) 24:172–6. doi: 10.2987/8756-971X(2008)24[172:AOITVA]2.0.CO;2

51. Hurst TP, Ryan PA, Kay BH. Efficacy of residual insecticide Biflex Aquamax applied as barrier treatments for managing mosquito populations in suburban residential properties in Southeast Queensland. *J Med Entomol*. (2014) 49:1021–6. doi: 10.1603/ME11278

52. Wang J-H, Wu J-W, Liu D. Benefit evaluation of dengue adult mosquito chemical control and its application. *Taiwan Epidemiol Bull*. (2009) 25:391–9.

53. Muzari OM, Adamczyk R, Davis J, Ritchie S, Devine G. Residual effectiveness of Λ-Cyhalothrin Harbourage sprays against foliage-resting mosquitoes in North Queensland. *J Med Entomol*. (2014) 51:444–9. doi: 10.1603/me13141

54. De Little SC, Williamson GJ, Bowman DM, Whelan PI, Brook BW, Bradshaw CJ. Experimental comparison of aerial Larvicides and habitat modification for controlling disease-carrying Aedes Vigilax mosquitoes. *Pest Manag Sci*. (2012) 68:709–17. doi: 10.1002/ps.2317

55. McGuinness SL, Lau CL, Leder K. The evolving Japanese encephalitis situation in Australia and implications for travel medicine. *J Travel Med*. (2023) 30:taad029. doi: 10.1093/jtm/taad029

56. Lobigs M, Larena M, Alsharifi M, Lee E. Pavy M. live chimeric and inactivated Japanese encephalitis virus vaccines differ in their cross-protective values against Murray Valley encephalitis virus. *J Virol*. (2009) 83:2436–45. doi: 10.1128/jvi.02273-08

57. Coroian M, Silaghi C, Tews BA, Baltag E, Marinov M, Alexe V, et al. Serological survey of mosquito-borne arboviruses in wild birds from important migratory hotspots in Romania. *Pathogens*. (2022) 11:1270. doi: 10.3390/pathogens11111270

58. Furlong M, Adamu A, Hickson RI, Horwood P, Golchin M, Hoskins A, et al. Estimating the distribution of Japanese encephalitis vectors in Australia using ecological niche modelling. *Trop Med Infect Dis*. (2022) 7:393. doi: 10.3390/tropicalmed7120393

59. Kretschmer M, Ruberto I, Townsend J, Zabel K, Will J, Maldonado K, et al. Unprecedented outbreak of West Nile virus - Maricopa County, Arizona, 2021. *MMWR Morb Mortal Wkly Rep*. (2023) 72:452–7. doi: 10.15585/mmwr.mm7217a1

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Phase III, randomized, double-blind, placebo-controlled clinical study: a study on the safety and clinical efficacy of AZVUDINE in moderate COVID-19 patients

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Background: In 2019, a highly pathogenic coronavirus named severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) surfaced and resulted in the outbreak of coronavirus disease 2019 (COVID-19). With the aim of finding effective drugs to fight against the disease, several trials have been conducted since COVID-19 can only be considered a treatable disease, from a clinical point of view, after the availability of specific and effective antivirals. AZVUDINE (FNC), initially developed for treating HIV, is a potential treatment for COVID-19 as it has the capability to lower the patient's viral load and promote recovery.

Methods: Volunteers infected with SARS-CoV-2 confirmed by reverse transcription polymerase chain reaction (RT-PCR), with good kidney and liver function, who were not using other antivirals or monoclonal antibodies were eligible. Samples from patients were assessed for viral load every 48 h during treatment using reverse transcription quantitative polymerase chain reaction (RT-qPCR) and droplet digital polymerase chain reaction (ddPCR).

Results: The study's primary outcome measure was the percentage of participants showing an improvement in clinical scores, while the secondary outcome measure was the percentage of participants with a clinical outcome of cure. These measures were used to assess the safety and efficacy of FNC for treating COVID-19. In the analysis of sociodemographic variables, no significant differences were detected between patients in the FNC and the placebo group for race, age group, or sex. The results showed a potential benefit to participants who received FNC during the study, as observed in the shorter hospital stay, shorter negative conversion time of SARS-CoV-2, and a significant reduction in viral load. Furthermore, the reduction in fever and chills were significant at D1, D2, and D3.

In this study, a total of 112 adverse events cases were noted, with 105 cases being categorized as non-serious and only 7 cases as serious adverse events.

Conclusion: The pandemic is not being effectively controlled and is causing multiple waves of infection that require extensive medical resources. However, FNC has demonstrated potential to reduce the treatment duration of moderate COVID-19 cases, thereby saving significant medical resources. This makes FNC a promising candidate for COVID-19 treatment.

Clinical trial registration: [clinicaltrials.gov], identifier [NCT04668235].

KEYWORDS

COVID-19, SARS-CoV-2, AZVUDINE, FNC, viral load

1. Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel coronavirus that causes coronavirus disease 2019 (COVID-19). The disease has rapidly spread around the world, with high transmission rates and substantial mortality rates (1–3). COVID-19 symptoms vary from mild respiratory illness to severe progressive pneumonia, multiple organ failure, and death (3, 4). As antivirals are key to treating COVID-19, trials have been conducted to identify effective drugs (4).

Several antiviral drugs have been investigated for the treatment of COVID-19, but some have shown adverse effects such as nephrotoxicity and hepatoxicity. For instance, remdesivir has been associated with these adverse events in patients with COVID-19 (5, 6). Additionally, drugs such as favipiravir and molnupiravir have been reported to significantly increase the number of mutations in the RNA structure (7).

Nucleoside antiviral drugs are known for their high efficacy in inhibiting the activity of virus DNA-dependent DNA polymerases (DdDps), RNA-dependent DNA polymerases (RdDps), and RNA-dependent RNA polymerases (RdRps), resulting in the inhibition of viral replication and a high drug resistance barrier (8). The use of FNC (AZVUDINE) in treating mild and common COVID-19 has shown promising results, as it has been found to potentially shorten the nucleic acid negative conversion (NANC) time compared with standard antiviral treatment, expedite viral elimination, and maintain the vital signs of the patients (9).

In the assessment of COVID-19, viral load progression is a crucial aspect. Liu et al. (10) observed that severe cases exhibited higher viral loads compared with mild cases, and a higher viral load corresponded to an increased risk of incubation and death (11). Furthermore, Fajnzylber et al. (12) demonstrated that viral load was associated with COVID-19 severity and mortality. A univariate survival analysis illustrated a significant difference in the probability of survival between individuals with high viral load and those with low viral load (13).

This study was one of the first studies to quantify viral load [absolute quantification by droplet digital polymerase chain reaction (ddPCR)], every 48 h, establishing information on viral load behavior and course of infection. The mean times of the NANC were measured in the FNC and the placebo groups, and the nephrotoxicity and hepatoxicity were monitored.

2. Results

2.1. Inclusion and exclusion criteria

The study enrolled patients who met specific criteria, including: (1) being at least 18 years old, regardless of gender; (2) testing positive for SARS-CoV-2 nucleic acid through RT-PCR of respiratory or blood samples or highly homologous with known SARS-CoV-2 through viral gene sequencing of respiratory or blood samples; and (3) confirmation of COVID-19 according to the diagnostic criteria outlined in the "latest clinical guidelines for novel coronavirus" issued by the World Health Organization (WHO) on 28 January 2020. All patients who met these criteria were required to sign informed consent forms (ICFs), and those with moderate COVID-19 were admitted to the hospital for treatment. After patients signed the informed consent forms, randomization was performed; thus, the treatment was initiated (D1 of the study), for both the FNC and placebo groups.

The exclusion criteria for this study encompassed several factors, including (1) any known or suspected allergy to the components of FNC tablets; (2) patients with malabsorption syndrome, gastrointestinal absorption issues, an inability to take oral medication, or who require intravenous nutrition; (3) patients currently undergoing anti-HIV treatment; (4) patients experiencing respiratory failure requiring mechanical ventilation, shock, or ICU monitoring/treatment for organ failures; (5) pregnant or lactating women, as well as those with plans for giving birth during the trial period or within 6 months after its completion; (6) individuals who participated in other clinical trials or used experimental drugs within 12 weeks prior to the study; and (7) patients deemed unsuitable for participation in the experiment based on the judgment of the researcher.

The definition of moderate COVID-19 was patients with fever, poor general condition, severe myalgia, persistent dry cough, diarrhea, moderate dyspnea without hypoxia (SpO_2 93–94%/TC <50%) or with

Abbreviations: ALT, Alanine aminotransferase; AST, Aspartate aminotransferase; BT, Bleeding time; COVID-19, Coronavirus disease 2019; FNC, AZVUDINE; GGT, Gamma-glutamyl Transferase; ICFs, Informed consent forms; ICU, Intensive care unit; NANC, Nucleic acid negative conversion; SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; WHO, World Health Organization.

hypoxia (SpO_2 92–93%/TC >50%), and with hospital admission recommended.

There was the presence of comorbidities among the participants, among them the most common were: arterial hypertension, obesity, type 2 diabetes, and alcohol consumption (Supplementary Table S3).

2.2. Demographic analysis

Between April 2021 and May 2022, a total of 476 individuals were considered for inclusion in this study. Among them, 296 participants were excluded due to various reasons, including not meeting the eligibility criteria, experiencing worsening symptoms prior to transfer to the research center ward, or withdrawing from the clinical trial before participation. Ultimately, 180 participants were randomized, with 172 successfully completing the treatment, while 8 individuals experienced serious adverse events during the course of the study. Of these cases, seven were due to disease progression (referred to the ICU) and one due to previous disease (mitral regurgitation with surgical indication) unrelated to FNC (Figure 1 and Tables 1, 2).

Patient demographics and baseline characteristics were well-matched between the FNC group and the control group at enrollment (Table 3). The median age was 48 years (IQR 41–58), and there was no significant difference between the age of participants who used the FNC and the placebo ($p = 0.135$). The largest number of participants was male, totaling 104 individuals (58%), there were no significant differences concerning gender ($p = 0.075$), indicating that the results obtained were not influenced by the age of the individuals or by gender (Table 3).

TABLE 1 Demonstration of aggravated cases during the study days.

	Treatments		
Proportion of ICU aggravations	Total	FNC	Placebo
Hospital discharge	172 (95.6%)	88 (96.7%)	83 (94.3%)
ICU aggravation	7 (3.9%)	3 (3.3%)	4 (4.5%)
Dropout	1 (0.6%)	0 (0%)	1 (1.1%)

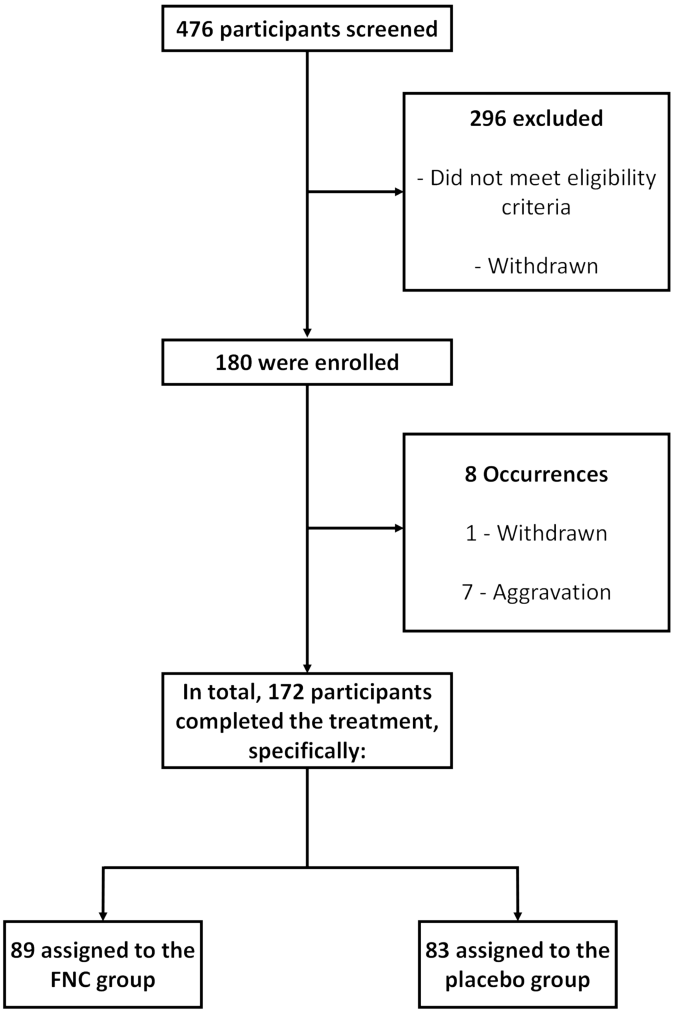

FIGURE 1
Trial profile.

TABLE 2 General data of cases of aggravation referred to the ICU.

ID	Hospitalization date	Aggravation date (ICU)	Result date	Final result
R19	12/05/2021	15/05/2021	02/06/2021	Death
R26	19/05/2021	20/05/2021	02/06/2021	Death
R41	29/05/2021	08/06/2021	20/06/2021	Death
R79	22/06/2021	26/06/2021	27/06/2021	Death
R115	24/07/2021	26/07/2021	15/08/2021	Death
R149	28/08/2021	29/08/2021	15/10/2021	Hospital discharge
R161	09/09/2021	22/09/2021	28/09/2021	Death

TABLE 3 Demographic and baseline characteristics of participants.

		Treatments		
Overall	N	FNC, $N = 91^1$	Placebo, $N = 88^1$	p-value[2]
Age	179	51 ± 13 (48)	48 ± 13 (48)	0.135
Race	179			0.417
White		48 (53%)	51 (58%)	
Black		18 (20%)	11 (12%)	
Brown		25 (27%)	26 (30%)	
Gender	179			0.075
F		44 (48%)	31 (35%)	
M		47 (52%)	57 (65%)	

[1]Mean ± SD (Median); n (%).
[2]Wilcoxon rank sum test; Pearson's Chi-squared test.

2.3. Clinical improvement

The data indicated that the initial clinical score of the participants who used the FNC was 4.42 ± 0.50 and for those in the control group it was 4.50 ± 0.50, with no significant difference in the clinical scores at which the participants entered the treatment ($p = 0.298$) (Supplementary Figures S1, S2).

Upon clinical discharge, the majority of participants achieved a clinical score of 0 or 1 on the WHO Ordinal Scale of Clinical (14) Improvement, with the exception of one patient who withdrew and seven patients who experienced worsening symptoms. Participants who used the FNC had a final score of 0.02 ± 0.15, while those who participated in the control group had a score of 0.11 ± 0.31, with a statistically significant difference between the groups ($p = 0.024$) (Table 4 and Supplementary Figures S1, S2).

2.4. Time to improvement of symptoms

During the study, the time required for participants to recover was determined by assessing the number of days they experienced symptoms. The characteristic symptoms of patients infected with the SARS-CoV-2 virus were evaluated (Table 4 and Supplementary Tables S1, S4). It was not possible to identify statistical differences in the time to the improvement of all symptoms between the two groups, FNC and placebo, of participants, except by the time of improvement of fever ($p < 0.01$) and chill ($p = 0.08$) symptoms.

TABLE 4 Overall results between FNC and placebo treatments on study outcomes.

	Treatments		
Objectives and outcomes	FNC	Placebo	p-value
Initial score	4.42 ± 0.50 (4.0)	4.50 ± 0.50 (4.5)	$p = 0.300$
Final Score	0.02 ± 0.15 (0.0)	0.11 ± 0.31 (0.0)	$\boldsymbol{p = 0.024}$
Temperature normalization—fever reduction (number of days)	0.13 ± 0.50 (0.00)	0.38 ± 0.68 (0.00)	$\boldsymbol{p < 0.001}$
Cure time/absence of viral RNA	7.7 ± 3.6 (6.5)	8.9 ± 3.5 (8.0)	$\boldsymbol{P = 0.028}$

In the analysis of curing time, it was observed that the FNC group had a shorter cure time/absence of viral RNA (6.5 days, $p = 0.028$) compared with the placebo group (8 days). There was a significant reduction in the length of hospital stay for the FNC group. Nine participants took more than 14 days for the first negative conversion.

2.5. Time of the nucleic acid negative conversion

The duration of negative nucleic acid conversion (NANC) is often used as an indicator of drug efficacy and clinical improvement. In this study, clinical discharge was achieved after two consecutive negative NANC results. The findings revealed that the FNC treatment group had a significantly shorter time to achieve the second negative NANC result (7.73 days, $p = 0.028$), compared with the placebo group (8.89 days) (as shown in Figure 2).

2.6. Detection of SARS-CoV-2 viral load by RT-PCR and ddPCR technique

In this study, the FNC group showed a more accentuated increase in cycles threshold (CTs)/day, although without showing significant differences compared with the control group (Supplementary Figure S3A). It was not possible to observe significant differences between the two groups on any of the treatment days. Since it was not possible to notice differences in the results of CTs, the same occurred for the viral load of the participants analyzed by the RT-PCR technique (Table 5 and Figure 3A).

It was not possible to identify a significant difference in viral load quantified through the RT-PCR technique between FNC and the control group. However, the viral load quantified by ddPCR showed a great difference between the groups (Table 6 and Figure 3B). The high sensitivity of the ddPCR confronts the variability obtained by calculating the viral load by RT-PCR (standard curve calculation due to the logarithmic variability) after treatment with FNC, showing a significant reduction in viral load at D3 ($p < 0.002$), D5, D7, and D9 ($p < 0.001$), and D11 ($p < 0.006$).

Notably, it was possible to observe significant differences in the time of improvement of fever at D1 ($p < 0.015$), D2 ($p < 0.040$), and D3 ($p < 0.026$), and chill ($p = 0.08$) symptoms (Table 4). More information can be found in the Supplementary material.

2.7. Sequencing of SARS-CoV-2 strains

Here, genetic sequencing was performed to demonstrate the distribution of strains between the FNC and placebo groups. The strain with the lowest prevalence was Alpha, which affected 7.8 and 18.8% of the volunteers who used FNC and the placebo, respectively (Figure 4). The Delta strain affected 37.7% of the volunteers who used FNC and the placebo (Figure 4). The strain with the highest incidence during the research was Gamma, which affected 54.5 and 43.8% of the volunteers who used FNC and the placebo, respectively (Figure 4).

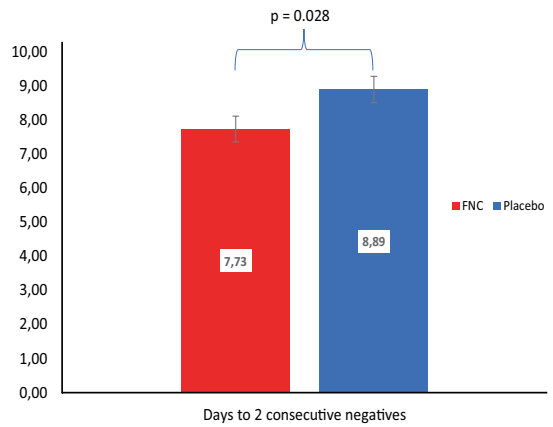

FIGURE 2
The mean (SD) number of days until the second nucleic acid testing showed negativity compared between the FNC group and the placebo group. The Mann–Whitney test was used to analyze the differences between the groups, with the FNC group represented by a red bar and the placebo group represented by a blue bar.

2.8. Changes in kidney and liver functions baselines

The renal function test results of the participants assigned to either the FNC or the placebo group, which included evaluations of creatinine and blood urea nitrogen, exhibited similar value profiles. These values remained within the normal parameters throughout the treatment, and no significant differences were observed between the two groups during the treatment period (Figures 5A,B).

The liver function test results of the participants assigned to either the FNC or the placebo group, which included assessments of aspartate aminotransferase (AST), alanine aminotransferase (ALT), glutamyl transpeptidase, and total bilirubin, revealed that all values were within the normal range. Both groups exhibited similar results profiles, and no statistically significant changes were observed during the course of the treatment. Additionally, the results obtained from the exams related to renal function (Figures 5C–F) were consistent with these findings.

2.9. Time and proportion of lung imaging improvement

It was not possible to observe significant differences regarding the improvement of lung images during the treatment days (Supplementary Table S2). All participants started the study with a clinical picture of 25–50% of pulmonary involvement; however, despite the clinical improvement that was observed, the improvement

TABLE 5 Estimated (RT-PCR) viral load values during the treatment days.

		Treatments		
Overall	N	FNC, $N = 91^1$	Placebo, $N = 88^1$	p-value^2
Viral load (D1)	178	10,398 (1,000; 11,613) [7,227]	10,456 (1,019; 11,324) [8,178]	0.635
Viral load (D3)	172	10,080 (533; 11,752) [6,730]	10,199 (546; 11,434) [7,394]	0.346
Viral load (D5)	166	101 (0; 10,219) [3,961]	970 (2; 10,199) [4,175]	0.331
Viral load (D7)	129	0 (0; 1,004) [2,335]	10 (0; 9,828) [3,173]	0.672
Viral load (D9)	97	5 (0; 1,010) [2,337]	10 (0; 1,026) [2,626]	0.120
Viral load (D11)	72	0 (0; 78) [1,146]	10 (0; 102) [1,677]	0.069
Viral load (D13)	50	0 (0; 102) [1,095]	0 (0; 0) [702]	0.655
CTs (D1)	178	0 (0; 0) [545]	0 (0; 0) [439]	0.686
CTs (D3)	172	24.2 (21.3; 27.0) [23.9]	23.9 (21.6; 26.6) [23.9]	0.299
CTs (D5)	166	24.7 (20.7; 28.1) [24.5]	24.8 (21.5; 27.8) [24.3]	0.334
CTs (D7)	129	28.8 (24.6; 31.0) [27.3]	27.9 (24.5; 30.9) [26.7]	0.685
CTs (D9)	97	31.00 (29.30; 31.00) [29.72]	30.40 (28.60; 31.00) [28.99]	0.119
CTs (D11)	72	31.00 (28.45; 31.00) [29.48]	31.00 (31.00; 31.00) [29.90]	0.062
CTs (D13)	50	31.00 (31.00; 31.00) [30.28]	31.00 (31.00; 31.00) [30.43]	0.655

[1]Median (25%; 75%) [Mean].
[2]Wilcoxon rank sum test.

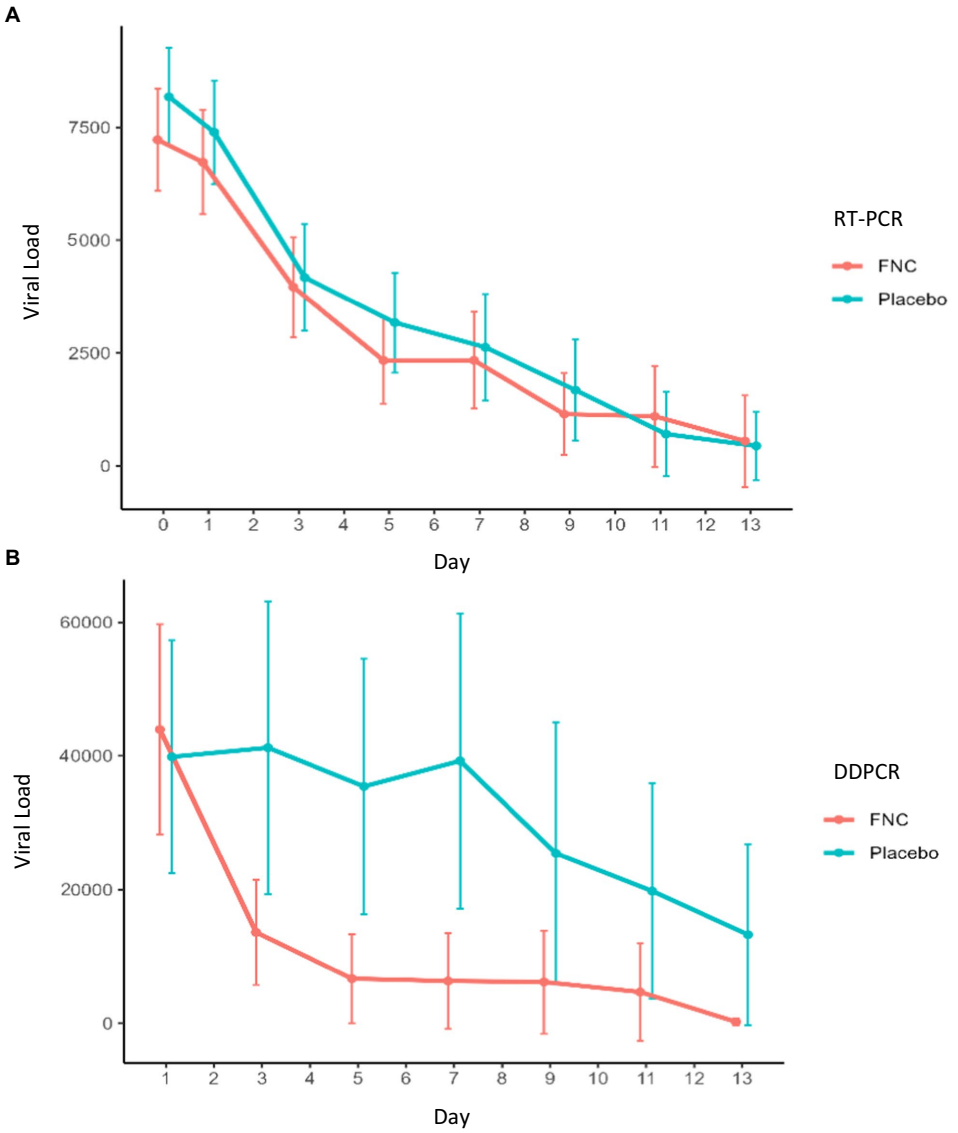

FIGURE 3
(A) Estimated (RT-PCR) and **(B)** absolute viral load analysis (ddPCR) of participants in the FNC group and the placebo group during the treatment days. Data are median (SD). (Red line, FNC; blue line, placebo).

of the lungs occurred slowly, so it was not possible to observe a difference in this parameter due to the short treatment period (14 days) and clinical follow-up at D28 and D60.

2.9.1. Adverse events and clinical safety of FNC

In this study, a total of 113 adverse events were recorded, with 105 categorized as non-serious and only 8 considered serious. Of these cases, seven were due to disease progression and one due to previous disease (mitral regurgitation with surgical indication) unrelated to FNC (Table 7).

The adverse events observed in this study were mainly related to the increase in ALT (45 cases), GGT (13 cases), AST (10 cases), all being grade 1 intensity, considering that they occurred within the normal range. It was also possible to observe an increase in ALT, GGT, and AST at the time of randomization, which is to be expected in infectious conditions. The adverse reactions observed in this study were the same as those related to antiviral drugs, with no unexpected adverse reactions occurring (Table 8).

Phlebitis that occurred during the study was due to the administration of intravenous antibiotics, which was later changed to oral administration. There was also no significant change in urinary phosphorus. In preliminary studies, vertigo (incidence ≥5%) has been attributed to FNC; however, in this study, there were only two reported cases of dizziness related to labyrinthitis (history) and hypoglycemia (due to loss of taste). It should also be considered that the participants were bedridden, which could potentiate these events.

There were seven exclusions due to the disease worsening and progression to the ICU. There were six deaths and one recovery where participants received adequate care and support during hospitalization. In the case of deaths, three participants arrived at the hospital with a worsening condition, and after admission they were transferred to the ICU within 1–3 days (Table 7).

TABLE 6 Absolute (ddPCR) viral load values during the treatment days.

		Treatments		
Overall	N	FNC, $N = 91^1$	Placebo, $N = 88^1$	p-value[2]
DDPCR (D1)	178	6,108 (362; 46,646) [43,988]	4,183 (141; 39,483) [39,861]	0.250
DDPCR (D3)	176	49 (0; 5,638) [13,629]	1,002 (34; 22,471) [41,221]	**0.002**
DDPCR (D5)	155	0 (0; 202) [6,682]	284 (14; 16,827) [35,440]	**<0.001**
DDPCR (D7)	116	0 (0; 0) [6,329]	1,120 (40; 25,230) [39,258]	**<0.001**
DDPCR (D9)	89	0 (0; 0) [6,176]	256 (0; 12,665) [25,426]	**<0.001**
DDPCR (D11)	65	0 (0; 0) [4,681]	0 (0; 1,673) [19,791]	**0.006**
DDPCR (D13)	44	0 (0; 0) [223]	0 (0; 0) [13,273]	0.111

[1]Median (25%; 75%).
[2]Wilcoxon rank sum test.

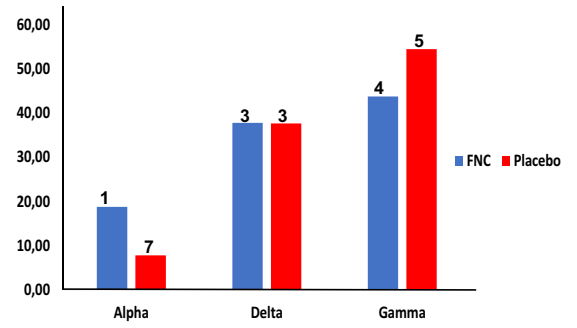

FIGURE 4
Percentage of volunteers infected by the different strains of SARS-COV-2 distributed among the treatments. (Red bar, FNC; blue bar, placebo).

There was also no significant change in urinary phosphorus as reported in the adverse events of special interest (Supplementary Table S5). Serum cholinesterase is decreased in hepatic parenchymal diseases (e.g., viral hepatitis and cirrhosis), congestive heart failure, abscesses, neoplasms, malnutrition, acute infections, anemia, myocardial infarction, and dermatomyositis. It may be increased in obese patients, diabetics, and those with nephrotic syndrome. We observed that the values evaluated were not significant and that the participants did not have other debilitating conditions (Supplementary Table S6).

There was no significant difference in the inflammatory marker values during the study days (Supplementary Table S7). There was a reduction of leukocytes and neutrophils within the normal range but significant on D1 and compatible with an initial stage of infection (Supplementary Table S8). Procalcitonin has good sensitivity and specificity for the diagnosis of secondary bacterial infections in patients with viral diseases and was not significant at D1, which meets the protocol's inclusion criteria, becoming significant at D7 for 102 participants, reducing up to D15 (medical discharge) and D28 (follow-up after medical discharge).

There was a significant increase in lymphocytes at D13 for 51 participants, but within the normal range (Supplementary Table S9). There was the significance of CD8 at D11 (elimination of infectious cells) for 63 participants. There was CD4 significance at D28 (specific for opportunistic infections) for 128 participants, which may explain post-covid symptoms.

Although there was an improvement in respiratory symptoms leading participants to hospital discharge, this improvement was not observed in the statistical analysis (Supplementary Table S10).

Although there was an improvement in O_2 saturation, this was not observed in the statistical analysis. There was an improvement in O_2 saturation in the two groups from D2 onwards (Supplementary Table S11). The improvement in respiratory rate was not significant and ventilatory support makes this parameter questionable (Supplementary Table S12).

Supplementary Table S13 shows the number of participants who entered the study without the need for supplemental oxygen supply (room air), or with the need for supplementary oxygen supply (nasal catheter and reservoir mask), with significance between groups, at D1. It also shows that the number of these participants who moved to room air practically doubled at D2 in the FNC group. The predominance of recovery to room air continued until D6, which coincided with the time when the viral load decreased with FNC on D3, D5, and D7. Also, the number of participants using a catheter or reservoir mask from D2 onwards was lower in the FNC group than in the placebo group. Also related to the frequency of supplemental oxygenation or non-invasive ventilation, we have the number of liters of O_2/min that tended to decrease in the FNC group (Supplementary Table S14).

There was no significance between the groups in the use of mechanical ventilation, although there was a predominance of worsening conditions in the placebo group. Another factor is the speed of evolution of the clinical condition due to the disease, since a moderate patient could progress in a few hours to a severe clinical condition, requiring admission to the ICU, which justifies entry into the study (moderate clinical condition) and subsequent worsening (admission to the ICU) (Supplementary Table S15).

3. Discussion

Patient demographics data indicated that the results obtained were not influenced by the age of the individuals or by gender (Table 3). The present study demonstrated no significant difference in the time to improvement of all symptoms between participants who received FNC and those who received the placebo. These findings are consistent with a previous pilot study by Ren et al. (9), which also reported no differences in symptoms and laboratory test results during

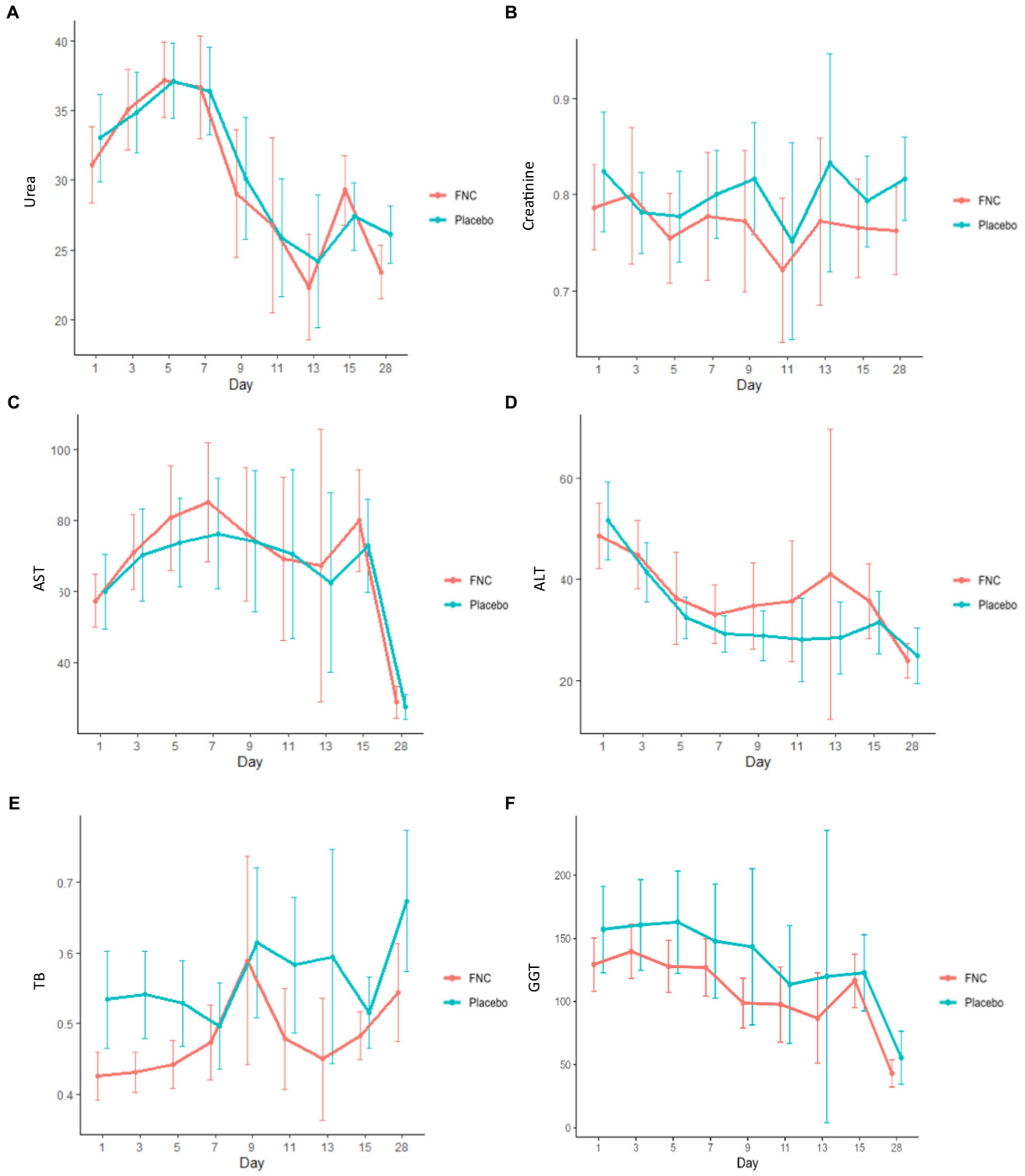

FIGURE 5
During the treatment, the dynamic changes in kidney and liver markers: **(A)** creatinine, **(B)** urea, **(C)** alanine aminotransferase (ALT), **(D)** aspartate aminotransferase (AST), and **(E)** total bilirubin (TB), and **(F)** gamma-glutamyl transpeptidase (GGT) of the patients in the FNC group and patients in the placebo group. Data are median (SD). (Red line: FNC; blue line: placebo).

screening between the FNC and control groups. However, it was possible to observe significant differences in the time of improvement of fever ($p < 0.01$) and chill ($p = 0.08$) symptoms. The initial sensation of coldness during fever may be attributed to vasoconstriction leading to a decrease in skin temperature (3); thus, chill and fever are correlated. Since fever attenuation was observed in the FNC group, this may be a consequence of the decline in the infection (4), which in turn is related to a possible reduction of the patient viral load.

Another point to be highlighted is that there was a significant reduction in the length of hospital stay for the FNC group, reducing the time of exposure to the virus action and the possibility of greater sequelae. Nine participants took more than 14 days for the first negative conversion. Concomitant with these data, the NANC time was significantly shorter in participants treated with FNC (7.73 days, $p = 0.028$) compared with those treated with the placebo (8.89 days), as shown in Figure 2, which is consistent with the findings of Ren et al.

TABLE 7 Global quantification of adverse events.

		Treatments			
N = 180	Total	FNC	Placebo	Subject (%)	Intensity
Adverse events	105	50	55	58.33	Grade 1 and 2
Frequency and intensity of serious adverse events	8	3	5	4.44	Grade 4
All-cause mortality rate during the study	7	3	4	3.88	4—Death
Frequency and intensity of unexpected adverse events	0	0	0	0	0
Occurrence of drug interactions	0	0	0	0	0

(9) which demonstrated that FNC treatment may shorten the NANC time in mild and common COVID-19 cases when compared with standard antiviral treatment. Thus, FNC treatment may reduce the treatment duration for mild patients and, consequently, save valuable medical resources.

Several studies have reported a relationship between viral loads and disease severity (15–18). For instance, Liu et al. (10) found that severe COVID-19 cases had higher viral loads than mild cases, and it has also been shown that higher viral loads are associated with an increased risk of incubation and death (11). Additionally, Fajnzylber et al. (12) reported that viral load is implicated in the severity and mortality of COVID-19. A significant difference in survival probability was observed between patients with high viral load and those with low viral load based on a univariate survival analysis (13). A recent randomized clinical trial investigated the effectiveness of FNC added to standard treatment compared with a placebo group for patients with mild COVID-19 (19). The findings suggest that FNC treatment may shorten the time of the nucleic acid negative conversion and reduce viral load in these patients (19).

In the present study, it was not possible to identify a significant difference in viral load quantified through the RT-PCR technique between the FNC and the control group. RT-PCR is considered the gold standard for the diagnosis of COVID-19, but its reliability has been questioned due to negative results in some clinically suspected patients and positive results in recovered patients (2, 20). Moreover, RT-PCR results can be influenced by viral RNA sequence variations, and sampling procedures can contribute to a high false-negative rate due to differences in viral load across anatomical sites (21). In real COVID-19 cases, one-time testing can result in a false-negative rate as high as 30–50% (21).

According to Yu et al. (18), although RT-PCR is sensitive and reliable for detecting SARS-CoV-2, ddPCR performs better in detecting low-viral-load samples. In their study, the results of RT-PCR and ddPCR were consistent in the 95 positive samples, and the Ct value of RT-PCR was highly correlated with the copy number value of ddPCR. However, when Ct values were between 34 and 38, the viral load of samples with the same Ct value was significantly different, indicating that the Ct value of RT-PCR may not sensitively reflect the level of viral load when the viral load is low. In our study, ddPCR quantified a significantly higher viral load than RT-PCR between the treatment groups (Table 6 and Figure 3B), which is consistent with previous studies that showed ddPCR's advantage of absolute quantification and higher sensitivity for virus detection than RT-PCR (18, 22, 23).

In addition, the sequencing of SARS-CoV-2 for the detection of potential lineages was performed. The distribution of strains between the FNC and the placebo groups showed that the strain with the highest incidence during the research was Gamma, which affected 54.5 and 43.8% of the volunteers who used the FNC and placebo, respectively (Figure 4). The variant omicron had not appeared during the period in which the study was carried out (24). This study had only six vaccinated participants, three in the placebo group and three in the FNC group. This study was carried out in a period when vaccines were not widely available for the population, and therefore vaccine interference may exist in only three vaccinated participants infected by the Delta strain variants AY.99.1, AY 0.99.1, and AY.99.2, respectively.

In this study, the treatment with FNC was well tolerated by patients. Vital signs, liver function, and kidney function in both groups were normal. These data reinforce what was observed in the pilot clinical trial previously performed with FNC, in which hepatic and renal functions did not change between the FNC and the control group, indicating the non-toxicity of the drug. This is not the case for many antivirals; in studies with remdesivir, for example, nephrotoxicity and hepatoxicity were reported as adverse drug events in patients with COVID-19 (5, 6). It was reported that similar types of antiviral drugs may cause mitochondrial injury in renal tubular epithelial cells (6, 21). Therefore, our results highlight the safety of FNC since no changes were observed in markers of kidney and liver damage when the two groups were compared.

The adverse reactions identified in this study were consistent with those commonly associated with antiviral medications, and no unexpected adverse reactions were reported (Table 8). The analysis of adverse events between the FNC and the placebo groups showed a similar incidence rate, indicating that adverse events observed were likely a result of the underlying disease and not due to the treatment.

The analysis of the viral load, every 48 h, served as a safety examination that could identify the intensity of infection of individuals, being a marker in the prevention of worsening (a condition that, when it occurs, excludes the participant from the study). Verifying viral load enabled patient management, preventing worsening and allowing safety parameters to be better assessed.

To summarize, administering FNC to moderate COVID-19 patients may lead to a faster conversion to nucleic acid negativity compared with the placebo group, which could potentially reduce hospitalization duration and improve clinical outcomes. FNC treatment accelerates viral clearance, leading to a significant decrease in viral load and symptom relief. These findings support the use of FNC in the treatment of moderate COVID-19 patients. Since FNC is an oral drug that is excreted within 24 h without integration into human genetic material, it offers a safe and effective treatment option that can help reduce the time and cost of COVID-19 treatment and control the pandemic's spread.

TABLE 8 Consolidated report of adverse events.

Classification	Grade 1		Grade 4		Grade 1		Grade 4	
	Case	Subject (%)	Case	Subject (%)	Case	Subject (%)	Case	Subject (%)
ALT elevation	**24**	26.37	0	0	**21**	23.59	0	0
GT range lifting	**6**	6.59	0	0	**7**	7.86	0	0
AST elevation	**4**	4.39	0	0	**6**	6.74	0	0
Headache	**2**	2.19	0	0	**6**	6.74	0	0
Phlebitis MS	**1**	1.09	0	0	**3**	3.37	0	0
GT gamma reduction	**1**	1.09	0	0	**2**	2.24	0	0
High fever	**2**	2.19	0	0	**1**	1.12	0	0
Dizziness	**0**	0	0	0	**2**	2.24	0	0
Sodium reduction	**0**		0	0	**2**	2.24	0	0
Potassium reduction	**1**	1.09	0	0	**2**	2.24	0	0
Hemoglobin reduction	**1**	1.09	0	0	**1**	1.12	0	0
Hyperglycemia	**1**	1.09	0	0	**1**	1.12	0	0
Hypoglycemia	**1**	1.09	0	0	**0**	0	0	0
Calcium reduction	**0**	0	0	0	**0**	0	0	0
Creatinine increase	**1**	1.09	0	0	**0**	0	0	0
CPK increase	**0**	0	0	0	**1**	1.12	0	0
Troponin I increase	**1**	1.09	0	0	**0**	0	0	0
Backache	**0**	0	0	0	**1**	1.12	0	0
Diarrhea	**1**	1.09	0	0	**0**	0	0	0
Tachycardia	**1**	1.09	0	0	**0**	0	0	0
Nauseas	**1**	1.09	0	0	**0**	0	0	0
Respiratory insufficiency	**0**	0	**3**	3.29	**0**	0	**4**	4.49
Severe mitral insufficiency	**0**	0	**1**	1.09	**0**	0	0	0
Leukopenia	**1**	1.09	0	0	**0**	0	0	0
Total	**50**	54.81	**4**	4.38	**56**	62.86	**4**	4.49

4. Methodology

4.1. Study design

This clinical trial was conducted at Santa Casa de Misericordia de Campos Hospital as a strategic decision to ensure the standardization and quality of molecular biology analyses. Each RT-PCR equipment and reagent kit used in RT-PCR has different sensitivities and performance, hence the need to concentrate the analyses. The study was a double-blind, placebo-controlled clinical trial with randomization and was approved by the institutional review board of the National Health Surveillance Agency (CE 0937457/21–4) and the National Council for Research Ethics (CAAE 52176421.8.0000.5244). The trial was registered on clinicaltrials.gov (NCT04668235) under the title "Study on Safety and Clinical Efficacy of AZVUDINE in COVID-19 Patients (SARS-CoV-2 Infected)." All participants provided written informed consent, and the inclusion and exclusion criteria, design, goals, and outcomes are detailed in Supplementary methodology.

Patients assigned to the FNC group received standard treatment along with oral FNC tablets at a dosage of 5 mg (five tablets administered once a night). This concentration was based in the previous randomized controlled clinic study of FNC tablets in the Treatment of Mild and Common COVID-19 (9). The mean half-life of FNC at this dosage is 13.8 h, with both the intact drug and its metabolites excreted in the urine within 24 h. In the control group, patients were administered a placebo in addition to standard treatment. Details of the inclusion and exclusion criteria can be found in the results and discussion section. The placebo tablets were physically identical to the FNC tablets, containing microcrystalline cellulose, hydrated lactose, polyvinylpyrrolidone K30, croscarmellose sodium, and magnesium stearate.

Standard treatment: All participants received the treatment for COVID-19 prescribed by the Ministry of Health in Brazil. Medications include ceftriaxone disodic, 1,000 mg/mL fras; omeprazole, 40 mg-vial-amp 10 mL inject.; ondansetron, chloridate, 2 mg/mL; dipiron sodic, 500 mg/mL ampoule 2 mL ii; formoterol fumarate 12 mcg + budesonide 400 mcg; dexamethasone, 4 mg/mL ampoule 2.5 mL in.; enoxaparin; 40 mg/0.4 mL inject. Syringe; captopril, 50 mg-tablet orally; losartan potassium, 50 mg tablets; clarithromycin, 500 mg tablet orally; clonazepam, 2 mg tablet orally; ceftriaxone

disodic, 1,000 mg/mL fras; omeprazole, 40 mg vial-amp 10 mL inject.; ondansetron, 2 mg/mL chloridate; dipiron sodica, 500 mg/mL ampoule 2 mL ii; formoterol fumarate 12 mg + budesoni; dexamethasone, 4 mg/mL ampoule 2.5 mL in; enoxaparin, 40 mg/0.4 mL inject. Syringe; captopril, 50 mg tablet orally.

Enrollment: Once patients provided their informed consent by signing the ICF, a throat swab was collected for RT-PCR nucleic acid testing to confirm the presence of COVID-19. The main investigator assessed whether the patient met the inclusion criteria, and eligible patients with laboratory-confirmed COVID-19 and moderate symptoms were transferred to the hospital for admission.

> First patient enrollment: 04/23/2021.
> Last patient enrollment: 03/04/2022.

Randomization: The main investigator conducted exams to assess whether the patients met the eligibility criteria after they signed the consent form. If the patients were found to be eligible, they were admitted to the hospital and randomly assigned to either the FNC group or the control group in a 1:1 ratio. Randomization was performed using Software Researcher IGZ v2.0, at the participant's hospitalization, randomly into the FNC and control groups. In the pharmacy, the already fractionated drug received a bar code, where the system only allowed the drug to be dispensed if the bar code matched the randomization of the participant.

Apart from monitoring the vital signs and performing routine hematology and biochemistry exams, the participants' SARS-CoV-2 nucleic acid levels were checked by RT-PCR after they commenced their medication. The nucleic acid detection tests were conducted every 48 h during the treatment period to obtain the optimal measurement of the participants' viral load. Clinical discharge was confirmed when two consecutive negative test results were obtained. These tests were utilized to obtain the average time taken for the nucleic acid to turn negative (NANC).

This study was carried out at the height of the pandemic, in 2021, and the beginning of vaccinations, hence the low vaccination rate in the participants (six people). During this period, the need for ICU care was frequent, as were deaths. And since there was no effective treatment, monitoring the viral load during the course of the disease (every 48 h) could establish viral behavior, the relationship with the clinic, and the efficacy of the experimental therapy. The trial ended on 10 August 2022.

Outcomes: The primary outcome was the proportion of participants with improved clinical status. The criterion for a participant to have an improvement in clinical status was a decrease in the WHO Ordinal Scale of Clinical Improvement by at least one category compared with that when screening. Time Frame: Day 1 to Day 15.

The study's secondary outcomes included the following: (1) the proportion of participants who achieved a clinical cure during the study, defined as the absence of viral RNA in collected samples and meeting the clinical criteria for hospital discharge; (2) the time to improvement of symptoms such as diarrhea, myalgia, fatigue, malaise, cough, dyspnea, and headache; (3) changes in liver and kidney function from baseline; (4) the comparison of SARS-CoV-2 viral load negative conversion time by RT-PCR between the FNC group and the control group; (5) length of hospital stay; (6) frequency and intensity of adverse events, unexpected adverse events, and serious adverse events; (7) the all-cause mortality rate during the study; and (8) the evaluation of the tolerability of AZVUDINE (FNC) at a dosage of 5 mg/day for up to 14 days.

The hospital discharge criterion was two consecutive negative results and an improvement in clinical status. However, the treated strains were aggressive (Alpha, Gamma and Delta), for this reason, participants needed to remain hospitalized until the second negative result, for safety reasons due the clinical conditions, in this period, in 2021, were not so simple, in addition to there being a lack of knowledge about the disease. Eleven participants failed to perform the second RT-PCR during hospitalization due to hospital discharge due to clinical improvement. Seven participants failed to perform the second RT-PCR due to being transferred to the ICU. In total, 18 participants skipped the second RT-PCR exam. All participants were included in the statistical analysis except one dropout.

The safety of the participants was continuously monitored throughout the study by tracking vital signs, changes in liver and renal function, and adverse events. The adverse events were evaluated based on their type, incidence, severity, time of occurrence, drug correlation, and severity assessment. Previous research has reported that the use of FNC did not result in any significant adverse events that were drug-related (9).

4.2. Statistical analysis

Initially, there were 342 participants in the study. However, due to the decrease in the number of COVID-19 cases in Brazil toward the end of 2021, the sample size was reevaluated and subsequently reduced to 180 participants. These participants were randomly assigned to two study groups, each consisting of 90 participants. All enrolled patients with moderate COVID-19 were hospitalized. The sample calculation was performed using the formula of "sample calculation for superiority studies using proportions," described by World Health Organization (14). To analyze demographic information and baseline eigenvalues, descriptive statistics such as mean, standard deviation, quartiles, and minimum and maximum values were calculated for numerical variables. Frequency and percentage were determined for categorical data. The appropriate statistical methods were employed to compare the two groups based on the type of indicator. The Mann–Whitney test was utilized to compare quantitative data, while Fisher's exact test was employed for categorical data. All statistical analyses were conducted using R-studio software.

4.3. Quantification of SARS-CoV-2 viral load by reverse transcription–polymerase chain reaction

The MagMAXTM Viral/Pathogen Nucleic Acid Isolation kit (Applied Biosystems) was employed to extract total RNA from nasal and throat swabs obtained from the participants of the clinical study. The extraction process was carried out in accordance with the manufacturer's guidelines.

Following the extraction of total RNA, RT-PCRs were conducted using the TaqPath™ COVID-19 CE-IVD RT-PCR kit (ANVISA Reg.:

10358940107) on the QuantStudio5 RT-PCR equipment from Applied Biosystems (ANVISA Reg: 10358940069), as per the instructions provided by the manufacturer. The primers and probes chosen were designed to target the ORF1ab and N genes.

To estimate the viral load of each sample, CTs obtained from RT-PCR were plotted on a standard curve created using serial dilutions of the positive control (TaqPath™ COVID-19 Control), which consists of SARS-CoV-2 viral RNA at a known concentration of 1 × 104 copies/μL.

An RT-PCR result is deemed positive when CT values are equal to or lower than 30.5. During the reaction, the specific probe utilized to detect the presence of SARS-CoV-2 is cleaved by DNA polymerase, causing the emission of fluorescence when viral RNA is present. Higher levels of viral RNA generate greater fluorescence, leading to an earlier appearance of the CT value in the reaction. Conversely, lower levels of viral RNA result in lower fluorescence, leading to a delayed appearance of the CT value. CT values above 30.5 are interpreted as negative. By constructing a concentration curve for viral RNA, we can generate a curve of CT values, which ranges from lower values (indicating higher copies of viral RNA) to higher values (indicating lower copies of viral RNA).

4.4. Quantification of SARS-CoV-2 viral load by droplet digital polymerase chain reaction

Nasal and throat swabs collected from clinical study participants were subjected to RNA extraction using the MagMAXTM Viral/Pathogen Nucleic Acid Isolation kit (Applied Biosystems) in accordance with the manufacturer's guidelines. Following the extraction of total RNA, ddPCR was conducted.

PCR amplification was carried out with primers and probes targeting the ORF1ab and N genes, along with a positive reference gene, following the manufacturer's guidelines for the reaction system and amplification conditions (Shanghai BioGem Medical Technology Co., Ltd., China).

The Targeting One Digital PCR System, which includes the COVID-19 digital PCR detection kit, droplet generation kit, and droplet detection kit, was utilized to conduct digital droplet PCR analyses. The kit was designed to detect the ORF1ab gene, the N gene, and a positive reference gene, with a detection limit of 10 copies/test. Targeting One Technology is authorized by the China Food and Drug Administration. A fractional number represents viral fragments that do not constitute a viral unit.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Comissão Nacional de Ética em Pesquisa (Conep). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

PC coordinated the project and supervised the writing of the manuscript. SS performed the analysis of the data. AV assisted in the acquisition of statistical data. RS, RA, SC, and AS assisted in the acquisition of data. PC, SS and RS wrote the manuscript. WD and AM performed the sequencing analyses. CS assisted the medical team that conducted the clinical research. JC and PL assisted in reviewing the manuscript. All authors read and approved the final version of the manuscript.

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Conflict of interest

The research was entirely developed by the High Complexity Center, Galzu Institute (study design, collection, analysis, interpretation of data, the writing of this article or the decision to submit it for publication).

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1215916/full#supplementary-material

References

1. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. (2020) 382:727–33. doi: 10.1056/NEJMoa2001017

2. Wu J, Liu J, Zhao X, Liu C, Wang W, Wang D, et al. Clinical characteristics of imported cases of COVID- 19 in Jiangsu Province: a multicenter descriptive study. *Clin Infect Dis*. (2020) 71:706–12. doi: 10.1093/cid/ciaa199

3. Wang Y, Kang H, Liu X, Tong Z. Combination of RT-qPCR testing and clinical features for diagnosis of COVID-19 facilitates management of SARS-CoV-2 outbreak. *J Med Virol*. (2020) 92:538–9. doi: 10.1002/jmv.25721

4. Zhang X, Horby P, Cao B. COVID-19 can be called a treatable disease only after we have antivirals. *Sci. Bull*. (2022) 67:999–1002. doi: 10.1016/j.scib.2022.02.011

5. Beigel JH, Tomashek KM, Dodd LE, Mehta AK, Zingman BS, Kalil AC, et al. Remdesivir for the treatment of Covid-19—final report. *N Engl J Med*. (2020) 383:1813–26. doi: 10.1056/NEJMoa2007764

6. Adamsick ML, Gandhi RG, Bidell MR, Elshaboury RH, Bhattacharyya RP, Kim AY, et al. Remdesivir in patients with acute or chronic kidney disease and COVID-19. *J Am Soc Nephrol*. (2020) 31:1384–6. doi: 10.1681/ASN.2020050589

7. Abdelnabi R, Foo CS, Kaptein SJ, Zhang X, Langendries L, Vangeel L, et al. The combined treatment of Molnupiravir and Favipiravir results in a marked potentiation of efficacy in a SARS-CoV2 hamster infection model through an in- creased frequency of mutations in the viral genome. *bioRxiv*. (2021):2020–12. doi: 10.1101/2020.12.10.419242

8. Jordheim LP, Durantel D, Zoulim F, Dumontet C. Advances in the development of nucleoside and nucleotide analogues for cancer and viral diseases. *Nat Rev Drug Discov*. (2013) 12:447–64. doi: 10.1038/nrd4010

9. Ren Z, Luo H, Yu Z, Song J, Liang L, Wang L, et al. A randomized, open-label, controlled clinical trial of AZVUDINE tablets in the treatment of mild and common COVID-19, a pilot study. *Adv Sci*. (2020) 7:e2001435. doi: 10.1002/advs.202001435

10. Liu Y, Yan L-M, Wan L, Xiang T-X, Le A, Liu J-M, et al. Viral dynamics in mild and severe cases of COVID-19. *Lancet Infect Dis*. (2020) 20:656–7. doi: 10.1016/S1473-3099(20)30232-2

11. Magleby R, Westblade LF, Trzebucki A, Simon MS, Rajan M, Park J, et al. Impact of SARS-CoV-2 viral load on risk of intubation and mortality among hospitalized patients with coronavirus disease 2019. *Clin Infect Dis*. (2020) 73:e4197–205. doi: 10.1093/cid/ciaa851

12. Fajnzylber J, Regan J, Coxen K, Corry H, Wong C, Rosenthal A, et al. SARS-CoV-2 viral load is associated with increased disease severity and mortality. *Nat Commun*. (2020) 11:5493. doi: 10.1038/s41467-020-19057-5

13. Pujadas E, Chaudhry F, McBride R, Richter F, Zhao S, Wajnberg A, et al. SARS-CoV-2 viral load predicts COVID-19 mortality. *Lancet Respir Med*. (2020) 8:E70. doi: 10.1016/S2213-2600(20)30354-4

14. World Health Organization. *Laboratory testing for 2019 novel coronavirus (2019-nCoV) in suspected human cases*. Available at: https://www.who.int/publications-detail/laboratory-testing-for-2019-novel-coronavirus-in-suspected-human-cases-20200117.

15. Fodha I, Vabret A, Ghedira L, Seboui H, Chouchane S, Dewar J, et al. Respiratory syncytial virus infections in hospitalized infants: association between viral load, virus subgroup, and disease severity. *J Med Virol*. (2007) 79:1951–8. doi: 10.1002/jmv.21026

16. Zheng S, Fan J, Yu F, Feng B, Lou B, Zou Q, et al. Viral load dynamics and disease severity in patients infected with SARS-CoV-2 in Zhejiang province, China, January–march 2020: retrospective cohort study. *BMJ*. (2020) 2020:369m1443. doi: 10.1136/bmj.m1443

17. Pan Y, Zhang D, Yang P, Poon LLM, Wang Q. Viral load of SARS-CoV-2 in clinical samples. *Lancet Infect Dis*. (2020) 20:411–2. doi: 10.1016/S1473-3099(20)30113-4

18. Yu F, Yan L, Wang N, Yang S, Wang L, Tang Y, et al. Quantitative detection and viral load analysis of SARS-CoV-2 in infected patients. *Clin Infect Dis*. (2020) 71:793–8. doi: 10.1093/cid/ciaa345

19. da Silva RM, Gebe Abreu Cabral P, de Souza SB, Arruda RF, Cabral SPF, de Assis ALEM, et al. Serial viral load analysis by DDPCR to evaluate FNC efficacy and safety in the treatment of mild cases of COVID-19. *Front Med*. (2023) 10:1143485. doi: 10.3389/fmed.2023.1143485

20. Winichakoon P, Chaiwarith R, Liwsrisakun C, Salee P, Goonna A, Limsukon A, et al. Negative nasopharyngeal and oropharyngeal swab does not rule out COVID-19. *J Clin Microbiol*. (2020) 58:e00297-20. doi: 10.1128/JCM.00297-20

21. Ackley TW, McManus D, Topal JE, Cicali B, Shah S. A valid warning or clinical Lore: an evaluation of safety outcomes of Remdesivir in patients with impaired renal function from a multicenter matched cohort. *Antimicrob Agents Chemother*. (2020) 65:e02290–20. doi: 10.1128/aac.02290-20

22. Huang JT, Liu YJ, Wang J, Xu ZG, Yang Y, Shen F, et al. Next generation digital PCR measurement of hepatitis B virus copy number in formalin-fixed paraffin-embedded hepatocellular carcinoma tissue. *Clin Chem*. (2015) 61:290–6. doi: 10.1373/clinchem.2014.230227

23. Gupta RK, Abdul-Jawad S, McCoy LE, Mok HP, Peppa D, Salgado M, et al. HIV-1 remission following CCR5Δ32/Δ32 haematopoietic stem-cell transplantation. *Nature*. (2019) 568:244–8. doi: 10.1038/s41586-019-1027-4

24. Ahmad SU, Kiani BH, Abrar M, Jan Z, Zafar I, Ali Y, et al. A comprehensive genomic study, mutation screening, phylogenetic and statistical analysis of SARS-CoV-2 and its variant omicron among different countries. *J Infect Public Health*. (2022) 15:878–91. doi: 10.1016/j.jiph.2022.07.002

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The epidemiology of *Aedes*-borne arboviral diseases in Zhejiang, Southeast China: a 20 years population-based surveillance study

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Objective: *Aedes*-borne arboviral diseases were important public health problems in Zhejiang before the coronavirus disease 2019 (COVID-19) pandemic. This study was conducted to investigate the characteristics and change of the epidemiology of *Aedes*-borne arboviral diseases in the province.

Methods: Descriptive analyses were conducted to summarize the epidemiology of *Aedes*-borne arboviral diseases during 2003–2022.

Results: A total of 3,125 cases, including 1,968 indigenous cases, were reported during 2003–2022. Approximately three-quarters of imported cases were infected from Southeast Asia. The number of annual imported cases increased during 2013–2019 ($R^2 = 0.801$, $p = 0.004$) and peaked in 2019. When compared with 2003–2012, all prefecture-level cities witnessed an increase in the annual mean incidence of imported cases in 2013–2019 (0.11–0.42 per 100,000 population vs. 0–0.05 per 100,000 population) but a drastic decrease during 2020–2022 (0–0.03 per 100,000 population). The change in geographical distribution was similar, with 33/91 counties during 2003–2012, 86/91 during 2013–2019, and 14/91 during 2020–2022. The annual mean incidence of indigenous cases in 2013–2019 was 7.79 times that in 2003–2012 (0.44 vs. 0.06 per 100,000 population). No indigenous cases were reported between 2020–2022. Geographical extension of indigenous cases was also noted before 2020—from two counties during 2003–2012 to 44 during 2013–2019.

Conclusion: Dengue, chikungunya fever, zika disease, and yellow fever are not endemic in Zhejiang but will be important public health problems for the province in the post-COVID-19 era.

KEYWORDS

dengue, chikungunya fever, zika, China, emergence

1. Introduction

Arboviral diseases are viral diseases transmitted by arthropods, predominantly mosquitoes, sandflies, and ticks. In the past five decades, there has been an unprecedented emergence of arboviral diseases, especially dengue, chikungunya, yellow fever, and zika, raising global concerns (1). Dengue, chikungunya, yellow fever, and zika are transmitted by *Aedes* mosquitoes and are similar in clinical symptoms, geographical and temporal distribution, prevention, and control strategy. These four viruses are single-stranded positive-sense RNA, of which dengue virus (DENV), yellow fever virus, and zika virus (ZIKV) belong to the genus *Flavivirus* in the family *Flaviviridae*, whereas chikungunya virus (CHIKV) is a member of the *Alphavirus* genus in the family *Togaviridae*. The clinical manifestations of these viruses are diverse, ranging from asymptomatic infection to mild and self-limited febrile illness, permanent severe disability, congenital anomalies, and early death, with no specific treatments that are currently available. Yellow fever can be prevented with vaccines, whereas the dengue vaccine is unsatisfactory, and no vaccine is currently available for chikungunya or zika.

Dengue is the most prevalent mosquito-borne disease and causes the heaviest health burden of any arbovirus. It was listed as one of 10 threats to global health in 2019 and a neglected tropical disease by the World Health Organization. Dengue is endemic in the tropics and subtropics, and now affects over half of the world's population (2). It is estimated that there are 390 million dengue infections per year, of which 96 million manifest clinically (3). Geographically, Southeast Asia and South Asia are most devastatingly affected by dengue with the highest incidence of cases, deaths, and number of disability-adjusted life years, followed by Latin America and the Caribbean (3, 4).

Chikungunya was first identified in Tanzania in 1953 during a large outbreak of the disease. Traditionally, it was endemic in Africa and Asia, and in America since 2013 (5). Most infected people recover after some days, with some even having no clinical symptoms at all. However, some who are infected experience long-term impacts (mainly post chikungunya rheumatism), which can last for years (5). In one study, the prevalence of long-term disabilities for follow-up times of 6–12 months, 12–18 months, and > 18 months were 39.70%, 35.85%, and 28.20%, respectively (5). The disease burden from chronic CHIKV infections is significantly larger than that of acute infections (6).

Yellow fever is endemic in the tropical areas of Africa, and Central and South America (7). Through mass vaccination campaigns, the disease was successful controlled by the middle of the 20th century. However, it re-emerged in the endemic region during 2016–2018, leading to the first ever confirmed case of yellow fever in Asia in 2016 (8). The cause of the re-emergence was complicated, partly attributed to waning vaccine-derived or naturally acquired immunity, dwindling international vaccine supplies, and unsatisfactory vaccine coverage (9). It was estimated that, globally, 393.7–472.9 million people still require vaccination within at-risk districts to achieve the 80% population coverage threshold recommended by the World Health Organization. To protect at-risk populations, prevent international spread, and contain outbreaks rapidly, the Eliminate Yellow Fever Epidemics Strategy was launched by the World Health Organization in 2017 (7).

ZIKV was first isolated from rhesus macaque monkey in Uganda in 1947. Subsequently, sporadic human cases were reported in Africa and Asia. In the past decades, it gradually spread from Africa and Asia to Oceania and the Americas (10). At first, little attention was paid to zika due to its extremely low incidence and mild symptoms. However, since 2013, concerns about the disease have increased because of its dramatic increase in incidence and its association with the development of neurological diseases such as microcephaly and Guillain–Barré syndrome. The prevalence of microcephaly is approximately 3% in infants of mothers with confirmed or probable ZIKV infection during pregnancy (11), and that of ZIKV-associated Guillain–Barré syndrome is approximately 1.23% (12).

China also experienced the emergence and re-emergence of *Aedes*-borne arboviral diseases. Major dengue fever epidemics occurred in China in 2014 with 46,864 reported cases and in 2019 with 22,407 reported cases. It was estimated that the cost of dengue fever prevention and control in China in 2019 was approximately 3 billion Chinese Yuan (13). Outbreaks of indigenous chikungunya were reported in Guangzhou province in 2010, Zhejiang province in 2017, and Yunnan province in 2019. In February of 2016, the first zika case was confirmed in a Chinese traveler came from Venezuela; since then, imported cases have been identified sporadically. In the same year, yellow fever was first reported in China among 11 Chinese workers from Angola, also making the first confirmed case of yellow fever in Asia. Zhejiang province, located in the southeastern coastal area of China, has the most active economy, and the highest social mobility and population density, rendering it vulnerable to communicable diseases, especially travel-related imported disease. This study aimed to uncover the epidemiological characteristics of *Aedes*-borne arboviral diseases and their change in the past two decades in Zhejiang to provide guiding information for their control and prevention in the post-coronavirus disease 2019 (COVID-19) era.

2. Materials and methods

2.1. Data sources

Dengue cases were defined according to the Diagnostic Criteria and Principles of Management for Dengue (WS 216–2001, before 2008) (14) or Diagnostic Criteria for Dengue (WS 216–2008, after 2008) (15), or Diagnostic Criteria for Dengue (WS 216–2018, after August 2018) (16). Chikungunya fever was diagnosed according to the Diagnostic and Treatment Scheme for Chikungunya Fever (before August 2018) (17) or Diagnosis for Chikungunya Fever (WS/T 590–2018, after August 2018) (18). The Prevention and Control Scheme for ZIKV Disease (first edition, before April 2016) (19) or the Prevention and Control Scheme for ZIKV Disease (second edition, after April 2016) were used to confirm the cases of ZIKV disease (20). Yellow fever cases were defined with the Diagnosis and Treatment Scheme for Yellow Fever (before April 2016) (21) or the Prevention and Control Scheme for Yellow Fever (after April 2016) (22). All the data about *Aedes*-borne arboviral diseases in Zhejiang were collected from the Chinese National Notifiable Disease Surveillance System. Imported and indigenous cases were defined according to their epidemiological history. All data were provided anonymously without individual identifying information. Cases were recognized as imported if they were infected in places other than Zhejiang province; otherwise, they were recorded as indigenous. The annual demographic data of the counties in Zhejiang from 2004 to 2022 were collected

from the Chinese National Notifiable Disease Surveillance System. A map of Zhejiang province was downloaded from National Earth System Science Data Sharing Infrastructure (23). The data were divided into three periods in this work: period one (2003–2012), period two (2013–2019), and period three (2020–2022).

2.2. Statistical analysis

The characteristics of the cases are presented as frequencies for categorical variables and median (inter-quartile range) or mean value ± standard deviations for quantitative variables. Continuous data were compared using the student's *t*-test or analysis of variance. Categorical variables were analyzed with the chi-squared or Fisher's exact tests. A significant difference was noted if $p < 0.05$. WPS Office 2016 (Kingsoft Software Service Co., Ltd., Beijing, China), SPSS software version 17.0 (SPSS Inc., Chicago, IL, United States), and R software (version 4.1.1) were used for all the descriptive and statistical analyses.

3. Results

3.1. General overview

A total of 3,125 cases, with 3,124 symptomatic infections and one asymptomatic ZIKV infection, were reported during 2003–2022, with no deaths or severe cases reported. In those cases, 1,968 were indigenous, 1,081 were infected overseas, 75 were infected in other provinces in the Chinese mainland, and one imported case's infection source was unidentified. Dengue was responsible for the overwhelming majority of *Aedes*-borne arboviral diseases in Zhejiang, with 1,965 indigenous and 1,125 imported cases, followed by chikungunya fever with three indigenous and 26 imported cases. No indigenous and six imported ZIKV infections were reported. No yellow fever was reported in Zhejiang during 2003–2022. Eight provinces in mainland China exported cases to Zhejiang, with 74 cases in 2013–2019 and one case in 2020. Yunnan and Guangzhou accounted for the majority of cases reported, as 41.33% and 40% of domestic imported cases were from those two provinces, respectively. According to the standard country or area codes for statistical use (M49), other than Europe, all five regions exported cases to Zhejiang, with Asia accounting for the vast majority (Table 1). At the sub-region level, cases from Southeast Asia ranked first in all three periods, and the proportion increased significantly over time ($Z = 3.063$, $p = 0.002$). Southern Asia was the second-most frequently reported infection source, but its proportion gradually decreased over time ($Z = -3.152$, $p = 0.002$). In total, 37 countries from the four regions exported cases to Zhejiang, and the five countries that exported the most cases were Cambodia (36.51% of imported cases), Thailand (11.51%), Vietnam (6.57%), the Philippines (6.14%), and India (5.71%), accounting for 71.05% of the overseas imported cases. The three countries that exported the most cases to Zhejiang were Cambodia (20% of imported cases), Bangladesh (8.57%), and Singapore (8.57%) during 2003–2012; Cambodia (38.01%), Thailand (11.85%), and Vietnam (6.40%) during 2013–2019; and the Philippines (21.74%), Cambodia (17.39%), and Singapore (17.39%) during 2020–2022. By year, for the imported cases from Cambodia, 80.33% (339/422) were reported in 2019. By disease, South-eastern Asia was the most common origin of overseas imported dengue and chikungunya in Zhejiang, accounting for 81.60% and 53.85% of cases, respectively (Figure 1). Southern Asia was also an important origin for chikungunya, as 38.46% of overseas imported cases were from this region. For zika, four out of six imported cases were from the Polynesian island nation of Samoa.

TABLE 1 The infection source of the imported *Aedes*-borne arboviral diseases in Zhejiang during 2003–2022.

Infection source		2003–2012 (*n*/%)	2013–2019 (*n*/%)	2020–2022 (*n*/%)	2003–2022 (*n*/%)
Asia	Southeast Asia	44 (62.86)	806 (75.82)	21 (91.30)	871 (75.35)
	South Asia	16 (22.86)	131 (12.32)	0 (0)	147 (12.72)
	West Asia	0 (0)	1 (0.09)	0 (0)	1 (0.087)
Africa	Sub-Saharan Africa	4 (5.71)	35 (3.29)	1 (4.35)	40 (3.46)
Americas	Latin America and the Caribbean	6 (8.57)	8 (0.75)	0 (0)	14 (1.21)
Oceania	Australia and New Zealand	0 (0)	1 (0.09)	0 (0)	1 (0.09)
	Melanesia	0 (0)	3 (0.28)	0 (0)	3 (0.26)
	Polynesia	0 (0)	4 (0.38)	0 (0)	4 (0.35)
Other provinces in China		0 (0)	74 (6.96)	1 (4.35)	75 (6.49)

3.2. Temporal distribution

Except in 2021, *Aedes*-borne infectious diseases were reported every year between 2003 and 2022 (Figure 2). The number of annual imported cases ranged from 2 to 10 during 2003–2012, with an annual mean incidence of 0.01 per 100,000 population. As a whole, the number of imported cases increased yearly during 2013–2019 [$R^2 = 0.801$, $\log(n) = 2.744 + 0.431$ (year-2012), $F = 25.2$, $p = 0.004$]. The annual imported case number exceeded 50 after 2016, and exceeded 100 after 2018 during this period. The annual mean incidence of imported cases during 2013–2019 was 0.27 per 100,000 population, 19.7 times that in 2003–2012. Since 2020, the annual imported case number drastically decreased due to the implementation of prevention and control measures to contain the spread of COVID-19. As a result, the annual mean incidence decreased by 95.67% and 14.85% in the period of 2020–2022 compared to that of 2013–2019 and 2003–2012, respectively. Approximately 70% of imported cases during 2020–2022 were reported between January and March 2020, before the implementation of the immigration control measures in China. Indigenous cases were reported in 2004, 2009, and 2014–2019; except for 2004, the annual case number in those years exceeded 50. The highest annual case number was recorded in 2017, with a total of 1,153 indigenous cases identified. The annual mean incidence of indigenous cases in 2013–2019 was 7.79 times that in 2003–2012 (0.44 vs. 0.06 per 100,000 population).

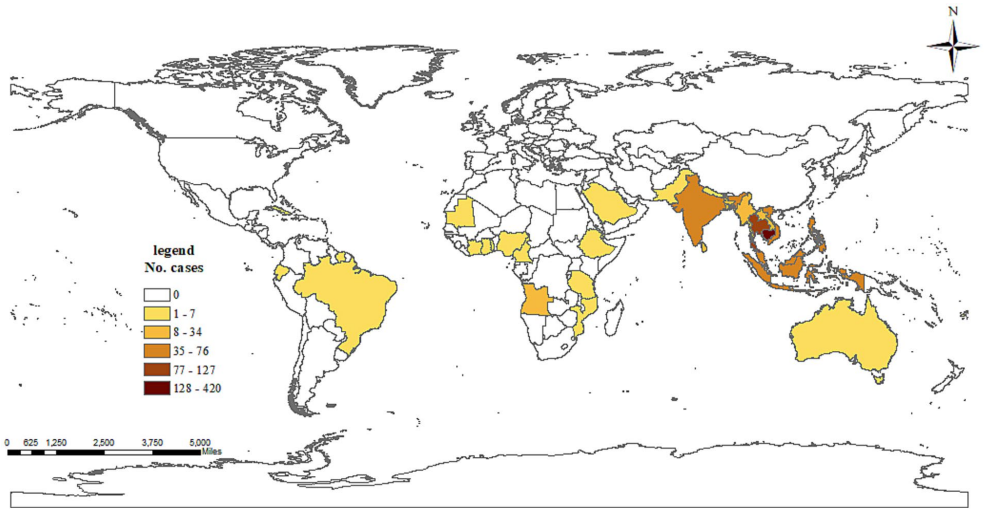

FIGURE 1
Number of dengue cases exported to Zhejiang, China, by country of origin during 2003–2022.

Imported cases were reported year-round, with a peak (70.70% of all notifications) between June and October (Figure 2). No significant difference in season distribution was noted between 2003–2012 and 2013–2019 ($\chi^2 = 0.841$, $p = 0.840$), in which the monthly peak was noted between July and October. During 2020–2022, more than half of the imported cases (52.17% of all notifications) were reported in January 2020. Indigenous cases were only reported in the months from July to November, and 59.76% cases were reported in September. The monthly distributions in 2003–2012 and 2013–2019 were significantly different ($\chi^2 = 27.377$, $p < 0.001$). No indigenous cases were identified in November during 2003–2012, the proportion of cases in September was higher than that in 2013–2019 (68.5% vs. 58.5%), and the proportion in October was lower (1.5% vs. 10.7%).

3.3. Spatial distribution

All 11 prefecture-level cities reported *Aedes*-borne infectious diseases in Zhejiang between 2003 and 2022, with Hangzhou (0.86 per 100,000 population), Jinhua (0.34 per 100,000 population), and Taizhou (0.22 per 100,000 population) reporting the highest annual mean incidence. The three prefecture-level cities with the top annual mean incidence were Jinhua (0.41 per 100,000 population), Ningbo (0.24 per 100,000 population), and Lishui (0.16 per 100,000 population) during 2003–2012; Hangzhou (2.33 per 100,000 population), Taizhou (0.60 per 100,000 population), and Wenzhou (0.53 per 100,000 population) during 2013–2019; and Hangzhou (0.03 per 100,000 population), Taizhou (0.03 per 100,000 population), and Jiaxing (0.02 per 100,000 population) during 2020–2022 (Figure 3). Nine out of the 11 prefecture-level cities identified *Aedes*-borne infectious diseases between 2003 and 2012, with no cases reported in Jiaxing or Zhoushan. All 11 prefecture-level cities reported *Aedes*-borne infectious diseases during 2013–2019. In the period of 2020–2022, only five prefecture-level cities (Hangzhou, Taizhou, Jiaxing, Lishui, and Jinhua) reported imported cases, and no indigenous cases were identified.

Imported cases of *Aedes*-borne infectious diseases were reported in all 11 prefecture-level cities in Zhejiang during 2003–2022. The three prefecture-level cities with the highest annual mean incidence of imported cases were Hangzhou (0.17 per 100,000 population), Jinhua (0.15 per 100,000 population), and Taizhou (0.13 per 100,000 population). The three prefecture-level cities with the highest annual mean incidence in the three study periods were notably different: Lishui (0.05 per 100,000 population), Ningbo (0.02 per 100,000 population), and Huzhou (0.02 per 100,000 population) during 2003–2012; Hangzhou (0.42 per 100,000 population), Jinhua (0.41 per 100,000 population), and Taizhou (0.34 per 100,000 population) during 2013–2019; and Hangzhou (0.03 per 100,000 population), Taizhou (0.03 per 100,000 population), and Jiaxing (0.02 per 100,000 population) during 2020–2022. All the prefecture-level cities witnessed an increase in the annual mean incidence of imported cases in 2013–2019 compared to 2003–2012, which was most notable in Hangzhou (0.42 vs. 0.02 per 100,000 population). In contrast, in 2020–2022, all the prefecture-level cities had a drastic decrease in the annual mean incidence of imported cases. The number of prefecture-level cities that reported imported cases was nine during 2003–2012, 11 during 2013–2019, and five during 2020–2022. The proportion of counties that reported imported cases was 33/91 during 2003–2012, 86/91 during 2013–2019, and 14/91 during 2020–2022. No imported cases were reported in Dongtou, Pan'an, Shengsi, Suichang, or Xihufengjingmingsheng across the whole study period (Figure 4). The top-five counties with the highest annual mean incidence of imported cases were totally different during the different periods: Qingtian (0.18 per 100,000 population), Beilun (0.10 per 100,000 population), Haishu (0.08 per 100,000 population), Liandu (0.08 per 100,000 population), and Wencheng (0.07 per 100,000 population) during 2003–2012; Yiwu (1.05 per 100,000 population), Binjiang (0.85 per 100,000 population), Xianju (0.78 per 100,000 population), Yuhang (0.72 per 100,000 population), and Cangnan (0.71 per 100,000 population) during 2013–2019; and Yuhuan (0.10 per 100,000 population), Chunan (0.10 per 100,000 population), Xihu (0.09 per 100,000 population), Jinyun (0.08 per 100,000 population) and Fuyang (0.08 per 100,000 population) during 2020–2022.

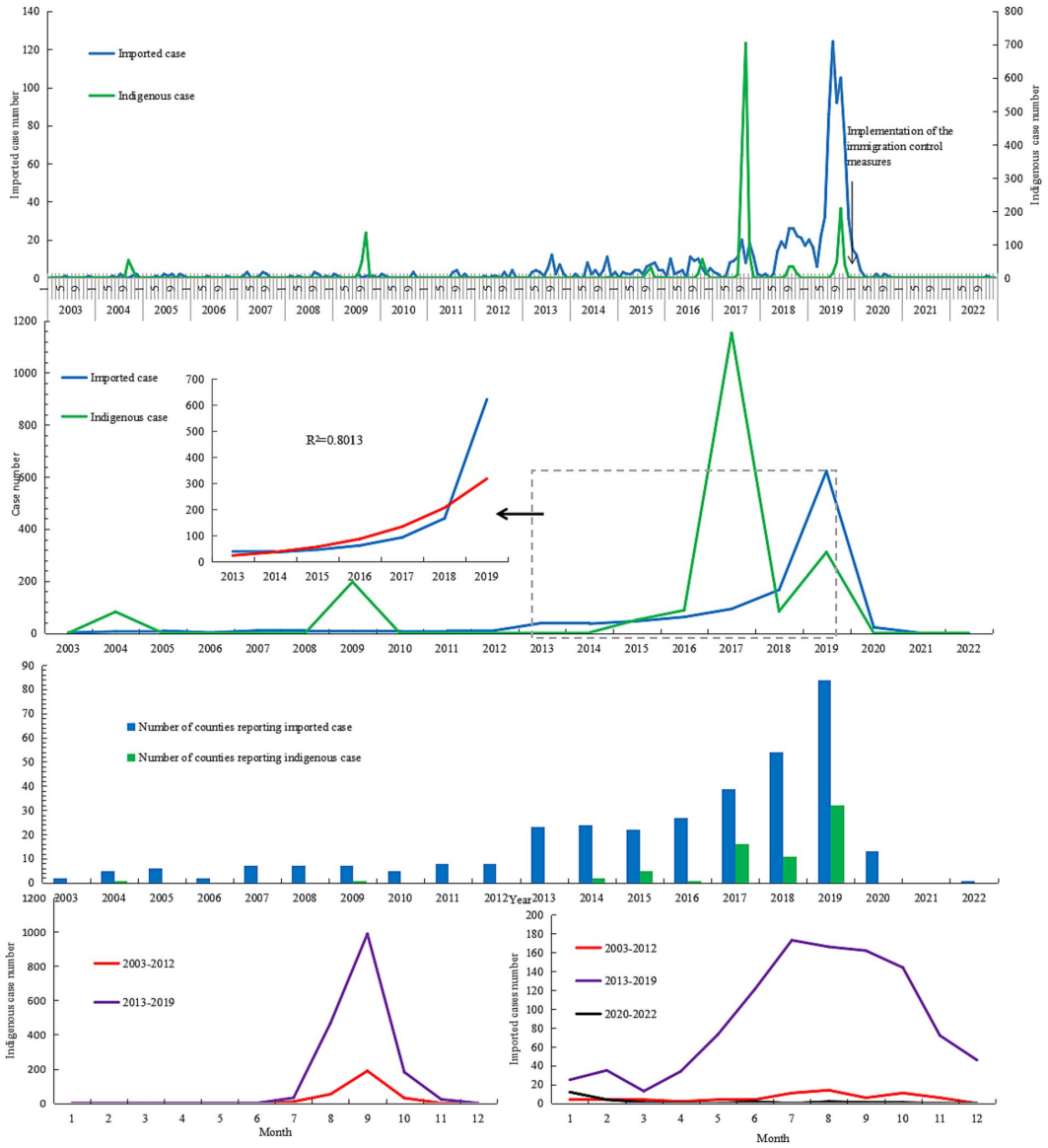

FIGURE 2
Temporal distribution of the *Aedes*-borne arboviral diseases in Zhejiang during 2003–2022.

Indigenous cases were recorded in 9/11 prefecture-level cities during 2003–2022. Hangzhou (0.69 per 100,000 population) reported the highest annual mean incidence of indigenous cases, followed by Jinhua (0.19 per 100,000 population) and Wenzhou (0.11 per 100,000 population). No indigenous cases were reported in Huzhou or Lishui. In the period of 2003–2012, indigenous cases were only reported in two counties: Cixi in Ningbo and Yiwu in Jinhua. The annual mean incidence during 2003–2012 was 0.13 and 0.40 per 100,000 population for these two prefecture-level cities, and 0.61 and 1.99 per 100,000 population for these two counties, respectively (Figure 4). During 2013–2019, 9/11 prefecture-level cities and 44/91 counties confirmed indigenous cases. The three cities with the highest annual mean incidence of indigenous cases during 2013–2019 were Hangzhou (1.91 per 100,000 population), Wenzhou (0.31 per 100,000 population), and Taizhou (0.26 per 100,000 population). The five counties with the highest annual mean incidence were Gongshu (8.04 per 100,000 population), Xihufengjingmingsheng (5.60 per 100,000 population), Shangcheng (4.29 per 100,000 population), Huangyan (1.95 per 100,000 population), and Xihu (1.84 per 100,000 population). Other than Jinhua, Huzhou, and Lishui, all the prefecture-level cities had a higher annual mean incidence of indigenous cases in the period of 2013–2019.

3.4. Demographic characteristics

There were 1,716 male and 1,409 female cases during 2003–2022, with a male:female gender ratio of 1.218:1. Males outnumbered females for imported cases, but the situation was reversed for indigenous cases. Compared with indigenous cases, there were significantly more male than female imported cases in both 2003–2012 and 2013–2019 ($p < 0.001$). No significant differences in the

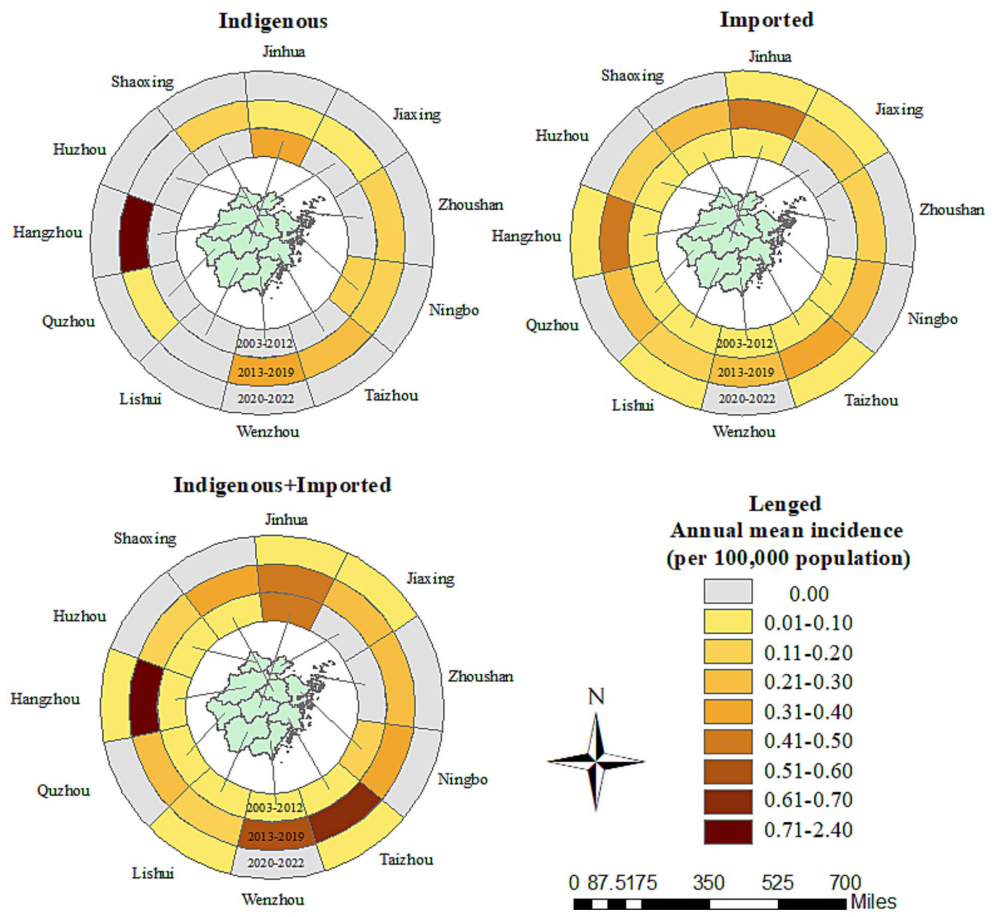

FIGURE 3
Prefecture-level city distribution of the *Aedes*-borne arboviral diseases in Zhejiang during 2003–2022.

gender distributions for imported cases were noted between the three periods ($\chi^2 = 0.150$, $p = 0.928$). For indigenous cases, the male proportion in 2013–2019 was significantly higher than that in 2003–2012 ($\chi^2 = 14.441$, $p < 0.001$).

The ages of the cases ranged from 9 months to 96 years, with a mean of 44.78 ± 17.103 years. On average, indigenous cases were older than the imported ones, regardless of gender and period ($p < 0.001$, Figure 5). As a whole, female cases were significantly older than male cases in 2013–2019 ($t = -3.271$, $p = 0.0011$), whereas male imported cases were significantly older than female imported cases during 2003–2002 ($t = 2.040$, $p = 0.042$). No significant difference in age was noted for cases of different genders from different periods and infectious origins ($p > 0.05$, Figure 6). For the female indigenous cases, those from 2013–2019 were significantly older than those from 2003–2012 ($t = -2.283$, $p = 0.023$, Figure 7). For the imported cases from different periods, a significant difference in age distribution was identified ($F = 3.188$, $p = 0.042$), whereas no difference was noted when subdivided into different genders ($p > 0.05$).

Occupational information was available for 2,983 cases, with businessperson (20.52%), farmer (15.59%), retiree (14.58%), worker (13.95%), and housework or unemployment (12.54%) as the five most frequently mentioned occupations (Figure 8). Overall, the occupation distribution was different for cases of different infectious origins ($\chi^2 = 304.128$, $p < 0.0001$), with indigenous cases mainly reporting occupations of retiree (21.17%), farmer (15.17%), housework or unemployment (14.20%), worker (13.67%), and businessperson (13.18%); and imported cases reporting occupations of businessperson (32.77%), farmer (6.29%), worker (14.41%), and housework or unemployment (9.76%). For the cases from 2003–2012 and 2013–2019, the occupation distribution was also significantly different ($\chi^2 = 503.889$, $p < 0.001$). The top-four occupations in 2003–2012 were farmer (61.28%), businessperson (13.16%), student (8.27%), and worker (6.02%), whereas the five most frequently reported occupations in 2013–2019 were businessperson (21.22%), retiree (15.99%), worker (14.81%), housework or unemployment (13.58%), and farmer (11.13%). Significant differences were also noted when cases were further subdivided into different infectious origins ($p < 0.05$). For the indigenous cases, in 2003–2012, the occupations of most reports were mainly farmer (82.23%) and student (7.61%), whereas retiree (23.61%), housework or unemployment (15.82%), worker (15.04%), and businessperson (14.50%) were the four most frequently reported occupations in 2013–2019. For the imported cases, the most common occupations were businessperson (44.93%) and worker (17.39%) for cases during 2003–2012; whereas businessperson (32.16%), farmer (17.45%), and worker (14.42%) were the most common in 2013–2019.

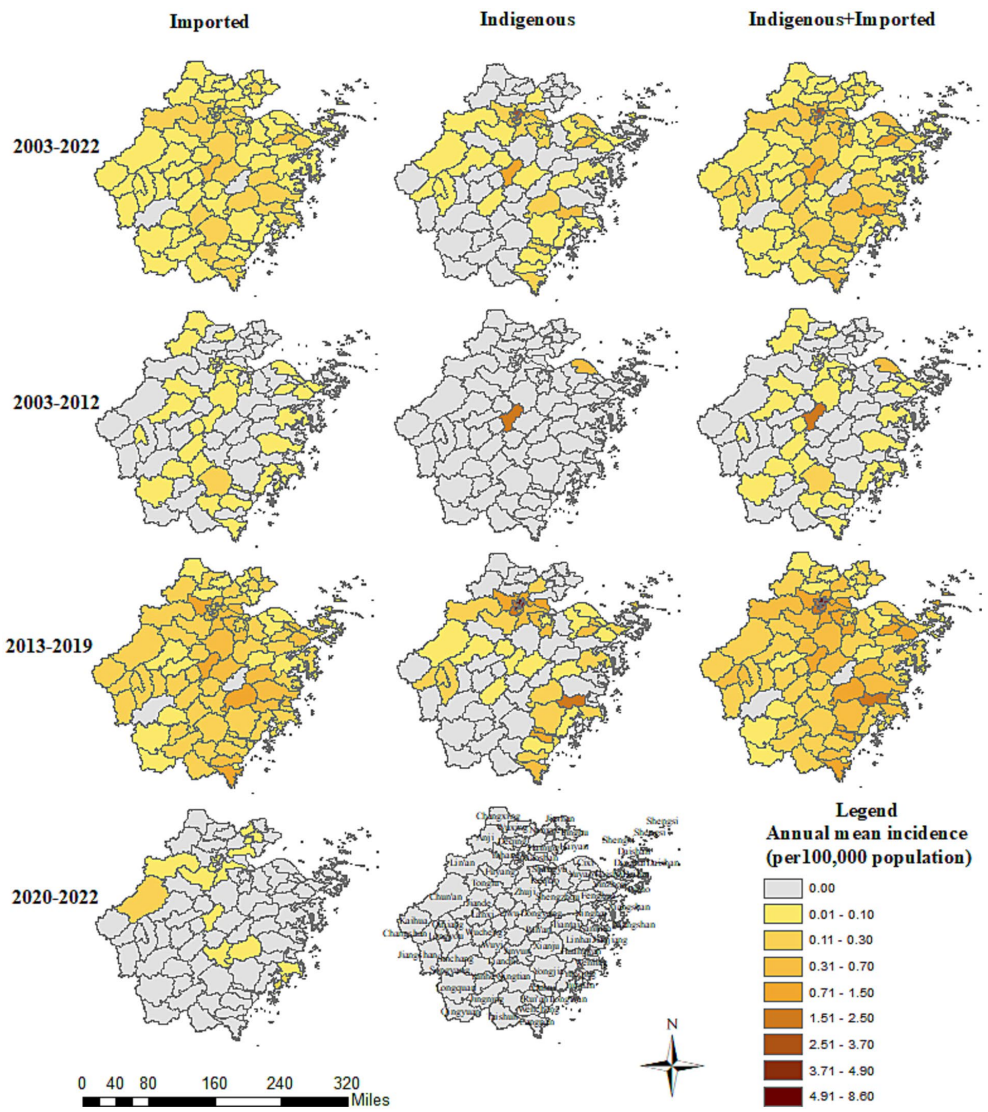

FIGURE 4
County distribution of the *Aedes*-borne arboviral diseases in Zhejiang during 2003–2022.

4. Discussion

The past decades have seen a global increase in the frequency, magnitude, and geographical expansion of *Aedes*-borne arboviral diseases. The cause of their emergence and re-emergence is complicated and includes many aspects, such as climate change, globalization, virus evolution, urbanization, insufficient mosquito control, and virus and vector adoption. The primary vector for *Aedes*-borne arboviral diseases is *Aedes aegypti*, which is concentrated in the tropical and subtropical parts of the world and has demonstrated suitability foci in 188 countries/territories (24). *Aedes albopictus* is the second-most important vector for DENV, ZIKV, and CHIKV. Its range extends from the tropics into the temperate parts of the world, with suitability foci in 197 countries/territories. In mainland China, the geographical distribution of *A. aegypti* is limited, as it is only found near the border or in coastal areas of Yunnan, Guangxi, Guangdong, and Hainan provinces (25). Vector surveillance has indicated that the geographic distribution of *A. aegypti* expanded in Yunnan province but contracted in Guangxi, Guangdong, and Hainan provinces in recent years (25). *A. albopictus* has been found throughout tropical, subtropical, and temperate zones in China, spanning most of the area from Hainan province to Liaoning province, and is the dominant mosquito species in residential areas. In Zhejiang province, *A. albopictus* is the primary vector of *Aedes*-borne arboviral diseases, and *A. aegypti* is not found. A study conducted in mainland China indicated that *A. aegypti* has its own unique ecological niches, and the influence factors for its spatial distribution include annual mean temperature, isothermality, temperature seasonality, rural residential land, and rivers (26).

Globally, dengue is the most prevalent and widely distributed *Aedes*-borne arboviral disease, as 111 countries/territories had reported the autochthonous transmission of DENV between 1952 and 2017 (24). In this period, the overall numbers of countries/territories reporting autochthonous occurrences of CHIKV, ZIKV, and yellow fever virus was 106, 85, and 43, respectively (24). Other than malaria, dengue accounted for the overwhelming majority of imported

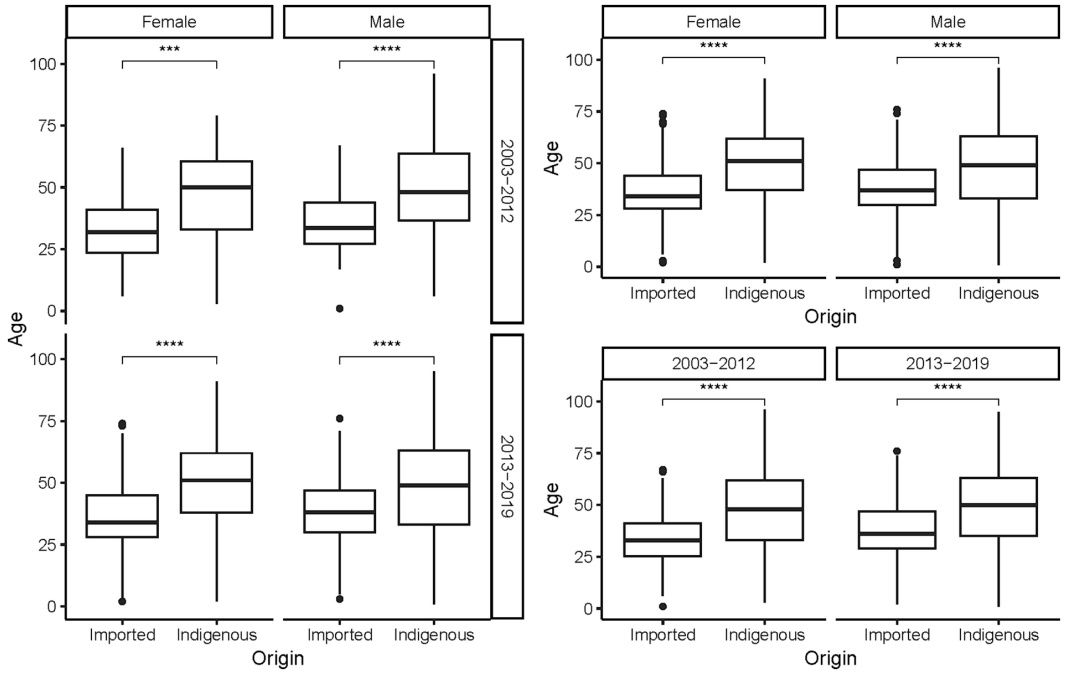

FIGURE 5
Age distribution of the *Aedes*-borne arboviral diseases with different infectious origins by different genders and periods. Ns $p > 0.05$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, and $****p \leq 0.0001$.

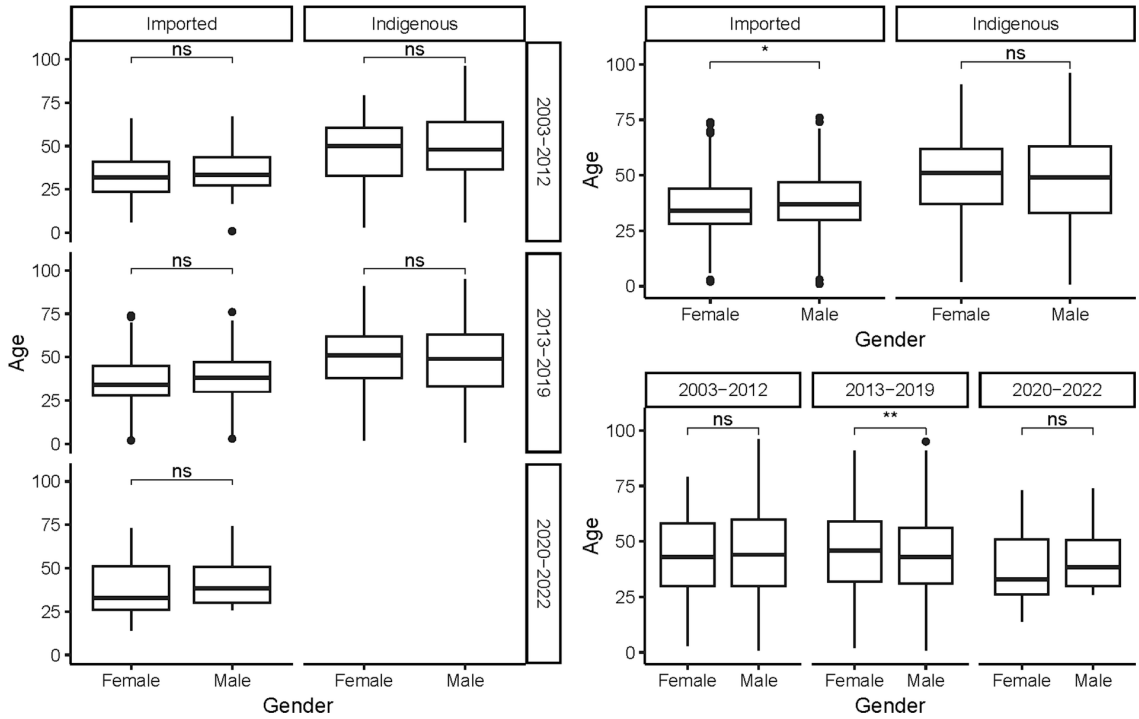

FIGURE 6
Age distribution of the *Aedes*-borne arboviral diseases of different genders by different infectious origins and periods. Ns $p > 0.05$, $*p \leq 0.05$, $**p \leq 0.01$, $***p \leq 0.001$, and $****p \leq 0.0001$.

infectious disease in mainland China (27), and the ratio of indigenous to imported cases was approximately 6.43:1 (25). Likewise, other than malaria, dengue was the most imported infectious disease in Zhejiang (28), but the ratio of indigenous to imported cases (1.75:1) was significantly lower than that in mainland China (25). The remarkably low indigenous-to-imported case ratio was attributed to the

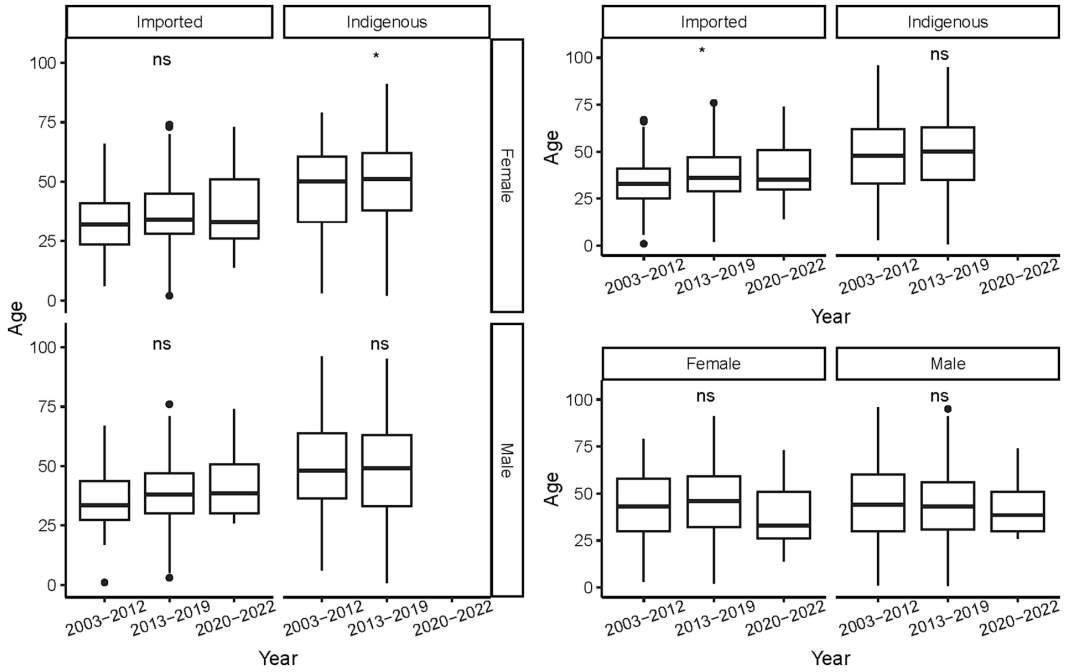

FIGURE 7
Age distribution of the *Aedes*-borne arboviral diseases of different periods by different genders and infectious origins. Ns $p > 0.05$, *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$, and ****$p \leq 0.0001$.

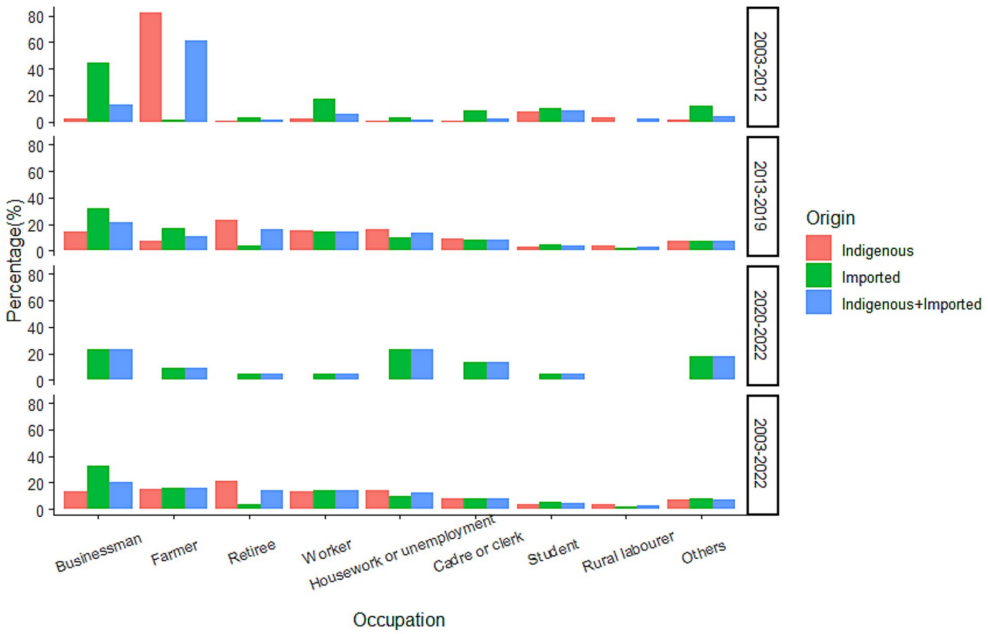

FIGURE 8
Occupational distribution of the *Aedes*-borne arboviral diseases in different periods.

advantages of early diagnose, social mobilization, health education, vector control, and quick emergency response that characterize disease control and prevention in Zhejiang (29). More than four-fifths of overseas imported cases in the province were infected in Southeast Asia. Globally, Southeast Asia was also a major source of imported dengue (30). Thailand, Myanmar, Indonesia, and the Philippines were the top-four countries from which dengue was imported, whereas the four countries that exported the most dengue cases to Zhejiang were Cambodia, Thailand, Vietnam, and the Philippines. A total of 337 cases were infected in Cambodia in 2019, accounting for 59.86% and 32.13% of overseas imported dengue cases in 2019 and 2003–2022, respectively. Cambodia was also the most common origin of overseas

imported dengue cases in mainland China in 2019, accounting for 55.9% of cases (31). To improve and strengthen cooperation in culture and tourism, Cambodia and China designated 2019 as the "China–Cambodia culture and tourism year," and a variety of activities were jointly organized to celebrate the year. There were 19 airlines operating some 500 direct flights per week between the two nations that year. Thus, in the first 10 months of 2019, Chinese tourists topped the list of foreign visitors coming to Cambodia at 2.02 million—a 24.4% year-on-year increase, accounting for 38% of all of Cambodia's international tourists. In same year, Cambodia had endured the most serious dengue outbreak in the past few years (32). All of these factors led to a significant increase in the number of imported dengue cases in China and specifically Zhejiang from Cambodia.

Although chikungunya was the second-most frequently reported *Aedes*-borne arboviral disease in Zhejiang, the number of cases was obviously lower than that of dengue. All three indigenous cases were reported in Quzhou in 2017, representing the second autochthonous CHIKV transmission in mainland China (33). The indigenous-to-imported case ratio in Zhejiang was also significantly lower than that of mainland China (0.12:1 vs. 4.52:1) but similar to that of Taiwan (33, 34). Southeast and South Asia were the largest sources of chikungunya in Zhejiang; Thailand, Bangladesh, and Myanmar were the countries that exported the most cases, similar to the situation for the whole nation (33). Southeast and South Asia were also a major source of chikungunya in Japan, but the top-three countries were Indonesia, India, and the Philippines (35). The imported zika cases in Zhejiang were mainly reported from a tour group traveling to Fiji and Samoa, whereas approximately two-thirds of the imported zika cases in mainland China were from Venezuela (36). No indigenous zika cases were reported in mainland China until now. However, in a retrospective study, ZIKV was isolated from a local man with a fever of unknown origin residing in Ruili, a China-Myanmar border city, Yunnan province, Southwest China, who did not travel overseas (37). In another study conducted in Guangxi province, Southwest China, healthy individuals with no overseas experience and negative for DENV and West Nile virus were found to be serologically positive for ZIKV and had micro-neutralization antibodies (38). Pigs, chickens, and sheep were also found to be seropositive for ZIKV in Guizhou province, Southwest China (39). Besides, strains of ZIKV were isolated from wild *Anopheles sinensis*, *Culex tritaeniorhynchus*, *Culex quinquefasciatus,* and *Armigeres subalbatus* in Southern China (40–42). A study of vector competence for ZIKV in China indicated that *A. aegypti* had the highest transmissibility, followed by *A. albopictus*, whereas *C. quinquefasciatus* had no transmission ability (43). Another study conducted in China indicated that *A. subalbatus* was a potential vector for ZIKV (44). All the above-mentioned studies suggested that there might be restricted autochthonous ZIKV transmission in Southern China, but further research was needed.

Coupled with the above-mentioned global emergence and re-emergence, Zhejiang witnessed an increase in the frequency, magnitude, and geographical distribution of *Aedes*-borne arboviral diseases, especial for dengue, only a few years before the COVID-19 pandemic. In the 3 years after the identification of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), no indigenous cases of *Aedes*-borne arboviral diseases were reported in Zhejiang, and the number of imported cases was drastically decreased, especially after the execution of immigration control measures to control and prevent the import of SARS-CoV-2. The results proved that Zhejiang is not an endemic province for dengue, zika, chikungunya, or yellow fever, and that the identified autochthonous transmissions were due to imported infected. Travelers played a key role in the introduction of viruses for *Aedes*-borne arboviral diseases in non-endemic areas. It was confirmed that passenger flows *via* airline travel from countries experiencing *Aedes*-borne arboviral diseases epidemics were positively correlated to the number of imported cases in China, Korea, and the United States (45–47). A 10% increase in the volume of air travelers from dengue-endemic countries was associated with a 5.9% increase in detected cases of imported dengue in China, and a 10% increase from chikungunya-endemic countries was associated with a 5.2% increase in imported chikungunya in the United States. A study in two dengue-high-risk areas of China indicated that one of the most important influence factors for dengue fever occurrence was the number of imported cases (48). Non-pharmaceutical interventions (NPIs) to mitigate the transmission of SARS-CoV-2 had different effects on vector-borne communicable diseases in different regions. For endemic diseases, the imposition of NPIs was related to increased of case numbers, such as tick-borne encephalitis in Germany, Ross River virus in Australia, and dengue fever in Peru (49–51). In contrast, for non-endemic vector-borne communicable diseases, NPIs were associated with a decline in case number, such as dengue and malaria cases in Australia and Germany, and dengue in China (49, 50, 52). The drop in the international passenger flight was believed to be the main reason for the decline of vector-bore communicable disease in non-endemic regions.

The determinants for the occurrence of vector-borne disease are complicated and numerous, including the presence and abundance of vectors, ecoclimatic conditions, the density of the human population, access of vectors to humans, and the underlying disease immunity of the population (53). As a non-endemic province, the introduction of the virus was the primary determinant for its transmission in Zhejiang. Regions with a higher frequency of overseas exchange and cooperation, larger population mobility, and denser population were at greater risk of *Aedes*-borne arboviral diseases in Zhejiang. Males who were 20–50 years of age, more physically active, and were more likely to travel overseas dominated the imported cases of the province, similar to the situation in mainland China and Korea (26, 47). Compared with imported cases, indigenous cases were older and more likely to be female on average, whereas in mainland China indigenous cases were younger than those in Zhejiang (54). The distribution of occupation, both for imported and indigenous cases, was similar between mainland China and Zhejiang province (54).

5. Conclusion

Dengue, chikungunya, zika and yellow fever were not endemic in Zhejiang province, and Southeast Asia was the major source of the imported cases. Before the COVID-19 pandemic, Zhejiang experienced a significant increase in the case number and an extension of the geographical distribution of *Aedes*-borne arboviral diseases, including imported and indigenous cases. Following the implementation of the NPIs to mitigate the transmission of SARS-CoV-2, only a few imported cases were reported during 2020–2022, and no indigenous cases were confirmed. In the post-COVID-19 era, with the recovery in international population mobility and global trade, there will be a worldwide emergence and re-emergence of

Aedes-borne arboviral diseases, and Zhejiang will witness a fast rise in case number, both imported and indigenous, and an extension in the geographical distribution of the diseases. Therefore, intensive surveillance, professional training, health education, vector control, and social mobilization are highly needed.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the Zhejiang Provincial Center for Disease Control and Prevention (No. 2020-021). The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because as an important part of ongoing public health surveillance, the collection and analysis of data on *Aedes*-borne arboviral diseases by public health workers in charge of risk assessment and policy proposals are exempt from written informed consent. It is authorized by the Law of the People's Republic of China on the Prevention and Treatment of Infectious Diseases. All of the collected data were supplied anonymously and no individual identifying information was provided.

Author contributions

JR: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. ZC: Data curation, Supervision, Writing – original draft. FL: Data curation, Supervision, Writing – original draft. YL: Data curation, Investigation, Writing – original draft. EC: Data curation, Investigation, Writing – original draft. XS: Investigation, Writing – original draft. SG: Investigation, Writing – original draft. RZ: Investigation, Writing – original draft. ZW: Conceptualization, Resources, Supervision, Writing – original draft. JS: Conceptualization, Resources, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Wilder-Smith A, Gubler DJ, Weaver SC, Monath TP, Heymann DL, Scott TW. Epidemic arboviral diseases: priorities for research and public health. *Lancet Infect Dis*. (2017) 17:e101–6. doi: 10.1016/S1473-3099(16)30518-7

2. Messina JP, Brady OJ, Golding N, Kraemer MUG, Wint GRW, Ray SE, et al. The current and future global distribution and population at risk of dengue. *Nat Microbiol*. (2019) 4:1508–15. doi: 10.1038/s41564-019-0476-8

3. Bhatt S, Gething PW, Brady OJ, Messina JP, Farlow AW, Moyes CL, et al. The global distribution and burden of dengue. *Nature*. (2013) 496:504–7. doi: 10.1038/nature12060

4. Yang X, Quam MBM, Zhang T, Sang S. Global burden for dengue and the evolving pattern in the past 30 years. *J Travel Med*. (2021) 28:taab146. doi: 10.1093/jtm/taab146

5. Noor FM, Hossain MB, Islam QT. Prevalence of and risk factors for long-term disabilities following chikungunya virus disease: a meta-analysis. *Travel Med Infect Dis*. (2020) 35:101618. doi: 10.1016/j.tmaid.2020.101618

6. Puntasecca CJ, King CH, LaBeaud AD. Measuring the global burden of chikungunya and Zika viruses: a systematic review. *PLoS Negl Trop Dis*. (2021) 15:e9055. doi: 10.1371/journal.pntd.0009055

7. Eliminate yellow fever epidemics (EYE): a global strategy, 2017–2026. *Wkly Epidemiol Rec*. (2017) 92:193–204.

8. Barrett ADT. The reemergence of yellow fever. *Science*. (2018) 361:847–8. doi: 10.1126/science.aau8225

9. Shearer FM, Moyes CL, Pigott DM, Brady OJ, Marinho F, Deshpande A, et al. Global yellow fever vaccination coverage from 1970 to 2016: an adjusted retrospective analysis. *Lancet Infect Dis*. (2017) 17:1209–17. doi: 10.1016/S1473-3099(17)30419-X

10. Agarwal A, Chaurasia D. The expanding arms of Zika virus: an updated review with recent Indian outbreaks. *Rev Med Virol*. (2021) 31:1–9. doi: 10.1002/rmv.2145

11. Martins MM, Alves da Cunha AJL, Robaina JR, Raymundo CE, Barbosa AP, Medronho RA. Fetal, neonatal, and infant outcomes associated with maternal Zika virus infection during pregnancy: a systematic review and meta-analysis. *PLoS One*. (2021) 16:e246643. doi: 10.1371/journal.pone.0246643

12. Barbi L, Coelho AVC, Alencar LCA, Crovella S. Prevalence of Guillain–Barré syndrome among Zika virus infected cases: a systematic review and meta-analysis. *Braz J Infect Dis*. (2018) 22:137–41. doi: 10.1016/j.bjid.2018.02.005

13. Xu M, Chang N, Tu T, Sun J, Jiang J, Xia Y, et al. Economic burden of dengue fever in China: a retrospective research study. *PLoS Negl Trop Dis*. (2022) 16:e0010360. doi: 10.1371/journal.pntd.0010360

14. Chinese Ministry of Health. The diagnostic criteria and principles of management for dengue (WS 216–2001). Available at: https://std.samr.gov.cn/hb/search/stdHBDetailed?id=8B1827F26727BB19E05397BE0A0AB44A. (2001). (Accessed March 1, 2023)

15. Chinese Ministry of Health. Diagnostic criteria for dengue (WS 216–2008). Available at: https://std.samr.gov.cn/hb/search/stdHBDetailed?id=8B1827F22E21BB19E05397BE0A0AB44A. (2008). (Accessed March 1, 2023)

16. Chinese National Health and Family Planning Commission. Diagnostic criteria for dengue (WS 216–2018). Available at: https://std.samr.gov.cn/hb/search/stdHBDetailed?id=8B1827F255FEBB19E05397BE0A0AB44A. (2018). (Accessed March 1, 2023)

17. Chinese Center for Disease Control and Prevention. The diagnostic and treatment scheme for chikungunya fever. Available at: https://www.chinacdc.cn/jkzt/crb/qt/jkkyr/. (2018). (Accessed March 1, 2023)

18. Chinese National Health and Family Planning Commission. Diagnosis for chikungunya fever (WS/T 590–2018). Available at: https://std.samr.gov.cn/hb/search/stdHBDetailed?id=8B1827F255FCBB19E05397BE0A0AB44A. (2018). (Accessed March 1, 2023)

19. Chinese National Health and Family Planning Commission. The prevention and control scheme for ZIKV disease (first edition). Available at: http://www.nhc.gov.cn/jkj/s3577/201602/97bc31cc1767485290529a281d11c901.shtml. (2003). (Accessed March 1, 2023)

20. Chinese National Health and Family Planning Commission. The prevention and control scheme for ZIKV disease (second edition). Available at: http://www.nhc.gov.cn/jkj/s3577/201604/a2481a0193ed4da9ac8c23f34d2fe8c7.shtml. (2003). (Accessed March 1, 2023)

21. Chinese Ministry of Health. Diagnosis and treatment scheme for yellow fever. Available at: http://www.nhc.gov.cn/bgt/pw10810/200807/24a950fca3b94ea4a5568aa934fdedda.shtml. (2008). (Accessed March 1, 2023)

22. Chinese National Health and Family Planning Commission. The prevention and control scheme for yellow fever. Available at: http://www.nhc.gov.cn/jkj/s3577/201604/328d68d317d647e086c4b0000d2507da.shtml. (2016). (Accessed March 1, 2023)

23. Nanjing Institute of Geography and Limnology, Chinese Academy of Sciences. The 1:100,000 county boundaries map of Zhejiang. Available at: http://www.geodata.cn/data/datadetails.html?dataguid=258898130861140&docid=6835. (2015). (Accessed March 1, 2023)

24. Leta S, Beyene TJ, De Clercq EM, Amenu K, Kraemer MUG, Revie CW. Global risk mapping for major diseases transmitted by *Aedes aegypti* and *Aedes albopictus*. *Int J Infect Dis*. (2018) 67:25–35. doi: 10.1016/j.ijid.2017.11.026

25. Yue Y, Liu Q, Liu X, Zhao N, Yin W. Dengue fever in mainland China, 2005–2020: a descriptive analysis of dengue cases and *Aedes* data. *Int J Environ Res Public Health*. 19:3910. doi: 10.3390/ijerph19073910

26. Wang T, Fan Z, Ji Y, Chen J, Zhao G, Zhang W, et al. Mapping the distributions of mosquitoes and mosquito-borne arboviruses in China. *Viruses*. (2022) 14:691. doi: 10.3390/v14040691

27. Wang Y, Wang X, Liu X, Ren R, Zhou L, Li C, et al. Epidemiology of imported infectious diseases, China, 2005–2016. *Emerg Infect Dis*. (2018) 25:33–41. doi: 10.3201/eid2501.180178

28. Ding Z, Wu C, Wu H, Lu Q, Lin J. The epidemiology of imported acute infectious diseases in Zhejiang province, China, 2011–2016: analysis of surveillance data. *Am J Trop Med Hyg*. (2018) 98:913–9. doi: 10.4269/ajtmh.17-0284

29. Ren J, Chen Z, Ling F, Huang Y, Gong Z, Liu Y, et al. Epidemiology of indigenous dengue cases in Zhejiang province, Southeast China. *Front Public Health*. (2022) 10:857911. doi: 10.3389/fpubh.2022.857911

30. Gwee XWS, Chua PEY, Pang J. Global dengue importation: a systematic review. *BMC Infect Dis*. (2021) 21:1078. doi: 10.1186/s12879-021-06740-1

31. Yue Y, Liu X, Ren D, Wu H, Liu Q. Spatial dynamics of dengue fever in mainland China, 2019. *Int J Environ Res Public Health*. (2021) 18:2855. doi: 10.3390/ijerph18062855

32. Togami E, Chiew M, Lowbridge C, Biaukula V, Bell L, Yajima A, et al. Epidemiology of dengue reported in the World Health Organization's Western Pacific Region, 2013–2019. *Western Pac Surveill Response J*. (2023) 14:1–16. doi: 10.5365/wpsar.2023.14.1.973

33. Chen B, Chen Q, Li Y, Mu D, Wang Z, Zhu M, et al. Epidemiological characteristics of imported chikungunya fever cases in China, 2010–2019. *Dis Surveill*. (2021) 36:539–43. doi: 10.3784/jbjc.202105080246

34. Chou YC, Hsieh CJ, Cheng CA, Wu DC, Wu WC, Lin FH, et al. Epidemiologic characteristics of imported and domestic chikungunya cases in Taiwan: a 13-year retrospective study. *Int J Environ Res Public Health*. (2020) 17:3615. doi: 10.3390/ijerph17103615

35. Nakayama E, Tajima S, Kotaki A, Shibasaki KI, Itokawa K, Kato K, et al. A summary of the imported cases of chikungunya fever in Japan from 2006 to June 2016. *J Travel Med*. (2018) 25:1–11. doi: 10.1093/jtm/tax072

36. Wang Y, Zhang X, Ren R, Li C, Xiang N, Tu W, et al. Epidemiological and clinical characteristics of 25 imported Zika cases in mainland China. *Chin J Vector Biol Control*. (2017) 28:535–7.

37. Tian J, Guo X, Zhou H, Liu Y, Yin X, Wu C, et al. Molecular epidemiological survey of mosquito-borne viral diseases in patients with a fever of unknown origin at the border between China and Myanmar. *J Pathog Biol*. (2021) 16:590–5.

38. Zhou CM, Liu JW, Qi R, Fang LZ, Qin XR, Han HJ, et al. Emergence of Zika virus infection in China. *PLoS Negl Trop Dis*. (2020) 14:e0008300. doi: 10.1371/journal.pntd.0008300

39. Li F, Zhou JZ, Zhou L, Fu SH, Tian ZZ, Wang Q, et al. Serological survey of Zika virus in humans and animals in Dejiang prefecture, Guizhou province, China. *Biomed Environ Sci*. (2019) 32:875–80. doi: 10.3967/bes2019.108

40. Wang J, Xu H, Song S, Cheng R, Fan N, Fu S, et al. Emergence of Zika virus in *Culex tritaeniorhynchus* and *Anopheles sinensis* mosquitoes in China. *Virol Sin*. (2021) 36:33–42. doi: 10.1007/s12250-020-00239-w

41. Song S, Li Y, Fu S, Liu H, Li X, Gao X, et al. Could Zika virus emerge in mainland China: virus isolation from nature in *Culex quinquefasciatus*, 2016. *Emerg Microbes Infect*. (2017) 6:e93. doi: 10.1038/emi.2017.80

42. Fu S, Song S, Liu H, Li Y, Li X, Gao X, et al. ZIKA virus isolated from mosquitoes a field and laboratory investigation in China, 2016. *Sci China Life Sci*. (2017) 60:1364–71. doi: 10.1007/s11427-017-9196-8

43. Liu Z, Zhou T, Lai Z, Zhang Z, Jia Z, Zhou G, et al. Competence of *Aedes aegypti*, ae. *Albopictus*, and *Culex quinquefasciatus* mosquitoes as Zika virus vectors, China. *Emerg Infect Dis*. (2017) 23:1085–91. doi: 10.3201/eid2307.161528

44. Yang W, Zhao S, Xie Y, Liu T, Kong L, Guo Y, et al. *Armigeres subalbatus* is a potential vector for Zika virus but not dengue virus. *Infect Dis Poverty*. (2022) 11:62. doi: 10.1186/s40249-022-00990-0

45. Findlater A, Moineddin R, Kain D, Yang J, Wang X, Lai S, et al. The use of air travel data for predicting dengue importation to China: a modelling study. *Travel Med Infect Dis*. (2019) 31:101446. doi: 10.1016/j.tmaid.2019.07.002

46. Nasserie T, Brent SE, Tuite AR, Moineddin R, Yong JHE, Miniota J, et al. Association between air travel and importation of chikungunya into the USA. *J Travel Med*. (2019) 26:taz028. doi: 10.1093/jtm/taz028

47. Cho KH, Park SY, Lee WC, Lee MJ, Lee JB. International travel and exotic dengue fever in South Korea from 2006 to 2015. *Jpn J Infect Dis*. (2018) 71:378–81. doi: 10.7883/yoken.JJID.2017.553

48. Liu K, Hou X, Wang Y, Sun J, Xiao J, Li R, et al. The driver of dengue fever incidence in two high-risk areas of China: a comparative study. *Sci Rep*. (2019) 9:19510. doi: 10.1038/s41598-019-56112-8

49. Ullrich A, Schranz M, Rexroth U, Hamouda O, Schaade L, Diercke M, et al. Impact of the COVID-19 pandemic and associated non-pharmaceutical interventions on other notifiable infectious diseases in Germany: an analysis of national surveillance data during week 1-2016–week 32-2020. *Lancet Reg Health Eur*. (2021) 6:100103. doi: 10.1016/j.lanepe.2021.100103

50. Jansen CC, Darbro JM, Birrell FA, Shivas MA, van den Hurk AF. Impact of COVID-19 mitigation measures on mosquito-borne diseases in 2020 in Queensland, Australia. *Viruses*. (2021) 13:1150. doi: 10.3390/v13061150

51. Plasencia-Dueñas R, Failoc-Rojas VE, Rodriguez-Morales AJ. Impact of the COVID-19 pandemic on the incidence of dengue fever in Peru. *J Med Virol*. (2022) 94:393–8. doi: 10.1002/jmv.27298

52. Xiao J, Dai J, Hu J, Liu T, Gong D, Li X, et al. Co-benefits of nonpharmaceutical intervention against COVID-19 on infectious diseases in China: a large population-based observational study. *Lancet Reg Health West Pac*. (2021) 17:100282. doi: 10.1016/j.lanwpc.2021.100282

53. Wilson ME. Arbovirus on board—predicting dengue importation into China. *Travel Med Infect Dis*. (2019) 31:101476. doi: 10.1016/j.tmaid.2019.101476

54. Yue Y, Liu X, Xu M, Ren D, Liu Q. Epidemiological dynamics of dengue fever in mainland China, 2014–2018. *Int J Infect Dis*. (2019) 86:82–93. doi: 10.1016/j.ijid.2019.06.015

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SARS-CoV-2 excretion kinetics in nasopharyngeal and stool samples from the pediatric population

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Background: The severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is responsible for serious respiratory infections in humans. Even in the absence of respiratory symptoms, gastrointestinal (GI) signs were commonly reported in adults and children. Thus, oral–fecal transmission was suspected as a possible route of infection. The objective of this study was to describe RNA shedding in nasopharyngeal and stool samples obtained from asymptomatic and symptomatic children and to investigate virus viability.

Methods: This study included 179 stool and 191 nasopharyngeal samples obtained from 71 children, which included symptomatic ($n = 64$) and asymptomatic ($n = 7$) ones. They were collected every 7 days from the onset of the infection until negativation. Viral RNA was detected by real-time RT-PCR, targeting the N and ORF1 genes. Whole-genome sequencing was performed for positive cases. Viral isolation was assessed on Vero cells, followed by molecular detection confirmation.

Results: All cases included in this study ($n = 71$) were positive in their nasopharyngeal samples. SARS-CoV-2 RNA was detected in 36 stool samples obtained from 15 out of 71 (21.1%) children; 13 were symptomatic and two were asymptomatic. Excretion periods varied from 7 to 21 days and 7 to 14 days in nasopharyngeal and fecal samples, respectively. Four variants were detected: Alpha ($n = 3$), B.1.160 ($n = 3$), Delta ($n = 7$), and Omicron ($n = 1$). Inoculation of stool samples on cell culture showed no specific cytopathic effect. All cell culture supernatants were negative for RT-qPCR.

Conclusion: Our study demonstrated nasopharyngeal and fecal shedding of SARS-CoV-2 RNA by children up to 21 and 14 days, respectively. Fecal shedding was recorded in symptomatic and asymptomatic children. Nevertheless, SARS-CoV-2 was not isolated from positive stool samples.

KEYWORDS

stool, pediatric population, infectious, variants, symptomatic, asymptomatic, SARS-CoV-2

1. Introduction

At the beginning of the coronavirus disease 2019 (COVID-19) pandemic, available data suggested that the severe acute respiratory syndrome coronavirus 2 virus (SARS-CoV-2) was able to affect adults more than children. Up to May 2020, pediatric infections were limited to 1–5% of total recorded cases (1–4). Rapidly, several waves of COVID-19 have occurred around the world as a consequence of the emergence of multiple variants of concern (VOCs) (5–9). An increase in the number of pediatric cases was recorded, especially with the emergence of the Delta and Omicron variants (4, 10–14).

COVID-19 is mainly characterized by severe upper and lower respiratory tract infections in humans (15–19). Pediatric disease was, in general, less severe; most of the cases were asymptomatic or developed mild signs (14). Nevertheless, severe cases were also reported, requiring hospitalization and intensive care unit admission (4, 14, 20–22). Gastrointestinal (GI) signs were frequently reported, even in the absence of respiratory symptoms (3), estimated between 2 and 79% of cases according to different studies (3, 23–26). Diarrhea, vomiting, and nausea were estimated at 8.8–49.5%, 4.2–15.9%, and 4.2–29.4% of cases, respectively (26–33). Indeed, SARS-CoV-2 S protein binding to the host cell angiotensin-converting enzyme 2 (ACE2) receptor mediates viral entry. Although ACE2 is present throughout the respiratory tract (34), its expression is relatively low compared to the gastrointestinal tract, kidney, and myocardium (35). The most common GI symptom was diarrhea, which was generally noticed during the first 8 days of the infection (33, 36). Regarding child infection, GI was described as having a higher incidence, especially diarrhea and vomiting, estimated at 8–35.6% and 6.5–66.7%, respectively (33, 37–40). Shedding of SARS-CoV-2 RNA in stool samples was reported (3, 19, 41, 42). Thus, fecal–oral transmission was considered a possible route for SARS-CoV-2 transmission (41). Nevertheless, little is known about the virus shedding according to different variants and the duration of excretion, especially in pediatric patients and in symptomatic and asymptomatic cases. Furthermore, data on virus viability in positive stool samples using real-time PCR (RT-qPCR) are controversial (43). Several studies proved the absence of the live SARS-CoV-2 virus in feces (44–47), while two studies reported the possible presence of an infectious virus (48, 49).

This study aimed to investigate the excretion of SARS-CoV-2 RNA in nasopharyngeal and stool samples obtained from symptomatic and asymptomatic COVID-19 pediatric cases. Additionally, the virus viability of positive stool samples was explored by cell culture and confirmed by specific molecular detection applied to the cell culture product.

2. Materials and methods

2.1. Ethics statement

This study was approved by the Medical Ethics Committee of the Bechir Hamza Children's Hospital of Tunis, Tunisia, under the reference "12/2021." It was performed in accordance with the ethical standards of the 1964 Declaration of Helsinki and its later amendments, or comparable ethical standards. Written consent was obtained from their parents or their legal tutors.

2.2. Studied samples

A total of 179 stool samples and 191 nasopharyngeal swabs were collected from 71 children between February 2021 and January 2022 at the Pasteur Institute of Tunis, in a pandemic context and after the obtention of their parents or their legal tutors' consent. Details of each collected sample are listed in Supplementary Table 1. The study included 28 boys and 43 girls, with a sex ratio equivalent to 0.65. Their age ranged from 1 month to 18 years old, with a median age of 15 years. Patients who tested positive for SARS-CoV-2 RNA in the nasopharyngeal swab further underwent stool sample collection from these patients; nasopharyngeal swabs and stools were collected every 7 days until negative results were obtained (Figure 1). Two groups were considered. Group 1 included 160 stool samples collected from COVID-19 symptomatic children ($n = 64$), and Group 2 included 19 stool samples collected from COVID-19 asymptomatic children ($n = 7$), among the contacts of symptomatic cases (Table 1). According to the WHO Living Guidance for Clinical Management of COVID-19, symptomatic cases were defined as patients with mild, moderate, severe, or critical signs of COVID-19. Asymptomatic cases were defined as COVID-19 patients, confirmed by SARS-CoV-2 real-time PCR on nasopharyngeal samples with no signs or symptoms of an illness or disease (50).

2.3. Nucleic acid extraction and detection by PCR

Nasopharyngeal and stool samples were processed in accordance with recommended good laboratory practices. Stool samples were treated with PBS/chloroform (1%) and centrifuged at 2500×*g* for 30 min, according to the WHO protocol for stool sample treatment (51–53). Viral RNA was extracted from 140 µL of the supernatants of nasopharyngeal and stool samples using the QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions (54). The presence of SARS-CoV-2 RNA was determined with RT-qPCR using HKU (55) and IPT2-IPT4 protocols (Institut Pasteur, Paris) (56) as previously described (57) (Figure 1).

2.4. Whole-genome sequencing (WGS)

The whole genome of SARS-CoV-2 was obtained by next-generation sequencing using the COVIDSeq Test (Illumina Inc., San Diego, CA, USA) as previously described (58, 59) (Figure 1). The library preparation process used validated protocols at the "National Reference Centre for Whole-Genome Sequencing of microbial pathogens at Istituto Zooprofilattico Sperimentale dell'Abruzzo e del Molise IZSAM," with the Hamilton Microlab STAR Liquid Handling System (Hamilton Robotics, Reno, NV, USA). NGS sequencing was achieved with the NextSeq 1,000 (Illumina Inc., San Diego, CA, USA), which provided read length data of 2 × 150 bp.

2.5. SARS-CoV-2 variant identification

Data analysis was automatically performed at the end of the sequencing run using the GENPAT platform at IZSAM in Teramo, as

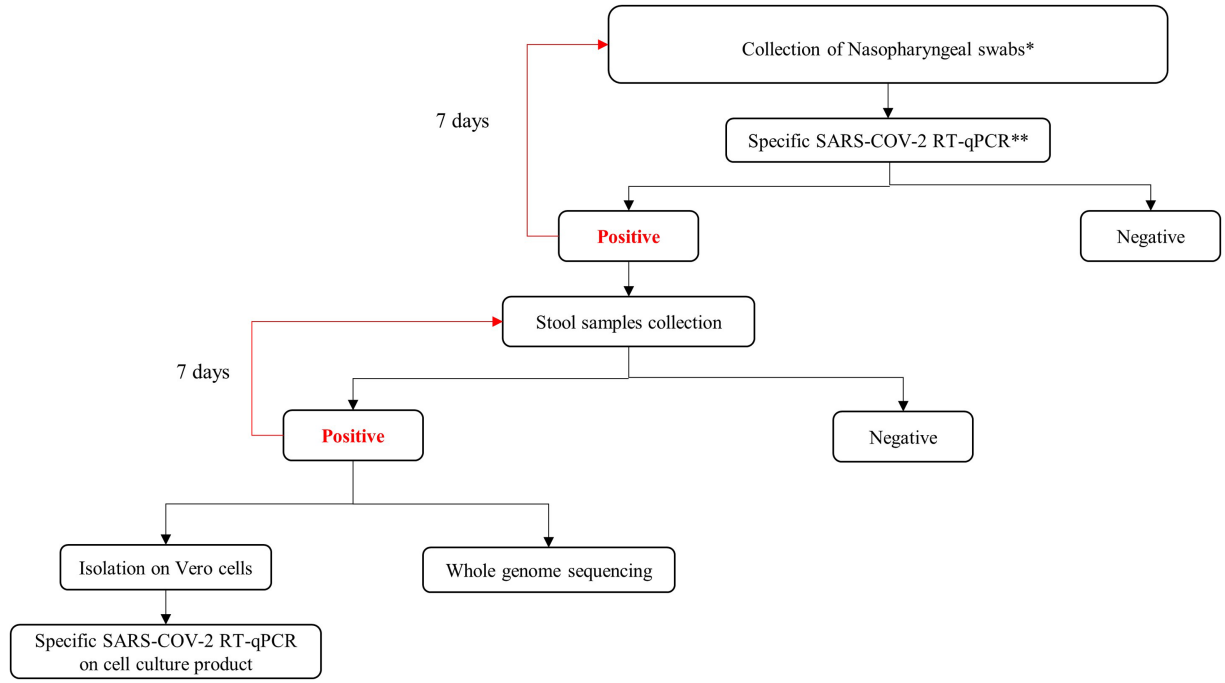

FIGURE 1
Workflow presenting the methodology used in this study. *Sample were collected from suspected COVID-19 pediatric cases and pediatric contact of confirmed adult cases; **WHO recommended protocol.

described in Molini et al. (59) and Di Pasquale et al. (60). Mapping to the Wuhan-Hu-1 reference genome (accession number NC_045512) was performed with the BWA tool (61), after quality control and trimming of the reads using FastQC and Trimmomatic (62). The consensus sequence was obtained using the iVar tool (63). The identification of SARS-CoV-2 lineage and sub-lineage was performed with the Pangolin (64) and Nextclade tools via the web[1, 2] (65). Boxplot was used to visualize our results (66).

2.6. Statistical analysis

A chi-square test was performed using R software (67), which evaluated whether there was a significant association between symptomatic and asymptomatic children. Statistical significance was determined using 95% confidence intervals.

2.7. Virus isolation

The stool sample was treated as previously described and inoculated on Vero cells (African green monkey kidney cells) obtained from ATCC (CCL-81) in a biosafety level 3 laboratory at the Pasteur Institute of Tunis (67). The inoculated cells were then maintained in Minimum Essential Medium (MEM) supplemented with 5% fetal bovine serum (FBS), incubated at 36°C and 5% CO2, and observed for cytopathic effect (CPE) for 7 days. In the absence of CPE, inoculated cells were harvested and clarified by centrifugation, and 200 µL of supernatant was inoculated onto a fresh cell culture monolayer and observed for an additional 7 days (53). Specimens were considered negative if no cytopathic effect was detected during 14 days after initial inoculation. For samples showing a cytopathic effect (CPE), the infected cells were then harvested and clarified by centrifugation. Virus suspensions were used for confirmation by SARS-CoV-2 real-time PCR.

3. Results

3.1. Positive SARS-CoV-2 RNA in nasopharyngeal swabs

All investigated cases presented an initial positive SARS-CoV-2 nasopharyngeal sample. Among them, 7 out of 71 (9.9%) children were asymptomatic, and 64 out of 71 (90.1%) were symptomatic.

They presented mild clinical signs, including fever ($n = 46$), cough ($n = 43$), headache ($n = 32$), loss of taste smell ($n = 36$), tiredness ($n = 21$), diarrhea ($n = 32$), vomiting ($n = 16$), muscle pain ($n = 29$), breathing difficulty ($n = 4$), conjunctivitis ($n = 2$), dyspnea ($n = 3$), urticaria ($n = 2$), and vertigo ($n = 1$). The duration of SARS-CoV-2 shedding in nasopharyngeal swabs varied between 7 and 21 days (average equal to 8.7 days). By days 7, 14, and 21, 42 (59%), 11 (15.5%), and 4 (5.6%) out of 71 children continued to shed SARS-CoV-2 RNA. All children stopped shedding SARS-CoV-2 RNA on day 28 (Figure 2A). The values of the threshold cycle (Ct values) varied from

1 https://pangolin.cog-uk.io/
2 https://clades.nextstrain.org/

13 to 34, 21 to 38, 22 to 37, and 28 to 35 on days 1, 7, 14, and 21, respectively. The median Ct values were 22, 27.3, 33.75, and 33.5 on days 1, 7, 14, and 21, respectively, as described in Table 2.

3.2. Positive SARS-CoV-2 RNA in stool samples

Among the studied population, SARS-CoV-2 RNA was detected in stool samples of 15 out of 71 (21.1%) children. They are constituted by 13 out of 64 (20.3%) symptomatic and 2 out of 7 (28.6%) asymptomatic patients, with no statistically significant difference between the two groups (value of $p > 0.05$; x squared = 0.00042451, df = 1, value of $p = 0.9836$; Table 3).

A total of 36 positive samples were detected, with a threshold cycle (Ct) varying between 23 and 37 (Table 2). For samples obtained from the symptomatic group, the Ct values were between 23 and 37, while samples obtained from the asymptomatic group presented Ct values varying between 24 and 32 (Table 3).

The RNA-shedding duration was between 7 and 14 days. On days 7 and 14, 12 and 5 of 71 (16% and 7%) children remained positive by RT-PCR, respectively. No one of them continued to shed the RNA in stool samples on day 21 (Figure 2B). In symptomatic children, the duration of SARS-CoV-2 RNA excretion in stool samples varied between 7 and 14 days (average equivalent to 9.4), while in asymptomatic cases, the duration of viral RNA excretion was 7 days (Table 2).

3.3. SARS-CoV-2 variant identification

With the aim of identifying variants of excreted viruses in stool samples, 14 full genome sequences were obtained from samples of 14 out of 15 children presenting fecal shedding (Table 2). For one child,

TABLE 1 Characteristics of studied samples obtained from symptomatic and asymptomatic children.

Groups	Number of patients	Number of stool samples	Number of nasopharyngeal swabs
Group 1: symptomatic children	64	160	171
Group 2: asymptomatic children	7	19	20
Total	71	179	191

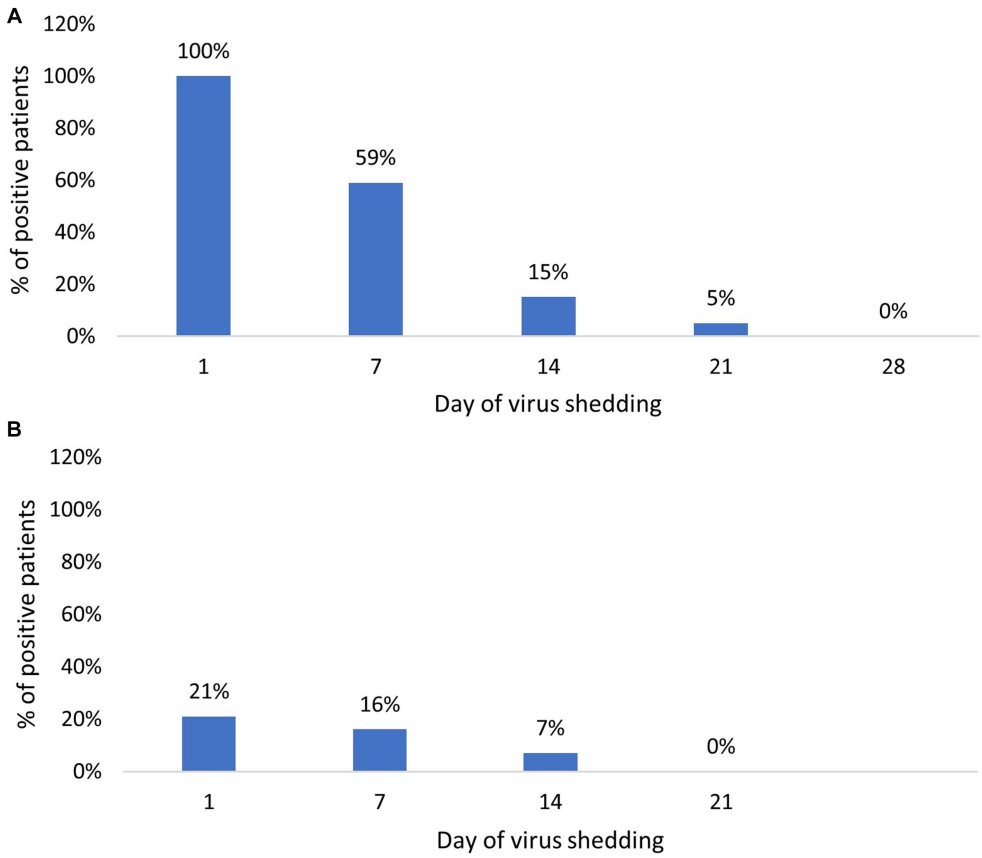

FIGURE 2
Percentage of SARS-CoV-2 positive patients over time: **(A)** Percentage of SARS-CoV-2 positive cases in nasopharyngeal samples; **(B)** Percentage of SARS-CoV-2 positive cases in stool samples.

TABLE 2 Shedding duration and Ct values according to SARS-CoV-2 variant.

Patients	Variants	Clinical status Asymptomatic (A) Symptomatic (S)	Duration of nasopharyngeal shedding (days)	CT values				Duration of stool shedding (days)	CT values			
				Day 1	Day 7	Day 14	Day 21		Day 1	Day 7	Day 14	Day 21
P1	Alpha (B.1.1.7)	A	7	34-34[a]	33-35[a]	NEG[a]		7	25-28[a]	26-28[a]	NEG[a]	
P2		S	7	28-29[b]	32-35[a]	NEG[a]		7	30-32[a]	34-33[a]	NEG[a]	
P3		S	7	28-28[a]	32-31[a]	NEG[a]		14	30-30[a]	31-30[a]	32-30[b]	NEG[a]
P4	B.1.160	S	14	17-18[a]	28-26[a]	34-31[a]	NEG[a]	7	32-34[a]	36-36[a]	NEG[a]	
P5		A	7	29-30[a]	36-37[a]	NEG[a]		7	30-31[a]	32-31[a]	NEG[a]	
P6		S	7	24-24[b]	23-22[a]	NEG[a]		14	30-30[b]	31-31[a]	35-34[a]	NEG[a]
P7	Delta (AY.122)	S	14	24-25[b]	25-25[a]	23-22[a]	NEG[a]	14	31-33[a]	32-33[a]	34-32[b]	NEG[a]
P8		S	7	30-32[a]	27-26[a]	NEG[a]		7	32-31[a]	33-33[a]	NEG[b]	
P9		S	14	22-24[b]	31-30[a]	28-29[a]	NEG[a]	14	28-30[a]	30-30[a]	30-29[b]	NEG[a]
P10		S	7	20-24[b]	18-22[a]	NEG[a]		14	23-24[a]	24-24[a]	29-26[b]	NEG[a]
P11		S	7	25-28[b]	32-34[a]	NEG[a]		7	31-33[a]	35-34[a]	NEG[a]	
P12		S	7	23-26[a]	35-36[a]	NEG[a]		7	31-30[b]	34-34[a]	NEG[a]	
P13		S	7	28-28[b]	24-25[a]	NEG[a]		14	32-33[b]	33-33[b]	37-37[a]	NEG[a]
P14	OMICRON (BA.1.1.1)	S	7	22-21[b]	23-21[d]	NEG[a]		7	30-30[b]	33-31[b]	NEG[b]	
P15	ND	S	7	30-30[b]	34-32[b]	NEG		7	30-31[b]	32-36[b]	NEG	

[a]HKU protocol; [b]IP2-IP4.
NEG, negative; ND, not determined.

TABLE 3 Prevalence of positive stool sample shedding cases among symptomatic and asymptomatic cases.

	Positive cases	Negative cases	Total cases	% of positive cases	Statistical test	ct value	Duration of virus shedding in stool samples
Symptomatic	13	51	64	20.3%	value of $p = 0.9836$	[23–37]	[7–14]
Asymptomatic	2	5	7	28.6%		[24–32]	7

sequences were not generated given the high real-time PCR Ct value. Four SARS-CoV-2 variants were detected: Alpha (B.1.1.7) ($n = 3$), B.1.160 ($n = 3$), Delta (AY.122 sub-variant) ($n = 7$), and Omicron (BA.1.1.1 sub-variant)($n = 1$). The Delta variant was the most excreted variant (value of $p < 0.05$). The duration of excretion of positive fecal samples was variable among those variants (Figure 3). Patients infected with the Alpha and B.1.160 variants presented positive RNA results during 7 ($n = 2$) to 14 ($n = 1$) days of the infection, for each one. On the other hand, 7 children infected with the Delta variant (AY.122 sub-variant) presented positive results up to 14 days ($n = 4$) and 7 days ($n = 3$). For the Omicron variant (BA.1.1.1 sub-variant), the RNA shedding was limited to 7 days.

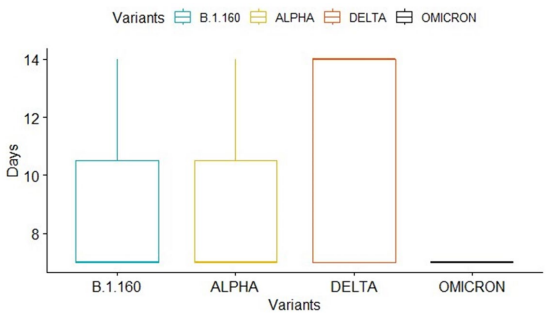

FIGURE 3
Presentation of SARS-CoV-2 RNA shedding duration for each variant using the boxplot package which describes data sets using 5 particular numbers: the minimum, first quartile, median, third quartile, and maximum. The box in the diagram begins with the first quartile and ends with the third quartile. Lines extend from the first quartile down to the minimum and from the third quartile up to the maximum.

3.4. Virus isolation

Virus isolation using Vero cells showed non-specific modification of the cell aspect in positive stool samples. The observed modifications appeared between 4 and 7 days, respectively. After passaging, slight modifications appeared after 5 days. Specific SARS-CoV-2 RT-qPCR detection was negative for all obtained cell culture supernatants.

4. Discussion

Since the beginning of the COVID-19 pandemic, the fecal–oral excretion of infectious SARS-CoV-2 has been a matter of debate. Transmission via the fecal–oral route was previously demonstrated for other coronaviruses, such as MERS-CoV and SARS-CoV-1 (3, 68). Thus, in this study, we investigated the SARS-CoV-2 RNA shedding in stool samples obtained from symptomatic and asymptomatic COVID-19 pediatric cases infected with different virus variants between February 2021 and January 2022, along the Alpha, B.1.160, Delta, and Omicron waves in Tunisia (58, 69). To support the hypothesis of possible fecal–oral transmission, the virus viability in positive samples was investigated by inoculation with cell culture.

A proportion of 21.1% of infected children showed SARS-CoV-2 RNA shedding. The shedding occurred similarly among symptomatic and asymptomatic children. Previous studies showed that children with fecal excretion of viral RNA may be asymptomatic or present with clinical respiratory or gastrointestinal signs (19, 41, 42). The rate of positive RNA in fecal specimens of COVID-19 patients was controversial among the different published studies. An overview of the gastrointestinal shedding of SARS-CoV-2 in infected children suggested an average of 20–30% of positive fecal shedding in infants with and without gastrointestinal signs (38). However, other studies, mainly achieved rapidly at the beginning of the pandemic, reported 83.3 to 91.4% of RNA shedding in stool samples from children (3, 42, 70, 71). Other authors reported rates between 47 and 69% (72, 73).

Moreover, several data points regarding the shedding duration in stool samples suggest prolonged periods of up to 70 days or more (3, 19, 42, 74). The prolonged shedding period was mainly related to severe cases requiring hospitalization or immunocompromised patients (39, 75). Many studies reporting asymptomatic and moderate cases showed a shedding period between 18 and 32 days through the digestive tract (3, 72). In our series of investigations, the shedding period was at least 21 days in nasopharyngeal samples and at least 14 days in stool samples. Indeed, they present mild clinical forms with moderate respiratory and gastrointestinal signs.

In our study, the excreted viral RNA belonged to four variants of SARS-CoV-2: Alpha (B.1.1.7), B.1.160, Delta (B.1.617.2), and Omicron (B.1.1.529). The Delta variant (sub-variant AY.122) appears to be the most excreted, during the longest period, and with the highest viral load. In the literature, limited information was available about the rate of SARS-CoV-2 RNA shedding by children and adults according to the variant. Available data suggested increasing SARS-CoV-2 infection in the pediatric population, especially during the Delta variant wave, with higher transmissibility and pathogenicity than other variants (4, 10–14). It was suggested that the Delta variant is 60% more transmissible than the Alpha variant (32, 76). Furthermore, disease duration in children infected with the Delta variant was reported to be longer, in some cases exceeding 29 days (7, 77, 78).

It is worth noting that the detection of SARS-CoV-2 RNA in the framework of wastewater-based genomic surveillance is of great interest for tracking SARS-CoV-2 variants and the early management of new waves of the infection. It can complement clinical surveillance efforts and also offer more details about the evolutionary dynamics of SARS-CoV-2 (79).

To investigate the hypothesis of possible fecal–oral transmission, SARS-CoV-2 RNA detection and virus isolation on cell culture, followed by molecular confirmation, were used. Indeed, RNA shedding in stool samples could not reflect systematically the possible fecal–oral transmission, as the detection of SARS-CoV-2 RNA might be the result of virus replication into the gastrointestinal tract (38). In our study, a total of 36 samples were inoculated, and none of them showed any specific CPE until 14 days. Moreover, SARS-CoV-2 RNA detection in the cell culture supernatant was also negative. In this regard, the data in the literature are conflicting. Many studies have demonstrated the absence of cytopathic effect (CPE) in Vero cells after inoculation with stool samples (44, 45), while others have suggested the presence of infectious particles by the use of cell culture and electron microscopy (EM) visualization (48, 49). Reasonably, isolation in cell culture and observation of virus particles in EM are not sufficient to confirm the presence of viable particles. The use of more specific methods, such as molecular detection of sub-genomic RNA, is therefore highly recommended for investigating virus viability in infected biological specimens (80).

In our setting, the presence of CPE or cell modifications generated with stool samples could be the result of the multiplication of other enteric viruses, especially on Vero cells, which are permissive for the majority of cultured viruses. In this case, the initial evidence of SARS-CoV-2 RNA from the stool sample reflects only the presence of SARS-CoV-2 genomic RNA.

Indeed, in our study, investigated stool samples were all showing high CT values, evidence that makes virus isolation unlikely.

Nevertheless, the different parameters used for cell culture isolation may impact the sensitivity of virus detection. Furthermore, it is worth mentioning that our findings are derived from a relatively small number of positive stool samples. Conducting an analysis on a larger dataset of fecal specimens, if accessible, would support our findings. It is worth highlighting that during the study, a significant

number of children, particularly adolescents, declined to provide stool samples.

From another point of view, it will be very interesting to investigate the potential correlations between fecal shedding patterns and variables such as the type of vaccine administered to participants and the severity of COVID-19 disease.

5. Conclusion

In conclusion, the exploration of SARS-CoV-2 shedding in stool samples bears significant relevance as it could contribute to a wider spread of the virus and environmental contamination. Our study has revealed that children can shed SARS-CoV-2 RNA in their nasopharyngeal and fecal samples for up to 21 and 14 days, respectively, particularly when infected with the Delta variant. However, none of the positive samples exhibited the presence of viable SARS-CoV-2 particles. Consequently, the likelihood of SARS-CoV-2 transmission via the fecal–oral route appears to be low. Further investigation involving a larger and more diverse population can provide additional support for our findings.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

The studies involving humans were approved by the local Medical Ethics Committee of Bechir Hamza Children's Hospital of Tunis, Tunisia, under the reference "12/2021." The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

SH-B and HTr: conceptualization. HKh, MG, and SH-B: data curation. HKh, MG, SBH, HKr, HTo, AC, AS, ZM, NH, CC, MA, AP, and RA: investigation. HKh, MG, SBH, HKr, HTo, AC, AS, ZM, NH, CC, MA, AP, and RA: resources. HTr, SH-B, CC, AL, and AP: funding acquisition. HTr, SH-B, MA, and AP: supervision. HTr, AL, and SH-B: validation. HKh, HTr, AL, and SH-B: visualization, original draft preparation, and writing—review and editing. HKh and SH-B: writing. All authors reviewed the manuscript and agreed to its submission to this journal.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer SM declared a past co-authorship with the author SH-B to the handling editor.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1226207/full#supplementary-material

References

1. Mannheim J, Gretsch S, Layden JE, Fricchione MJ. Characteristics of hospitalized pediatric coronavirus disease 2019 cases in Chicago, Illinois, march-April 2020. *J Pediatr Infect Dis Soc*. (2020) 9:519–22. doi: 10.1093/jpids/piaa070

2. Tsabouri S, Makis A, Kosmeri C, Siomou E. Risk factors for severity in children with coronavirus disease 2019: a comprehensive literature review. *Pediatr Clin N Am*. (2021) 68:321–38. doi: 10.1016/j.pcl.2020.07.014

3. Xu CLH, Raval M, Schnall JA, Kwong JC, Holmes NE. Duration of respiratory and gastrointestinal viral shedding in children with SARS-CoV-2: a systematic review and synthesis of data. *Pediatr Infect Dis J*. (2020) 39:e249–56. doi: 10.1097/INF.0000000000002814

4. Khemiri H, Ayouni K, Triki H, Haddad-Boubaker S. SARS-CoV-2 infection in pediatric population before and during the Delta (B.1.617.2) and Omicron (B.1.1.529) variants era. *Virol J*. (2022) 19:144. doi: 10.1186/s12985-022-01873-4

5. World Health Organization, *COVID-19 variants*. (2023). Available at: https://www.who.int/westernpacific/emergencies/covid-19/information/covid-19-variants, Accessed 28 june 2022

6. Dubey A, Choudhary S, Kumar P, Tomar S. Emerging SARS-CoV-2 variants: genetic variability and clinical implications. *Curr Microbiol*. (2021) 79:20. doi: 10.1007/s00284-021-02724-1

7. Bard JD, Bootwalla M, Leber A, Planet P, Moustafa AM, Harris R, et al. Emergence of SARS-CoV-2 variants of concern in the pediatric population of the United States. *bioRxiv*. (2021). doi: 10.1101/2021.05.22.21257660

8. Mascola JR, Graham BS, Fauci AS. SARS-CoV-2 viral variants—tackling a moving target. *JAMA*. (2021) 325:1261–2. doi: 10.1001/jama.2021.2088

9. Kunal S, Ish P, Aditi GK. Emergence of COVID-19 variants and its global impact In: S Adibi, P Griffin, M Sanicas, M Rashidi and F Lanfranchi, editors. *Frontiers of COVID-19: Scientific and clinical aspects of the novel coronavirus 2019 [internet]*. Cham: Springer International Publishing (2022). 183–201.

10. Torjesen I. Covid-19: Delta variant is now UK's most dominant strain and spreading through schools. *BMJ*. (2021) 373:n1445. doi: 10.1136/bmj.n1445

11. Molteni E, Sudre CH, Canas LDS, Bhopal SS, Hughes RC, Chen L, et al. Illness characteristics of COVID-19 in children infected with the SARS-CoV-2 Delta variant. *Children*. (2022) 9:652. doi: 10.3390/children9050652

12. Bittmann S. Role of Omicron variant of SARS-CoV-2 in children in Germany. *World J Pediatr WJP*. (2022) 18:283–4. doi: 10.1007/s12519-021-00511-3

13. Torjesen I. Covid-19: Omicron variant is linked to steep rise in hospital admissions of very young children. *BMJ*. (2022) 376:o110. doi: 10.1136/bmj.o110

14. Alteri C, Scutari R, Costabile V, Colagrossi L, Yu la Rosa K, Agolini E, et al. Epidemiological characterization of SARS-CoV-2 variants in children over the four COVID-19 waves and correlation with clinical presentation. *Sci Rep*. (2022) 12:10194. doi: 10.1038/s41598-022-14426-0

15. Lotfi M, Rezaei N. SARS-CoV-2: a comprehensive review from pathogenicity of the virus to clinical consequences. *J Med Virol*. (2020) 92:1864–74. doi: 10.1002/jmv.26123

16. Hindson J. COVID-19: faecal–oral transmission? *Nat Rev Gastroenterol Hepatol*. (2020) 17:259. doi: 10.1038/s41575-020-0295-7

17. Rathore JS, Ghosh C. Severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2), a newly emerged pathogen: an overview. *Pathog Dis*. (2020) 78:ftaa042. doi: 10.1093/femspd/ftaa042

18. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan. *China Lancet Lond Engl*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5

19. Zhang T, Cui X, Zhao X, Wang J, Zheng J, Zheng G, et al. Detectable SARS-CoV-2 viral RNA in feces of three children during recovery period of COVID-19 pneumonia. *J Med Virol*. (2020) 92:909–14. doi: 10.1002/jmv.25795

20. Callaway E. Delta coronavirus variant: scientists brace for impact. *Nature*. (2021) 595:17–8. doi: 10.1038/d41586-021-01696-3

21. Delahoy MJ, Ujamaa D, Whitaker M, O'Halloran A, Anglin O, Burns E, et al. Hospitalizations associated with COVID-19 among children and adolescents - COVID-NET, 14 states, march 1, 2020-august 14, 2021. *MMWR Morb Mortal Wkly Rep*. (2021) 70:1255–60. doi: 10.15585/mmwr.mm7036e2

22. Wang L, Berger NA, Kaelber DC, Davis PB, Volkow ND, Xu R. COVID infection severity in children under 5 years old before and after Omicron emergence in the US. *MedRxiv Prepr Serv Health Sci*. (2022):2022.01.12.22269179. doi: 10.1101/2022.01.12.22269179

23. Yeo C, Kaushal S, Yeo D. Enteric involvement of coronaviruses: is faecal-oral transmission of SARS-CoV-2 possible? *Lancet Gastroenterol Hepatol*. (2020) 5:335–7. doi: 10.1016/S2468-1253(20)30048-0

24. Ong J, Young BE, Ong S. COVID-19 in gastroenterology: a clinical perspective. *Gut*. (2020) 69:1144–5. doi: 10.1136/gutjnl-2020-321051

25. Ng SC, Tilg H. COVID-19 and the gastrointestinal tract: more than meets the eye. *Gut*. (2020) 69:973–4. doi: 10.1136/gutjnl-2020-321195

26. Jin X, Lian JS, Hu JH, Gao J, Zheng L, Zhang YM, et al. Epidemiological, clinical and virological characteristics of 74 cases of coronavirus-infected disease 2019 (COVID-19) with gastrointestinal symptoms. *Gut*. (2020) 69:1002–9. doi: 10.1136/gutjnl-2020-320926

27. Lu X, Zhang L, Du H, Zhang J, Li YY, Qu J, et al. SARS-CoV-2 infection in children. *N Engl J Med*. (2020) 382:1663–5. doi: 10.1056/NEJMc2005073

28. Lin L, Jiang X, Zhang Z, Huang S, Zhang Z, Fang Z, et al. Gastrointestinal symptoms of 95 cases with SARS-CoV-2 infection. *Gut*. (2020) 69:997–1001. doi: 10.1136/gutjnl-2020-321013

29. Young BE, Ong SWX, Kalimuddin S, Low JG, Tan SY, Loh J, et al. Epidemiologic features and clinical course of patients infected with SARS-CoV-2 in Singapore. *JAMA*. (2020) 323:1488–94. doi: 10.1001/jama.2020.3204

30. Fang D, Ma J, Guan J, Wang M, Song Y, Tian D, et al. Manifestations of digestive system in hospitalized patients with novel coronavirus pneumonia in Wuhan, China: a single-center, descriptive study. *Chin J Dig*. (2020):E005–5.

31. Gu J, Han B, Wang J. COVID-19: gastrointestinal manifestations and potential fecal-Oral transmission. *Gastroenterology*. (2020) 158:1518–9. doi: 10.1053/j.gastro.2020.02.054

32. Cheung KS, Hung IFN, Chan PPY, Lung KC, Tso E, Liu R, et al. Gastrointestinal manifestations of SARS-CoV-2 infection and virus load in fecal samples from a Hong Kong cohort: systematic review and Meta-analysis. *Gastroenterology*. (2020) 159:81–95. doi: 10.1053/j.gastro.2020.03.065

33. Matthai J, Shanmugam N, Sobhan P. Indian Society of Pediatric Gastroenterology Hepatology and Nutrition, pediatric gastroenterology chapter of Indian academy of pediatrics. Coronavirus disease (COVID-19) and the gastrointestinal system in children. *Indian Pediatr*. (2020) 57:533–5. doi: 10.1007/s13312-020-1851-5

34. Hou YJ, Okuda K, Edwards CE, Martinez DR, Asakura T, Dinnon KH, et al. SARS-CoV-2 reverse genetics reveals a variable infection gradient in the respiratory tract. *Cells*. (2020) 182:429–446.e14. doi: 10.1016/j.cell.2020.05.042

35. Puray-Chavez M, LaPak KM, Schrank TP, Elliott JL, Bhatt DP, Agajanian MJ, et al. Systematic analysis of SARS-CoV-2 infection of an ACE2-negative human airway cell. *Cell Rep*. (2021) 36:109364. doi: 10.1016/j.celrep.2021.109364

36. Park SK, Lee CW, Park DI, Woo HY, Cheong HS, Shin HC, et al. Detection of SARS-CoV-2 in fecal samples from patients with asymptomatic and mild COVID-19 in Korea. *Clin Gastroenterol Hepatol*. (2021) 19:1387–1394.e2. doi: 10.1016/j.cgh.2020.06.005

37. Tian Y, Rong L, Nian W, He Y. Review article: gastrointestinal features in COVID-19 and the possibility of faecal transmission. *Aliment Pharmacol Ther*. (2020) 51:843–51. doi: 10.1111/apt.15731

38. Chiappini E, Licari A, Motisi MA, Manti S, Marseglia GL, Galli L, et al. Gastrointestinal involvement in children with SARS-COV-2 infection: an overview for the pediatrician. *Pediatr Allergy Immunol*. (2020) 31:92–5. doi: 10.1111/pai.13373

39. Santos VS, Gurgel RQ, Cuevas LE, Martins-Filho PR. Prolonged fecal shedding of SARS-CoV-2 in pediatric patients: a quantitative evidence synthesis. *J Pediatr Gastroenterol Nutr*. (2020) 71:150–2. doi: 10.1097/MPG.0000000000002798

40. Wang J, Cui HR, Tang HB, Deng XL. Gastrointestinal symptoms and fecal nucleic acid testing of children with 2019 coronavirus disease: a systematic review and meta-analysis. *Sci Rep*. (2020) 10:17846. doi: 10.1038/s41598-020-74913-0

41. Xu Y, Li X, Zhu B, Liang H, Fang C, Gong Y, et al. Characteristics of pediatric SARS-CoV-2 infection and potential evidence for persistent fecal viral shedding. *Nat Med*. (2020) 26:502–5. doi: 10.1038/s41591-020-0817-4

42. Hua CZ, Miao ZP, Zheng JS, Huang Q, Sun QF, Lu HP, et al. Epidemiological features and viral shedding in children with SARS-CoV-2 infection. *J Med Virol*. (2020) 92:2804–12. doi: 10.1002/jmv.26180

43. Puhach O, Adea K, Hulo N, Sattonnet P, Genecand C, Iten A, et al. Infectious viral load in unvaccinated and vaccinated individuals infected with ancestral, Delta or Omicron SARS-CoV-2. *Nat Med*. (2022) 28:1491–500. doi: 10.1038/s41591-022-01816-0

44. Jeong HW, Kim SM, Kim HS, Kim YI, Kim JH, Cho JY, et al. Viable SARS-CoV-2 in various specimens from COVID-19 patients. *Clin Microbiol Infect*. (2020) 26:1520–4. doi: 10.1016/j.cmi.2020.07.020

45. Kim JM, Kim HM, Lee EJ, Jo HJ, Yoon Y, Lee NJ, et al. Detection and isolation of SARS-CoV-2 in serum, urine, and stool specimens of COVID-19 patients from the Republic of Korea. *Osong Public Health Res Perspect*. (2020) 11:112–7. doi: 10.24171/j.phrp.2020.11.3.02

46. Xiao F, Sun J, Xu Y, Li F, Huang X, Li H, et al. Infectious SARS-CoV-2 in feces of patient with severe COVID-19. *Emerg Infect Dis*. (2020) 26:1920–2. doi: 10.3201/eid2608.200681

47. Fumian TM, Malta FC, Dos Santos DRL, Pauvolid-Corrêa A, Fialho AM, Leite JPG, et al. SARS-CoV-2 RNA detection in stool samples from acute gastroenteritis cases. *Brazil J Med Virol*. (2021) 93:2543–7. doi: 10.1002/jmv.26786

48. Zhang Y, Chen C, Song Y, Zhu S, Wang D, Zhang H, et al. Excretion of SARS-CoV-2 through faecal specimens. *Emerg Microbes Infect*. (2020) 9:2501–8. doi: 10.1080/22221751.2020.1844551

49. Wang W, Xu Y, Gao R, Lu R, Han K, Wu G, et al. Detection of SARS-CoV-2 in different types of clinical specimens. *JAMA*. (2020) 323:1843–4. doi: 10.1001/jama.2020.3786

50. World health organization. (2021). WHO-2019-nCoV-clinical-2021.2-eng clinical manifestation.Pdf. Accessed 8 May 2023

51. Haddad-Boubaker S, Joffret ML, Pérot P, Bessaud M, Meddeb Z, Touzi H, et al. Metagenomic analysis identifies human adenovirus 31 in children with acute flaccid paralysis in Tunisia. *Arch Virol*. (2019) 164:747–55. doi: 10.1007/s00705-018-04141-5

52. World health Organization. *Manuel for virological investigation of poliomyelitis*. Geneva: World Health Organization (2004).

53. Haddad-Boubaker S, Yahia AB, Rezig D, Farès W, Touzi H, Triki H. Enterovirus detection in stool specimen: relevance for poliovirus and enterovirus surveillance. *Arch Inst Pasteur Tunis*. (2007) 84:3–9.

54. Chu DKW, Pan Y, Cheng SMS, Hui KPY, Krishnan P, Liu Y, et al. Molecular diagnosis of a novel coronavirus (2019-nCoV) causing an outbreak of pneumonia. *Clin Chem*. (2020) 66:549–55. doi: 10.1093/clinchem/hvaa029

55. World health Organization. (2020). Available at: https://www.who.int/docs/default-source/coronaviruse/peiris-protocol-16-1-20.pdf. Accessed 8 May 2023

56. World health Organization. (2020). Available at: https://www.who.int/docs/default-source/coronaviruse/real-time-rt-pcr-assays-for-the-detection-of-sars-cov-2-institut-pasteur-paris.pdf Accessed 8 May 2023

57. Gdoura M, Abouda I, Mrad M, Ben Dhifallah I, Belaiba Z, Fares W, et al. SARS-CoV2 RT-PCR assays: in vitro comparison of 4 WHO approved protocols on clinical specimens and its implications for real laboratory practice through variant emergence. *Virol J*. (2022) 19:54. doi: 10.1186/s12985-022-01784-4

58. Haddad-Boubaker S, Arbi M, Souiai O, Chouikha A, Fares W, Edington K, et al. The Delta variant wave in Tunisia: genetic diversity, spatio-temporal distribution and evidence of the spread of a divergent AY.122 sub-lineage. *Front Public Health*. (2023) 10:990832. doi: 10.3389/fpubh.2022.990832

59. Molini U, Coetzee LM, Engelbrecht T, de Villiers L, de Villiers M, Mangone I, et al. SARS-CoV-2 in Namibian dogs. *Vaccine*. (2022) 10:2134. doi: 10.3390/vaccines10122134

60. Di Pasquale A, Radomski N, Mangone I, Calistri P, Lorusso A, Cammà C. SARS-CoV-2 surveillance in Italy through phylogenomic inferences based on hamming distances derived from pan-SNPs, -MNPs and -InDels. *BMC Genomics*. (2021) 22:782. doi: 10.1186/s12864-021-08112-0

61. Li H. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. (2014), *arXiv*, doi: 10.48550/arXiv.1303.3997

62. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinforma Oxf Engl*. (2014) 30:2114–20. doi: 10.1093/bioinformatics/btu170

63. Grubaugh ND, Gangavarapu K, Quick J, Matteson NL, Jesus JGD, Main BJ, et al. An amplicon-based sequencing framework for accurately measuring intrahost virus diversity using PrimalSeq and iVar. *bioRxiv*. (2018):383513. Available at: https://www.biorxiv.org/content/10.1101/383513v1

64. O'Toole Á, Scher E, Underwood A, Jackson B, Hill V, McCrone JT, et al. Assignment of epidemiological lineages in an emerging pandemic using the pangolin tool. *Virus Evol*. (2021) 7:veab064. doi: 10.1093/ve/veab064

65. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics*. (2018) 34:4121–3. doi: 10.1093/bioinformatics/bty407

66. Frigge M, Hoaglin DC, Iglewicz B. Some implementations of the boxplot. *Am Stat*. (2012) 43:50–4. doi: 10.1080/00031305.1989.10475612

67. The R foundation. R: What is R? (2002). Available at: https://www.r-project.org/about.html

68. Valleriani F, Mancuso E, Vincifori G, Teodori L, Di Marcantonio L, Spedicato M, et al. Neutralization of SARS-CoV-2 variants by serum from BNT162b2 vaccine recipients. *Viruses*. (2021) 13:2011. doi: 10.3390/v13102011

69. Chouikha A, Fares W, Laamari A, Haddad-Boubaker S, Belaiba Z, Ghedira K, et al. Molecular epidemiology of SARS-CoV-2 in Tunisia (North Africa) through several successive waves of COVID-19. *Viruses*. (2022) 14:624. doi: 10.3390/v14030624

70. Cai J, Wang X, Zhao J, Ge Y, Xu J, Tian H, et al. Comparison of clinical and epidemiological characteristics of asymptomatic and symptomatic SARS-CoV-2 infection in children. *Virol Sin*. (2020) 35:803–10. doi: 10.1007/s12250-020-00312-4

71. Jiehao C, Jin X, Daojiong L, Zhi Y, Lei X, Zhenghai Q, et al. A case series of children with 2019 novel coronavirus infection: clinical and epidemiological features. *Clin Infect Dis*. (2020) 71:1547–51. doi: 10.1093/cid/ciaa198

72. Tam I, McNamara C, Dunbar J, O'Connor K, Manzano G, Cabana MD, et al. SARS-CoV-2 gastrointestinal shedding in hospitalized children. *Hosp Pediatr*. (2022) 12:e78–85. doi: 10.1542/hpeds.2021-006304

73. Benvari S, Mahmoudi S, Mohammadi M. Gastrointestinal viral shedding in children with SARS-CoV-2: a systematic review and meta-analysis. *World J Pediatr*. (2022) 18:582–8. doi: 10.1007/s12519-022-00553-1

74. Holm-Jacobsen JN, Vonasek JH, Hagstrøm S, Donneborg ML, Sørensen S. Prolonged rectal shedding of SARS-CoV-2 in a 22-day-old-neonate: a case report. *BMC Pediatr*. (2021) 21:506. doi: 10.1186/s12887-021-02976-7

75. Dergham J, Delerce J, Bedotto M, La Scola B, Moal V. Isolation of viable SARS-CoV-2 virus from feces of an immunocompromised patient suggesting a possible fecal mode of transmission. *J Clin Med*. (2021) 10:2696. doi: 10.3390/jcm10122696

76. Tian D, Sun Y, Xu H, Ye Q. The emergence and epidemic characteristics of the highly mutated SARS-CoV-2 Omicron variant. *J Med Virol*. (2022) 94:2376–83. doi: 10.1002/jmv.27643

77. Kim KW, Wang X, Adhikari A, Yeang M, Jenkins F, Naing Z, et al. Persistent high-level shedding of cultivable SARS-CoV-2 Delta virus 33 days after onset of COVID-19 in a hospitalized patient with pneumonia. *J Med Virol*. (2022) 94:4043–6. doi: 10.1002/jmv.27832

78. Sun W, Yang N, Mao Y, Yan D, Song Q, Qian G. Duration of viral shedding of the Omicron variant in asymptomatic and mild COVID-19 cases from Shanghai, China. *medRxiv*. (2022):2022.12.08.22283272. doi: 10.1101/2022.12.08.22283272v1

79. Pilapil JD, Notarte KI, Yeung KL. The dominance of co-circulating SARS-CoV-2 variants in wastewater. *Int J Hyg Environ Health*. (2023) 253:114224. doi: 10.1016/j.ijheh.2023.114224

80. Kim JY, Bae JY, Bae S, Cha HH, Kwon JS, Suh MH, et al. Diagnostic usefulness of subgenomic RNA detection of viable SARS-CoV-2 in patients with COVID-19. *Clin Microbiol Infect*. (2022) 28:101–6. doi: 10.1016/j.cmi.2021.08.009

Glossary

SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
GI	Gastrointestinal
RNA	Ribonucleic acid
RT-qPCR	Real-time quantitative reverse transcription polymerase chain reaction
ORF	Open reading frame
NGS	Next-generation sequencing
COVID-19	Coronavirus disease of 2019
VOCs	Variants of concern
ACE2	Angiotensin-converting enzyme 2
WHO	World Health Organization
PCR	Polymerase chain reaction
PBS	Polybutylene succinate
HKU	Hong Kong University
WHS	Whole-genome sequencing
BWA	Burrows-Wheeler Alignment
MEM	Minimum Essential Medium
FBS	Fetal bovine serum
CPE	Cytopathic effect
CT	Threshold cycle
EM	Electron microscopy

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External quality assessment for laboratories in pan-India ILI/SARI surveillance for simultaneous detection of influenza virus and SARS-CoV-2

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Introduction: The Indian Council of Medical Research has set up a nationwide network of 28 laboratories for simultaneous surveillance of influenza virus and SARS-CoV-2 in ILI/SARI patients, using an in-house developed and validated multiplex real-time RTPCR assay. The aim of this study was to ensure the quality of testing by these laboratories by implementing an external quality assessment program (EQAP).

Methods: For this EQAP, a proficiency test (PT) panel comprising tissue-culture or egg-grown influenza virus and SARS-CoV-2 was developed. The PT panel was distributed to all the participant laboratories, which tested the panel and submitted the qualitative results online to the EQAP provider. The performance of the laboratories was evaluated on qualitative criteria but cycle threshold (Ct) values were also gathered for each sample.

Results: On a qualitative basis, all the laboratories achieved the criteria of 90% concordance with the results of the PT panel provider. Ct values of different samples across the laboratories were within $\leq \pm 3$ cycles of the corresponding mean values of the respective sample. The results of this EQAP affirmed the quality and reliability of testing being done for simultaneous surveillance of influenza virus and SARS-CoV-2 in India.

KEYWORDS

EQAP, influenza, SARS-CoV-2, surveillance, ILI, SARI

Introduction

Respiratory infections are one of the two infections that are the topmost contributors to global disability-adjusted life years, the other being enteric infections (1). Respiratory infections have a significant impact on public health and the economy (2, 3). Amongst acute respiratory infections, the influenza virus is an important aetiology, especially in children and older adults, accounting for 3 to 5 million cases of severe disease annually (4). The COVID-19 pandemic added another pathogen to this group of infections. Over the last three years, since the advent of Severe Acute Respiratory Syndrome Corona Virus-2 (SARS-CoV-2), more than 656 million confirmed cases with 6.6 million mortalities have been reported globally up to 1 Jan, 2023 (5).

Owing to their RNA genome, both the influenza virus and SARS-CoV-2 are highly prone to mutations, resulting in the evolution of newer strains (6, 7). This characteristic grants both these viruses an ability to cause frequent outbreaks and occasional epidemics or pandemics. Annual outbreaks of influenza are well documented (4). Similarly, the frequent mutations in the SARS-CoV-2 genome have resulted in multiple variants of interest and variants of concern that kept the COVID-19 pandemic ongoing for nearly three years (8). This ability of both these viruses, coupled with a similar clinical picture, emphasizes the need for continuous simultaneous surveillance of these respiratory viruses. The World Health Organization (WHO) in Nov 2020 advised that surveillance for both influenza virus and SARS-CoV-2 be integrated, and the data be reported through the WHO Global Influenza Surveillance and Response System (GISRS) (9). GISRS has been in use for global influenza surveillance since 1952 and has played an important role in the timely detection of globally circulating influenza strains. This platform has facilitated the identification of emerging or reemerging influenza strains. To strengthen the influenza surveillance being done until recently in India in a limited manner by the Indian Council of Medical Research-National Institute of Virology (ICMR-NIV), and to initiate the simultaneous detection of influenza virus and SARS-CoV-2, ICMR established a pan-India surveillance network for influenza virus and SARS-CoV-2 by real-time reverse transcriptase Polymerase Chain Reaction (rRT-PCR) (10). Between 4 July 2021 and 31 October 2022, the network tested 34,260 samples, of which 12.84% samples were positive for one of the two viruses tested along with 37 dual/co-infections (11). Given the multitudinous number of samples being tested by this wide network of laboratories, it is essential to ensure the reliability of testing. This communication describes the external quality assessment program (EQAP) for all the participating laboratories, to establish a quality-assured system for simultaneous qualitative detection and differentiation of influenza virus and SARS-CoV-2 in ILI/SARI patients. EQAP has played a pivotal role in establishing the quality of molecular testing for influenza virus (12) and SARS-CoV-2 (13–15) as individual viruses. To our knowledge, this is the first report on simultaneous EQAP for influenza virus and SARS-CoV-2 virus.

Methods

EQAP organization

The pan-India surveillance network for influenza virus and SARS-CoV-2 is a three-tiered structure that has 28 laboratories across the length and breadth of the country (10, 11). These laboratories participated in the surveillance of these two respiratory viruses in samples collected from ILI & SARI patients from hospital and defined community settings (11). To ensure the reliability of testing, an EQAP was administered by the ICMR-NIV Pune which is a WHO-NIC for influenza and SARS-CoV-2 reference centre. The program was coordinated by the ICMR Headquarters (ICMR-HQ) to maintain confidentiality and transparency of the results. All testing laboratories barring two participated. The complete flow of the EQAP is depicted in Figure 1.

Panel composition

EQAP comprised a proficiency test (PT) panel of 12 coded samples. A group of external experts (clinical microbiologists) was constituted by the ICMR-HQ to deliberate on the composition of the PT panel and decide on the scoring and passing criteria. Based on the recommendations of the experts, the PT panel included six contemporary influenza strains, including one avian A(H9N2) virus, two SARS-CoV 2 virus and two negative controls. There were two samples each of influenza A(H1N1)pdm09 and SARS-CoV-2 to represent different viral loads of the same virus type. The panel also included two samples for allelic discrimination by rRT-PCR for testing antiviral susceptibility. The composition of the panel is depicted in Table 1. Each virus sample, except A(H9N2), was an *in-vitro* grown virus whereas A(H9N2) was an egg grown virus. All the viruses were inactivated prior to aliquoting. The PT panel was divided into two sections – section 1, for diagnosis of influenza and SARS CoV 2, comprised of the first ten samples and section 2, for detection of the antiviral susceptibility of A(H1N1)pdm09, comprised of the last two samples. The PT panel was jumbled in different combinations, before shipping out, to avoid any biasness between the laboratories. Each participating laboratory was asked to process samples following the exact procedure that would be used for the patient's sample. The laboratories had to complete the testing of the PT panel by rRT-PCR using the in-house developed multiplex molecular assay, the testing method used in the pan-India ILI/SARI surveillance network (10), within 10 days from the date of receipt of the PT panel.

Panel preparation

For preparing the PT panel, early passage Madin-Darby canine kidney (MDCK) cells were infected with previously isolated seasonal influenza viruses. The viruses used for infection had a HA titer of 8–16. After infection, the cells were incubated at 37°C for 3 days in 5% CO_2 or until the CPE appeared. The culture supernatant was harvested and clarified of cell debris by centrifugation at 10,000 rpm at 4°C. Each virus harvest was antigenically characterized by Haemagglutination assay (HA) and Haemagglutination inhibition assay (HI), as per the protocol described in the WHO manual (16), to determine the HA titer and confirm the subtype of each virus. Once characterized, each virus was heat-inactivated at 50°C for 30 min. The confirmation of complete inactivation was done by three passages of the inactivated virus in MDCK cells. Once confirmed, the viruses were aliquoted in 1 mL aliquots and stored frozen, until shipped, at −80°C. In addition to seasonal influenza viruses, avian influenza A(H9N2) was grown in eggs and gamma irradiated to inactivate the virus (17).

Abbreviations: EQAP, External quality qssurance program; ILI, Influenza like illness; SARI, Severe acute respiratory infection; SARS-CoV-2, Severe Acute Respiratory Syndrome Corona Virus-2; PT, Proficiency test; CLIA, Clinical Laboratory Improvement Amendments; CPE, Cytopathic effect; Ct, Cycle threshold.

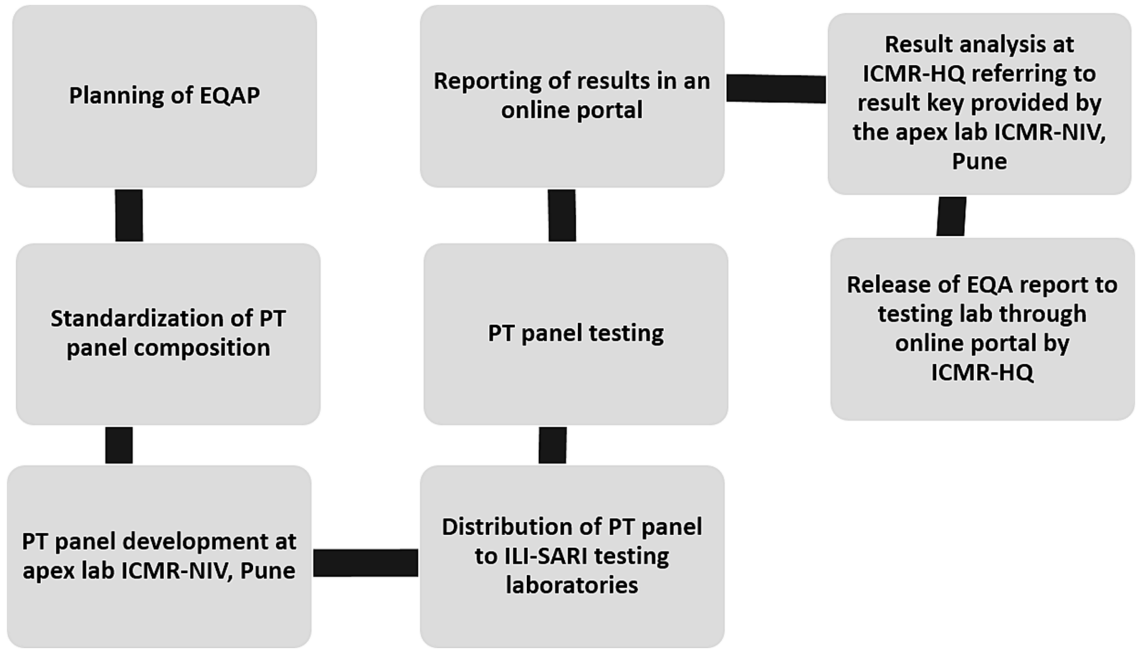

FIGURE 1
EQAP process flow.

TABLE 1 Composition of proficiency test panel used for EQAP.

EQA sample code	Sample type	Virus content
Sample_1	Influenza	Influenza A(H1N1)pdm09
Sample_2	Influenza	Influenza B/Victoria
Sample_3	Negative	Cell culture supernatant
Sample_4	Influenza	Influenza A(H3N2)
Sample_5	SARS-CoV-2	SARS-CoV-2
Sample_6	Influenza	Influenza B/Yamagata
Sample_7	Negative	Cell culture supernatant
Sample_8	Influenza	Influenza A(H1N1)pdm09
Sample_9	SARS-CoV-2	SARS-CoV-2
Sample_10	Influenza	Inf A unsubtypable (Unusual subtype – H9N2)
Sample_11	Influenza	A(H1N1)pdm09 virus wild type (sensitive)
Sample_12	Influenza	A(H1N1)pdm09 virus mutant type (resistant)

The SARS-CoV-2 (Delta variant B.1.617.2) was received as an inactivated virus from the high containment lab at NIV-Pune (personal communication).

Result submission and scoring

The key of each combination, based on the results assigned by the apex laboratory ICMR-NIV, was with ICMR-HQ. Each participating laboratory entered results online at the ILI/SARI surveillance portal that is in routine use to capture the surveillance data (10). The ICMR-HQ decoded the results submitted by the laboratories and computed the concordance scores. The scoring was done based only on the results of 10 samples of section one. Each sample was worth a score of 10. For samples no. 1, 2, 4, 6, 8, and 10 (influenza positive), each sample was assessed on two parameters: differentiation of influenza A and influenza B, and identification of subtypes of influenza A or influenza B (each correct reporting fetched a score of 5 each). The sample that had an unusual subtype was to be identified and reported as influenza A un-subtyped. Performance on antiviral susceptibility samples (sample no. 11 and 12) was not considered while computing the scores. These samples

TABLE 2 HA and HI titers of *in-vitro* propagated seasonal influenza viruses that were used for PT panel preparation.

Virus subtype	HA titer	HI titer
Influenza A(H1N1)pdm09	1:32	1:320
Influenza A(H3N2)	1:16	1:1280
Influenza B/Yamagata	1:64	320
Influenza B/Victoria	1:64	640

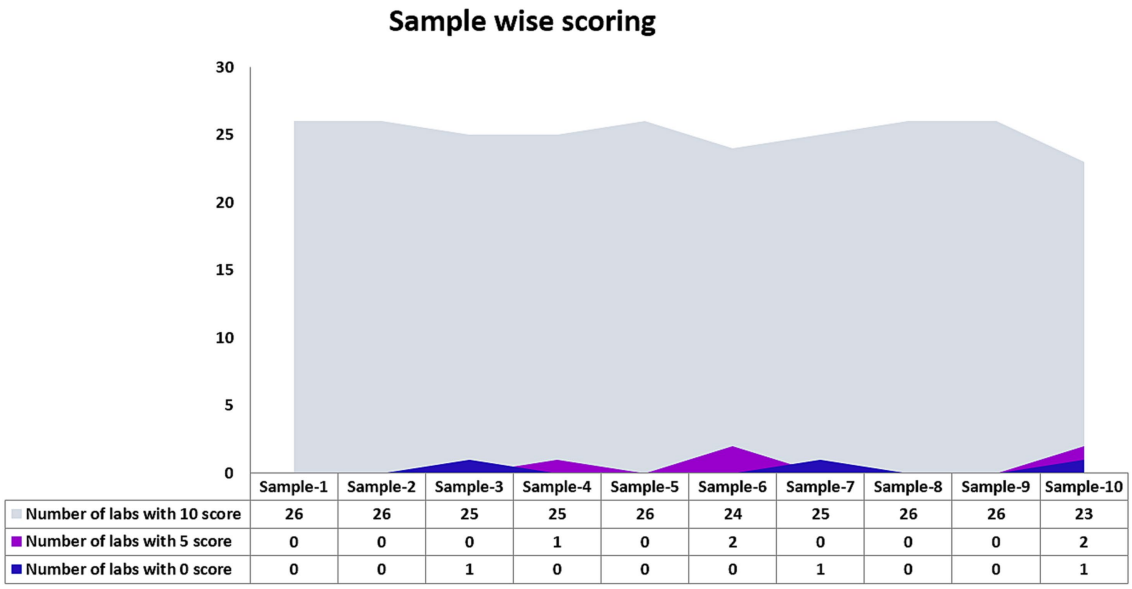

FIGURE 2
Number of laboratories that detected each sample in section 1 of the PT panel.

were used only to provide feedback on the quality of testing of the laboratory. Each laboratory that scored 90% concordance or more was considered as passing the EQAP.

Results

This was a qualitative EQAP. Semi-quantitative results (Ct value) were used for reference only.

Panel characterization at ICMR-NIV

The *in-vitro* propagated isolates of seasonal influenza viruses underwent antigenic characterization. The HA titer of each isolate and their HI titer against respective homologous reference serum is shown in Table 2. Further, each *in-vitro* propagated isolate of seasonal influenza viruses underwent sequencing to check if there have been any changes in genes of the respective viruses due to passage in cell culture. The M and HA gene sequences of the seasonal influenza viruses used in the PT panel aligned completely with the primers and probes used in the multiplex molecular assay, that is used in the pan-India ILI/SARI surveillance network (results not shown), confirming the suitability of the kit for the panel.

PT panel results of the testing laboratories

Twenty-six laboratories that participated in the EQAP tested all the samples in the panel and reported results within the stipulated time. The number of laboratories that detected each sample correctly is shown in Figure 2. Except for one sample by one laboratory, none of the laboratories reported false-negative results and all the laboratories could correctly identify the influenza A, influenza B, or SARS-CoV-2 sample. While subtyping, all the laboratories correctly identified A(H1N1)pdm09 and B/Victoria lineage. A(H3N2) was reported as untypable by one lab and the B/Yamagata lineage was missed by two laboratories. Two laboratories reported influenza A unusual subtype sample as H1N1. False positives were reported by two laboratories (one sample in each lab). Despite these odd reports, all the participating laboratories passed the EQAP by scoring 90% concordance or more (Figure 3).

Semi-quantitative performance of the laboratories

Figure 4 shows the Ct value of each sample (except the negative samples) within section 1 of the PT panel for differentiation of influenza A, influenza B, and SARS-CoV-2 samples. Most of the

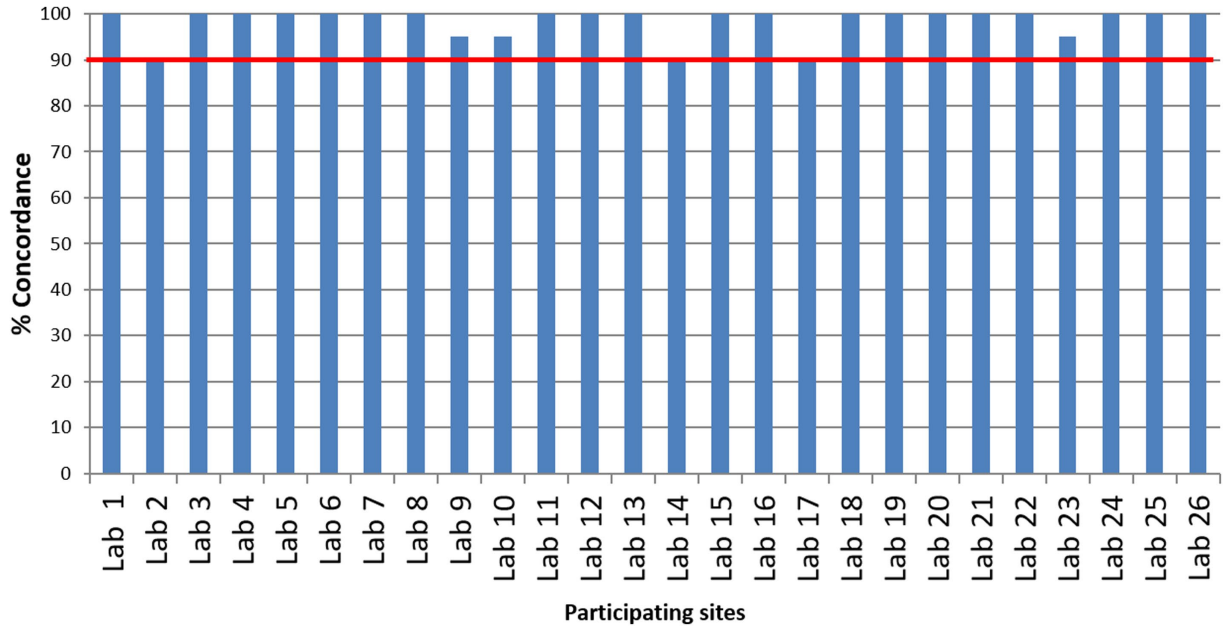

FIGURE 3
Overall performance of the laboratories.

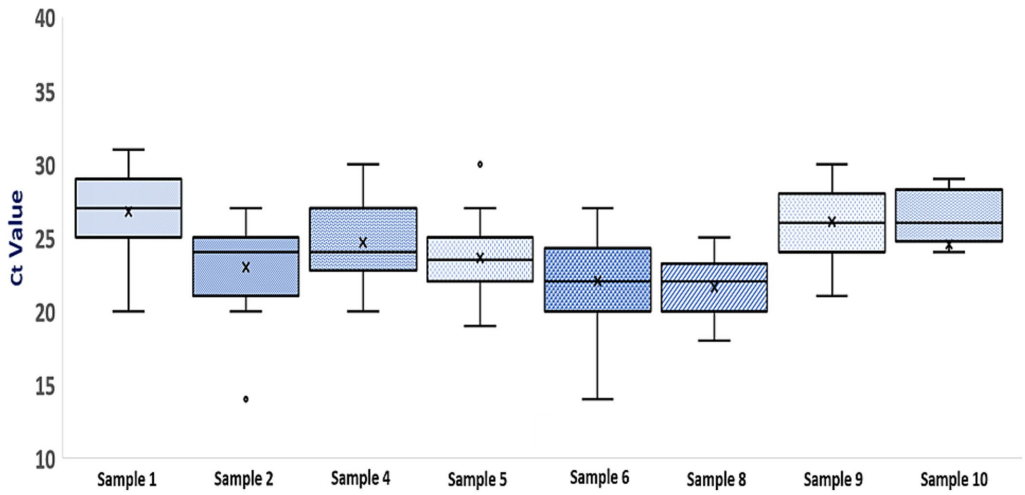

FIGURE 4
Boxplot of Ct values for samples in section 1 of the PT panel (Each box represents an individual sample. The top and bottom lines of each boxplot represent the third and first quartiles, respectively. The horizontal bar and X within each box represent the median Ct value and mean Ct value for that sample. For each sample, minimum and maximum Ct values are represented by error bars and outliers are represented by O).

laboratories (73–92%) reported Ct values for individual samples that are within $\leq \pm 3$ cycles of corresponding mean values of the respective sample. When analyzed in terms of interquartile spread, there was an occasional outlier for sample numbers 2 and 5. Nevertheless, most of the laboratories reported Ct values for individual samples that were within the lower and upper quartile of the respective sample. All the laboratories could also discriminate between different viral loads of influenza A(H1N1)pdm09 and SARS-CoV-2, as is seen by the corresponding Ct values, except one lab which failed to do so for high titred SARS-CoV-2 sample (sample number 5).

Discussion

India initiated surveillance for viral aetiologies of acute respiratory infections by leveraging the network of laboratories that was created under the VRDL scheme (10). The pan-India

ILI/SARI surveillance network provided insight into the prevalence of different influenza viruses and SARS-CoV-2 in India over a period of about 15 months, which varied depending on the time of the year and seasonality (11). Given the numerous number of tests being done, it was imperative to gain confidence in the reliability of the test results. EQAP, through PT panel testing, is a time-tested method in laboratory medicine to address discrepancies amongst laboratories when the same analyte is measured by the same method (18). EQAP permits simultaneous testing of multiple samples that vary from negative to strong positive samples. This allows covering the entire spectrum of samples that a laboratory can potentially test. Furthermore, EQAP is run in a coded manner where neither the panel composition nor the probable results are known to the participating laboratories. This simulates the everyday operations of the lab. Independent third-party monitoring of results reported by the testing laboratories, in comparison to the results reported by the EQAP provider, enhances the confidence of all the participating laboratories in the process. EQAP is a tool that has been extensively used as a measure of the quality of testing in multiple test methods (14, 15, 19–23) including molecular testing for influenza virus and SARS-CoV-2 (12, 13). EQAP for influenza virus and SARS-CoV-2 have been standalone activities (12–15) and there has been no published report on a simultaneous EQAP for both these viruses. Therefore, an EQAP was developed and administered by the national reference laboratory at ICMR-NIV Pune to ascertain the quality of integrated testing for influenza virus and SARS-CoV-2 in the pan-India ILI/SARI surveillance network.

Qualitatively, the performance of all the participating laboratories was satisfactory as they all met the qualifying criteria. The qualitative results when analyzed semi-quantitatively, showed small discrepancies in Ct values for the same sample amongst laboratories. Nevertheless, the laboratories could correctly discriminate different viral loads of the same virus (sample 1 vs. 8 and sample 5 vs. 9). Variation in Ct values for the same sample across laboratories has been reported in other EQAPs too (14, 24, 25). Variation in Ct could be attributed to the lack of adherence to good laboratory practices such as the use of standardized test protocol, regular calibration of equipment, and technical ability of staff familiar with the protocol. Such practices are known to impact the performance of laboratories. The performance of the laboratories that follow CLIA regulations in the U.S.A. has been shown to be better (26), thus emphasizing the need to follow certain minimum standards. Caution is advised while relying on Ct values for patient management. However, some clinicians opine that Ct values are helpful in decision-making (27) and thus request laboratories to report Ct values. For reporting comparable Ct values across the pan-India network, laboratories are advised to locally standardize and validate the test protocol and emphasis needs to be laid on the calibration of test equipment. A limitation of our PT panel was that the panel comprised of cell-culture or egg-grown viruses and thus did not represent an actual clinical sample. We resorted to this strategy due to the lack of availability of clinical samples in sufficient volumes. Our panel could not accommodate samples of all the viruses that represented high, medium, and low viral loads due to logistics issues. Therefore, we used two samples with varying viral load and the rest of the samples had medium viral load, for a fair performance assessment of the laboratories. Another limitation was that no reference standard was included in the panel to address the standardization of the test protocol. The EQAP indicated that integrated influenza virus and SARS-CoV-2 diagnosis, on a qualitative level, by all the laboratories participating in the pan-India ILI/SARI surveillance network is accurate and the in-house developed multiplex molecular assay performs reliably. From an epidemiological perspective, this is significant as co-circulation of influenza and SARS-CoV-2 has been documented in India (10). Thus, it becomes important that assays in use detect the underlying pathogen, accurately and reliably, without raising false positives. To our knowledge, this is the first national-level EQAP to assess the capability of testing laboratories to simultaneously detect influenza virus and SARS-CoV-2 using a multiplex rRT-PCR.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

VP: Methodology, Resources, Writing – review & editing. NV: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Project administration, Writing – review & editing. SJ: Methodology, Resources, Validation, Writing – review & editing. VV: Methodology, Resources, Writing – review & editing. NG: Conceptualization, Supervision, Writing – review & editing. NA: Conceptualization, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1274508/full#supplementary-material

References

1. Murray CJ, Vos T, Lozano R, Naghavi M, Flaxman AD, Michaud C, et al. Disability-adjusted life years (DALYs) for 291 diseases and injuries in 21 regions, 1990-2010: a systematic analysis for the global burden of disease study 2010. *Lancet*. (2012) 380:2197–223. doi: 10.1016/S0140-6736(12)61689-4

2. Troeger C, Blacker B, Khalil IA, Rao PC, Cao J, et al. GBD 2016 lower respiratory infections collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet Infect Dis*. (2018) 18:1191–210. doi: 10.1016/S1473-3099(18)30310-4

3. Putri WCWS, Muscatello DJ, Stockwell MS, Newall AT. Economic burden of seasonal influenza in the United States. *Vaccine*. (2018) 36:3960–6. doi: 10.1016/j.vaccine.2018.05.057

4. World Health Organization. Influenza (Seasonal). (2023). Available at: https://www.who.int/news-room/fact-sheets/detail/influenza-(seasonal).

5. World Health Organization. Weekly epidemiological update on covid-19 (2023). Available at: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---4-january-2023.

6. Petrova VN, Russell CA. The evolution of seasonal influenza viruses. *Nat Rev Microbiol*. (2018) 16:47–60. doi: 10.1038/nrmicro.2017.118

7. Harvey WT, Carabelli AM, Jackson B, Gupta RK, Thomson EC, Harrison EM, et al. SARS-CoV-2 variants, spike mutations and immune escape. *Nat Rev Microbiol*. (2021) 19:409–24. doi: 10.1038/s41579-021-00573-0

8. Centre for Disease Control and Prevention. SARS-CoV-2 Variant classification and definitions (2023). Available at: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html.

9. End-to-end integration of SARS-CoV-2 and influenza sentinel surveillance: revised interim guidance. (2023). Available at: https://www.who.int/publications/i/item/WHO-2019-nCoV-Integrated_sentinel_surveillance-2022.1.

10. Aggarwal N, Potdar V, Vijay N, Mukhopadhyay L, Borkakoty B, Manjusree S, et al. SARS-CoV-2 and influenza virus co-infection cases identified through ILI/SARI sentinel surveillance: a Pan-India report. *Viruses*. (2022) 14:627. doi: 10.3390/v14030627

11. Potdar V, Vijay N, Mukhopadhyay L, Aggarwal N, Bhardwaj SD, Choudhary ML, et al. Pan-India influenza-like illness and severe acute respiratory illness: epidemiological, clinical and genomic analysis. *Front Public Health*. (2023) 11:1218292. doi: 10.3389/fpubh.2023.1218292

12. Popowich MD, Brunt SJ, Bennett RT, George KS. Development of a proficiency testing program for molecular diagnosis of influenza viruses. *J Clin Virol*. (2012) 54:245–50. doi: 10.1016/j.jcv.2012.04.001

13. Lau KA, Kaufer A, Gray J, Theis T, Rawlinson WD. Proficiency testing for SARS-CoV-2 in assuring the quality and overall performance in viral RNA detection in clinical and public health laboratories. *Pathology*. (2022) 54:472–8. doi: 10.1016/j.pathol.2022.01.006

14. Sung H, Han MG, Yoo CK, Lee SW, Chung YS, Park JS, et al. Nationwide external quality assessment of SARS-CoV-2 molecular testing, South Korea. *Emerg Infect Dis*. (2020) 26:2353–60. doi: 10.3201/eid2610.202551

15. Kaur H, Mukhopadhyay L, Gupta N, Aggarwal N, Sangal L, Potdar V, et al. External quality assessment of COVID-19 real time reverse transcription PCR laboratories in India. *PLoS One*. (2022) 17:e0263736. doi: 10.1371/journal.pone.0263736

16. WHO manual on animal influenza diagnosis and surveillance. *World Health Organization*. (2002). Available at: https://apps.who.int/iris/handle/10665/68026

17. Pawar SD, Tandale BV, Raut CG, Parkhi SS, Barde TD, Gurav YK, et al. Avian influenza H9N2 seroprevalence among poultry workers in Pune, India, 2010. *PLoS One*. (2012) 7:e36374. doi: 10.1371/journal.pone.0036374

18. Miller WG, Jones GR, Horowitz GL, Weykamp C. Proficiency testing/external quality assessment: current challenges and future directions. *Clin Chem*. (2011) 57:1670–80. doi: 10.1373/clinchem.2011.168641

19. Donoso Mantke O, McCulloch E, Wallace PS, Yue C, Baylis SA, Niedrig M. External quality assessment (EQA) for molecular diagnostics of Zika virus: experiences from an international EQA Programme, 2016–2018. *Viruses*. (2018) 10:491. doi: 10.3390/v10090491

20. Pu T, Shui R, Shi J, Liang Z, Yang W, Bu H, et al. China anticancer association professional Committee of Tumour Pathology. External quality assessment (EQA) program for the immunohistochemical detection of ER, PR and Ki-67 in breast cancer: results of an interlaboratory reproducibility ring study in China. *BMC Cancer*. (2019) 19:978. doi: 10.1186/s12885-019-6210-3

21. Secchiero S, Fogazzi GB, Manoni F, Epifani M, Plebani M. The Italian external quality assessment (EQA) program on urinary sediment by microscopy examination: a 20 years journey. *Clin Chem Lab Med*. (2020) 59:845–56. doi: 10.1515/cclm-2020-1656

22. Mögling R, Colavita F, Reimerink J, Melidou A, Leitmeyer K, Keramarou M, et al. External quality assessment of SARS-CoV-2 serology in European expert laboratories, April 2021. *Euro Surveill*. (2022) 27:2101057. doi: 10.2807/1560-7917.ES.2022.27.42.2101057

23. Zhou W, Luo W, Yu S, Li H, Wang D, Zhang J, et al. Performance of HDL-C measurements assessed by a 4-year trueness-based EQA/PT program in China. *Clin Chem Lab Med*. (2022) 60:1586–97. doi: 10.1515/cclm-2020-0658

24. Buchta C, Görzer I, Chiba P, Camp JV, Holzmann H, Puchhammer-Stöckl E, et al. Variability of cycle threshold values in an external quality assessment scheme for detection of the SARS-CoV-2 virus genome by RT-PCR. *Clin Chem Lab Med*. (2021) 59:987–94. doi: 10.1515/cclm-2020-1602

25. Li RH, Wang QY. A localized small-scale external quality assessment (EQA) for PCR testing of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in the molecular laboratories. *J Virol Methods*. (2022) 301:114441. doi: 10.1016/j.jviromet.2021.114441

26. James D, Ames D, Lopez B, Still R, Simpson W, Twomey P. External quality assessment: best practice. *J Clin Pathol*. (2014) 67:651–5. doi: 10.1136/jclinpath-2013-201621

27. Service RF. A call for diagnostic tests to report viral load. *Science*. (2020) 370:22. doi: 10.1126/science.370.6512.22

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Genomic evolution of BA.5.2 and BF.7.14 derived lineages causing SARS-CoV-2 outbreak at the end of 2022 in China

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Since the end of 2022, when China adjusted its COVID-19 response measures, the SARS-CoV-2 epidemic has rapidly grown in the country. It is very necessary to monitor the evolutionary dynamic of epidemic variants. However, detailed reports presenting viral genome characteristics in China during this period are limited. In this study, we examined the epidemiological, genomic, and evolutionary characteristics of the SARS-CoV-2 genomes from China. We analyzed nearly 20,000 genomes belonging to 17 lineages, predominantly including BF.7.14 (22.3%), DY.2 (17.3%), DY.4 (15.5%), and BA.5.2.48 (11.9%). The Rt value increased rapidly after mid-November 2022, reaching its peak at the end of the month. We identified forty-three core mutations in the S gene and forty-seven core mutations in the ORF1ab gene. The positive selection of all circulating lineages was primarily due to non-synonymous substitutions in the S1 region. These findings provide insights into the genomic characteristics of SARS-CoV-2 genomes in China following the relaxation of the 'dynamic zero-COVID' policy and emphasize the importance of ongoing genomic monitoring.

KEYWORDS

SARS-CoV-2, lineage, selection pressure, mutation, China

1 Introduction

The coronavirus disease 2019 (COVID-19) pandemic, caused by the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been ongoing for over three years, posing an unprecedented challenge to global public health (1). As of August 1, 2023, there have been over 768 million confirmed cases and approximately 7 million cumulative deaths recorded as a result of SARS-CoV-2 infection (https://covid19.who.int/). Moreover, the number of patients infected with the virus continues to rise (2). The genome of SARS-CoV-2 undergoes mutations during viral replication, leading to the emergence of mutated viruses that are subjected to selective pressures. During its rapid spread and explosive radiation, SARS-CoV-2 has accumulated mutations on a genome-wide scale, continuously evolving into new variants that spread quickly to different parts of the world (3–6). To date, five variants of concern (VOCs) have emerged and been designated for monitoring: alpha, beta, gamma, delta, and omicron (7). Since its emergence in November 2021, the Omicron variants has acquired a significant number of new mutations, with 80% of them

accumulating in the S protein. This has resulted in the development of more than 700 Omicron lineages, including five main lineages (BA.1, BA.2, BA.3, BA.4, and BA.5) (8). The Omicron variant, with over 30 mutations in the S protein, has spread worldwide within a few months. It is believed to be more infectious and capable of immune escape than previous VOCs (9, 10). The evolution of SARS-CoV-2 is continuing, which leads to the expected generation of new variants. As of July 30, 2023, two Variants of Interest (VoIs) (i.e., XBB.1.5 and XBB.1.16) and seven Variants under Monitoring (VuMs) (i.e., BA.2.75, CH.1.1, XBB, XBB.1.9.1, XBB.2.3, and EG.5) were listed by the World Health Organization (WHO) (https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---3-august-2023).

Variants of SARS-CoV-2, especially those linked to epidemiological events, are currently being closely monitored by the WHO and other public health agencies worldwide (11). Whole-genome sequencing (WGS) greatly aids in tracking viral genomic changes and helps in comprehending phenotypic changes (12). In the last three years, a significant volume of genomic data has been generated, informing local and international communities about crucial aspects of the pandemic. This data has served as a foundation for adjusting prevention and control strategies (13).

At the end of 2022, China discontinued the 'dynamic zero-COVID' policy (14). Subsequently, China has entered a new phase, where the number of people infected with Omicron has surged (15). To monitor the evolutionary process of epidemic variants in China is very important (16). This study primarily concentrates on examining the genomic and evolutionary features of SARS-CoV-2 genomes in China during this period.

2 Materials and methods

2.1 Sample selection and nucleic acid test

From December 2022 to January 2023, nasopharyngeal samples were collected from individuals at the Clinical Laboratory of Sonya People's Hospital and preserved in 3 mL of inactivated viral sample preservation solution. A 200 μL sample was taken from each specimen to extract total RNA using the QIAamp Viral RNA Mini Kit (Qiagen, Germany), which was then eluted with RNase-free water. The concentration of total RNA was determined using the Qubit 2.0 fluorometer (Invitrogen, United States). The clinical one-step real-time PCR was conducted to detect SARS-CoV-2 by targeting the ORF1ab and N genes, respectively. This was done using the 2019-nCoV detection kit (PCR-Fluorescence) in accordance with the manufacturer's instructions (17). The total RNA from positive samples was stored at −80°C until further analysis.

2.2 Whole-genome sequencing and assembly

The total RNA from SARS-CoV-2 positive samples with a Ct value of 35 or less in both the ORF1ab and N genes was randomly selected for next-generation sequencing (NGS). The library was prepared using the ATOPlex RNA Multiplex PCR-based Library Preparation Set V3.1, following the manufacturer's instructions (940–000132-00, MGI, China). The process included reverse transcription, purification, end repair, and adaptor addition. The obtained library underwent further processing using the DNBSEQ One-step DNB Preparation Kit (1,000,026,466, MGI, China) and was subsequently sequenced on the MGISEQ-2000RS platform using SE100 technology.

The raw data (fastq reads) from each sample were filtered using FastQC tool v0.11.9 (18). Trimmomatic v0.39 (19) was then used to remove adapter sequences and low-quality base calls ($Q < 30$). The filtered reads, which were mapped to the Wuhan-Hu-1 genome (NC_045512.2), were utilized to acquire the consensus sequence for each sample through SPAdes v3.15.4 (20).

2.3 Variant calling and phylogenetic analysis

All complete SARS-CoV-2 genomes that belonged to the dominant lineages circulating in mainland China after the implementation of 10 new measures were downloaded from the GISAID and NCBI database (as of April 27, 2023). Genomes with incomplete collection dates and low coverage were excluded. The PANGO lineages of all genomes were determined according to the pangolin nomenclature. An in-house script was used to parse genomes with "N" and low coverage in order to identify the single nucleotide polymorphisms (SNPs) (21). The mutations in each genome were identified using Wuhan-Hu-1 as the reference. The frequency of each variant site was calculated by dividing the number of genomes containing the site by the total number of genomes in the lineage. A mutation with a frequency between 0.8 and 1.0 was considered a core mutation. The alignment was obtained using Nextalign, and phylogenetic analysis was performed using Nextstrain pipelines v12 under the SARS-CoV-2 workflow (22). The resulting tree was visualized using the online tool auspice (https://auspice.us/).

2.4 Non-synonymous (Ka) and synonymous (Ks) calculation

The nucleotides and amino acids from ORFs of each genome were predicted and obtained using ORFfinder (https://www.ncbi.nlm.nih.gov/orffinder/). The protein-coding DNA alignments were constructed using Parallel Alignment and Back-Translation (ParaAT v2.0) (23). The rates of non-synonymous substitutions (Ka), synonymous substitutions (Ks), and Ka/Ks ratio between the subject genome and the reference genome were calculated using the PAML-yn00 pipeline with the Yang and Nielsen (YN) method (24).

2.5 Statistical analysis

The instantaneous effective reproduction number (*Rt*) is estimated using the R package EpiEstim v0.1 (25). The estimation is based on the number of genomes reported per day, with generation time of 3.0 days and incubation periods of 4.0 days (26). Statistical plots were generated using Origin Pro 2021 version. The significance of differences was assessed using the χ^2, Mann–Whitney U test, or Wilcoxon test, with $p < 0.05$ indicating statistical significance.

3 Results

3.1 Genomic epidemiology of variants circulating in China

From December 2022 to January 2023, specimens that tested positive for SARS-CoV-2 were collected in Sonya, of which 90 specimens were randomly selected for whole genome sequencing. To analyze the genomic epidemiology after ending the zero-COVID policy in the Chinese mainland, the genomes of circulating lineages and their corresponding metadata were downloaded from the GISAID and NCBI database. A total of 19,749 genomes, including those from this study, were used to investigate genomic epidemiology. The study showed that these viruses were prevalent in 30 provincial-level administrative regions in the Chinese mainland (Figure 1A), with the highest number of genomes reported in Beijing (1640), Chongqing (1575), Fujian (1506), Gansu (1280), Guangdong (1122), and Guangxi (1017). The dominant pangolin lineages were composed of BF.7.14 (4,396, 22.3%), DY.2 (3,425, 17.3%), DY.4 (3,070, 15.5%), BA.5.2.48 (2,358, 11.9%), DY.1 (1832, 9.3%), and DY.3 (1,505, 7.6%) (Figure 1B and Supplementary Figure S1). The number of confirmed cases in the SARS-CoV-2 outbreak increased rapidly from December 2022 to February 2023, with most new additions in January 2023 (Figure 1C and Supplementary Figure S1). The number of new cases decreased to low levels in April 2023. The frequencies of the different lineages varied across regions.

The *Rt* is estimated based on the number of genomes reported per day (Figure 2). From October to mid-November 2022, the circulating

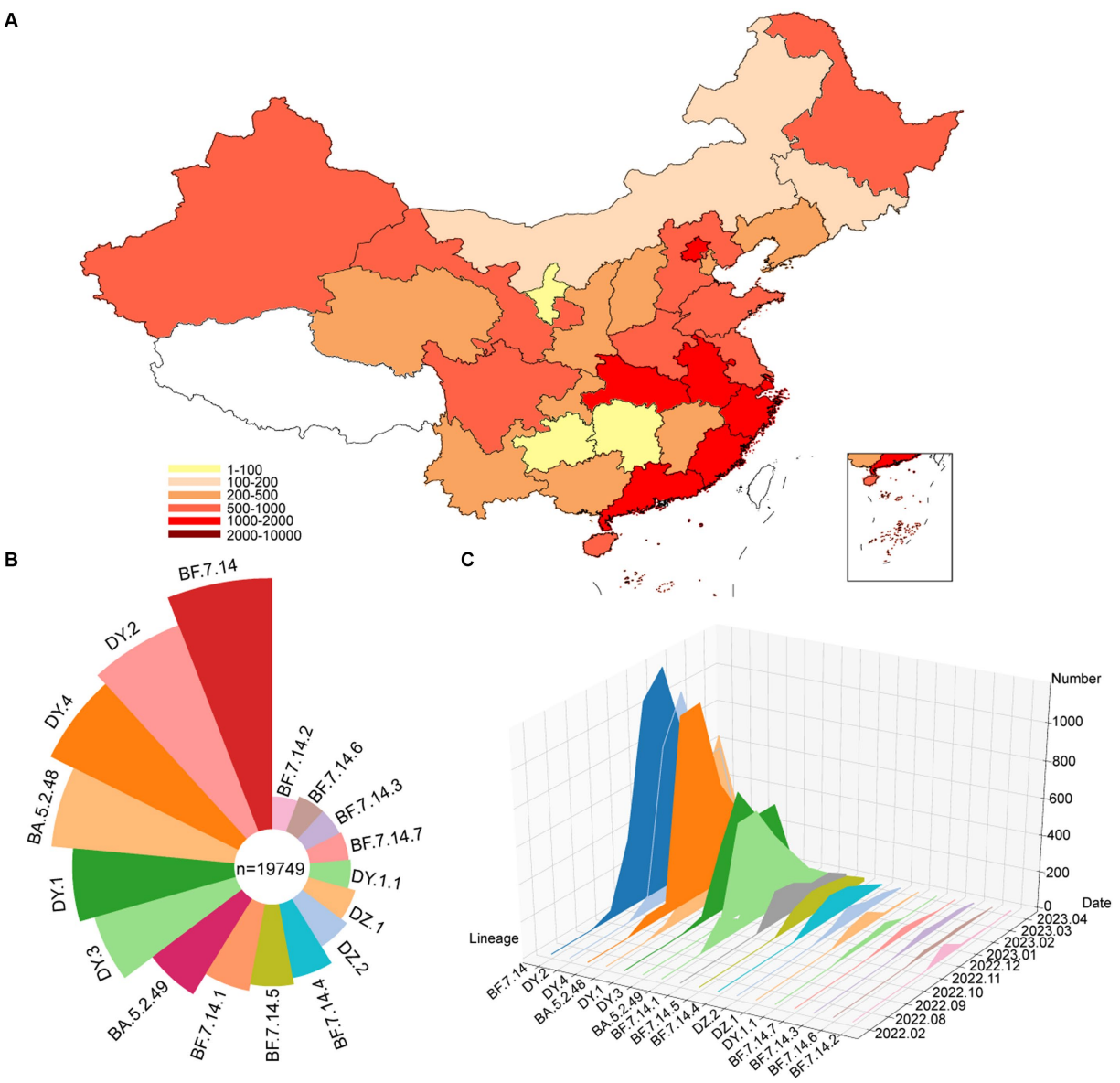

FIGURE 1
Temporal contribution of circulating SARS-CoV-2 lineages after ending the dynamic zero-COVID policy. **(A)** Map showing the genome contribution in Chinese mainland. The number of SARS-CoV-2 genomes are defined by the color-bar. **(B)** Overall SARS-CoV-2 lineages contribution in Chinese mainland. The lineages are represented by corresponding colors. The size represents the proportion of cases of each lineage out of 19,749 genomes. **(C)** The genome number of each circulating SARS-CoV-2 lineages changes over month. A total of 17 circulating lineages are presented from first detected to April 2023.

lineages (BA.5.2, BF.7.14 and their descendant lineages) were observed in China and underwent local transmission. The estimated *Rt* was increased rapidly after mid-November 2022, and peaked at the end of the month. However, there was an unexpected decrease in the *Rt*, which fell below 1 in early December, which may result from under-sequencing of SARS-CoV-2 during this period. Nevertheless, a second peak was observed in mid-December. From January to April, 2023, *Rt* decreased gradually under 1 and showed a fluctuation around 1 during late January and early February.

3.2 Evolutionary relationship

After removing low-quality genomes, a total of 10,474 SARS-CoV-2 genomes were utilized to construct a phylogenetic tree. The results, shown in Figure 3A, indicated that these sequences formed three main clades (L1-L3). Clade L1 represented BA.5.2.48 and its descendant lineages (DY.1, DY.1.1, DY.2, DY.3, and DY.4), L2 represented BA.5.2.49 and its descendant lineages (DZ.1 and DZ.2), and L3 represented BF.7.14 and its descendant lineages (BF.7.14.1, BF.7.14.2, BF.7.14.3, BF.7.14.4, BF.7.14.5, and BF.7.14.7). Except for DY.3, BA.5.2.49, and BF.7.14, all of the lineages, were first detected in the Chinese mainland.[1]

The evolutionary origins of these dominant lineages have been estimated. The temporal signal test results (Supplementary Figure S2) showed a significant correlation between the sampling dates and the root-to-tip distance ($R^2 = 0.818$). The estimated rate was 31.338 substitutions per year (Supplementary Figure S2). According to Figure 3B, the estimated date for the most recent common ancestor (TMRCA) of BF.7.14 and its descendant lineages was March 29, 2022, with a confidence interval of October 18, 2021, to April 13, 2022. The emergence date of BA.5.2.49 and its descendant lineages was inferred to be July 29, 2022, with a confidence interval from June 26, 2022, to August 21, 2022. The projected date for the emergence of DY.4 is estimated to be July 19, 2022, with a confidence interval from June 5, 2021, to July 26, 2022.

1 https://github.com/cov-lineages/pango-designation/blob/master/lineages.csv

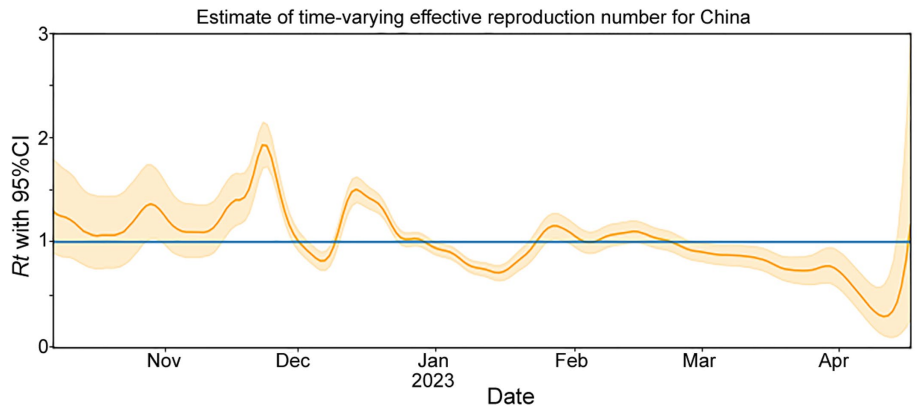

FIGURE 2
The effective reproduction number (*Rt*) for COVID-19 in China during November 2022 to April 2023. The solid yellow line represents the maximum likelihood estimates. The yellow background indicates their 95% confidence intervals (CI) based on the 2.5% quantile and 97.5% quantile. The blue line represents *Rt* = 1.

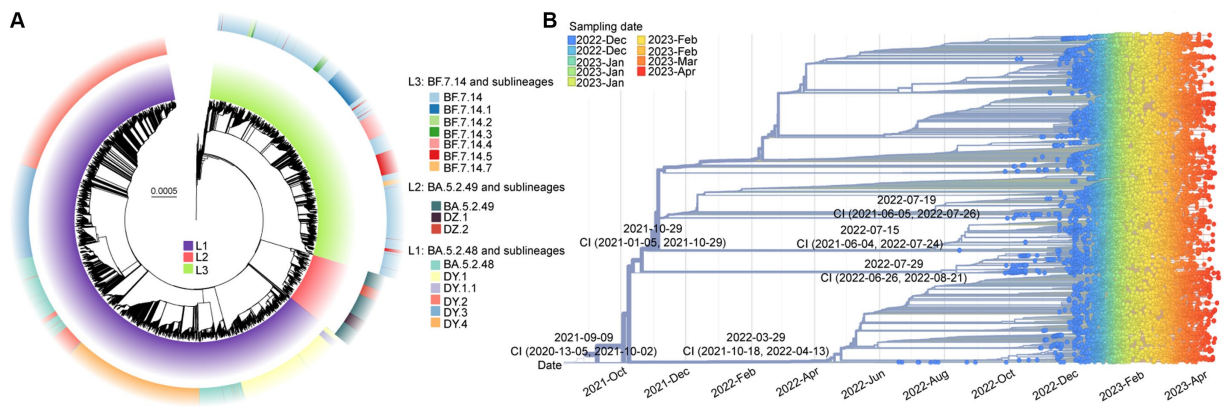

FIGURE 3
The molecular evolutionary relationships of circulating SARS-CoV-2 lineages during ending the dynamic zero-COVID policy. **(A)** The Maximum likelihood phylogenetic tree based on 19,749 SARS-CoV-2 genomes with Wuhan-Hu-1 as reference. The lineages and its descendant lineages were colored with corresponding colors. **(B)** The Nextstrain's phylodynamic analysis. The circle with different colors were labeled with sampling date. The phylogenetic tree was visualized by the Auspice online tool. The tMRCA with confidence interval (CI) are labeled on the branches.

3.3 Mutational analysis

A total of 43 core mutation profiles were detected in S proteins, including five deletions and one synonymous mutation (Figure 4A). Each lineage shared 33 core mutations, with 34–37 mutations in the S protein, respectively, indicating that the mutation frequency of the other 10 core mutations varied across lineages (Figure 4A). The BF.7.14 and its descendant lineages had R346T and C1243F mutations in the S protein, which was absent in the BA.5.2 and its descendant lineages (Figure 4A). The study also identified several unique core mutations in the S protein, including G75V in DZ.2, K147E in DY.1.1, A626V in BF.7.14.3, and S1021F in BF.7.14.7. The T883I mutation was unique to the S protein of BA.5.2.49 and its descendant lineages, compared to BA.5.2.48 and its descendant lineages, as well as BF.7.14 and its descendant lineages.

Meanwhile, 47 core mutation profiles were identified in the ORF1ab gene, including twenty synonymous mutations and one deletion (Figure 4B). All circulating lineages shared 22 core mutations, with 26–31 core mutations in the ORF1ab (Figure 4B). BF.7.14 and its descendant lineages contained three marker mutations in the ORF1ab

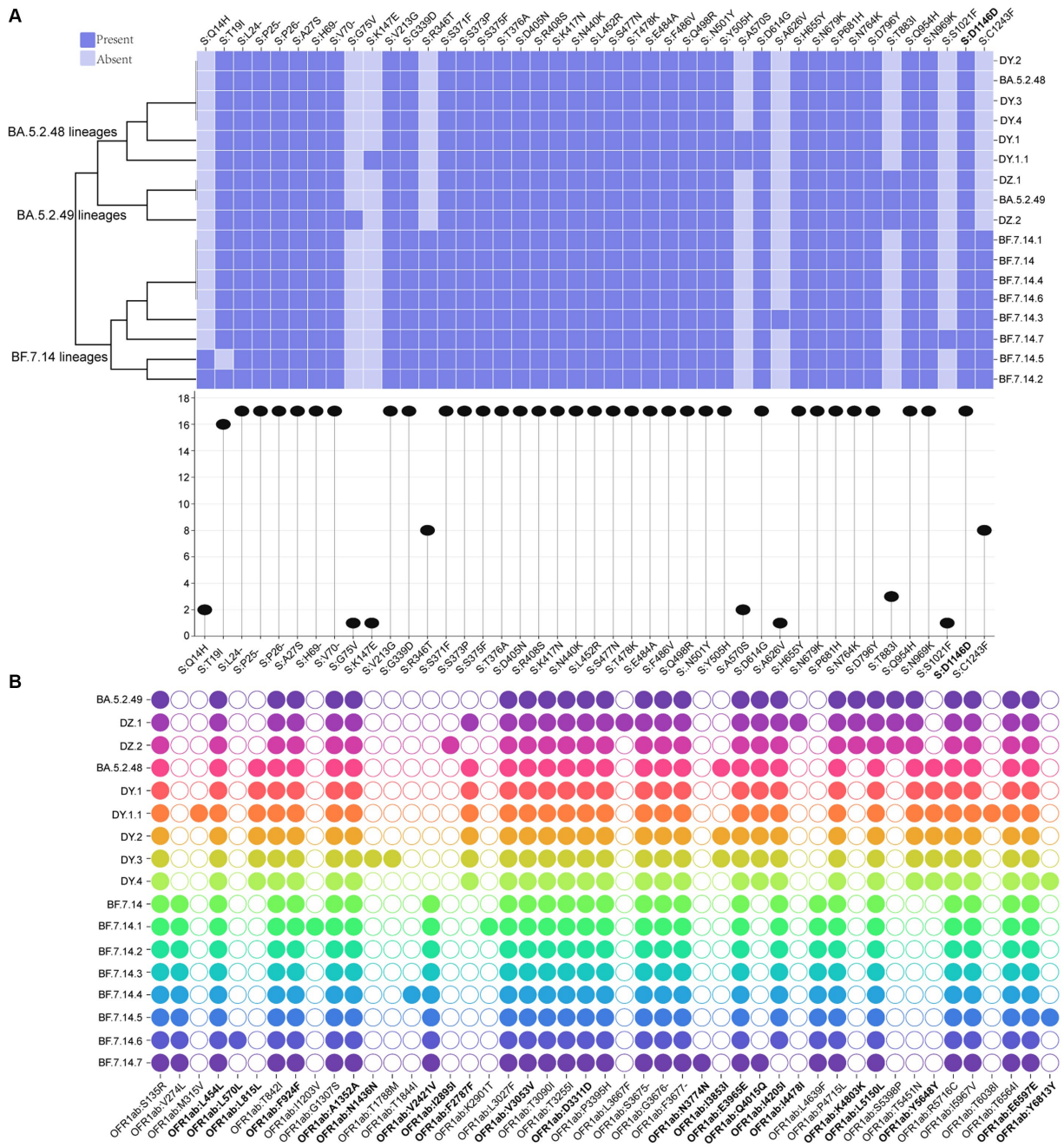

FIGURE 4
Core amino acid substitutions. **(A)** The core amino acid substitutions are presented or absented in S proteins of all circulating lineages. The substitution profiles are clustered by lineages. The plot on the bottom indicates the frequencies of individual substitutions across all lineages. **(B)** The core amino acid substitutions are presented or absented in ORF1ab of all circulating lineages. The substitutions with bold represented synonymous substitutions.

gene: V274L, V2421V, and L4639F, which were distinct from those found in BA.5.2 and its descendant lineages. Unique mutations (L815L, F2787F, and Y5648Y) in the ORF1ab gene were presented in all BA.5.2.48 and its descendant lineages, but were absent in the other two lineages (BF.7.14 and BA.5.2.49) and their corresponding descendant lineages (Figure 4B). The synonymous mutation (K4803K) in ORF1ab of BA.5.2.49 and its descendant lineages was a distinct mutation when compared to that of BA.5.2.48 and its descendant lineages, as well as BF.7.14 and its descendant lineages. Additionally, unique mutations were found only in certain lineages, including M315V and T6038I for DY.1.1, L570L for BF.7.14.6, I1203V and K2901T for BF.7.14.1, N1436N and T1788M for DY.3, I2895I for DZ.2, L3667F and I4478I for DZ.1, and N3774N for BF.7.14.7.

3.4 Selection analysis

To identify positive selection signals in the ongoing evolution of SARS-CoV-2, we utilized the high-quality SARS-CoV-2 genomes to calculate the Ka (non-synonymous substitutions per non-synonymous site) and Ks (synonymous substitutions per synonymous site) values between the genomes from different lineages and the reference genome (Wuhan-Hu-1, NC_045512.2). The results (Figure 5A) revealed that the Ka/Ks value for the S gene in all lineages was significantly higher than 1, with a median ranging from 3.80 to 4.10 (Supplementary Table S1). On the other hand, the Ka/Ks value for the remaining concatenated (non-S) genes was significantly less than 1 for all lineages, with the median ranging from 0.31 to 0.52 (Supplementary Table S1). Furthermore, the Ka/Ks value for the S gene was significantly higher ($p < 0.001$) than that of the non-S gene in all circulating lineages. This suggests that positive selection is the primary evolutionary force driving the evolution of the S protein, while purifying selection is acting on the protein sequences of the non-S genes in all circulating SARS-CoV-2 (Figure 5A).

To determine if selective pressure is consistent across all regions of the S gene, we calculated the Ka/Ks values for both the S1 and S2 regions in comparison to the reference genome. The Ka/Ks values for the S1 region of all circulating lineages were significantly higher than 1, with a median range from 6.22 to 6.67 (Supplementary Table S1). The Ka/Ks values for the S2 region of all circulating lineages were slightly higher than 1, with a median range from 1.05 to 1.37 (Supplementary Table S1). Importantly, the Ka/Ks values for the S1 region of all circulating lineages were significantly higher than those of the S2 region ($p < 0.01$) (Figure 5B). These results indicated that the positive selections of all

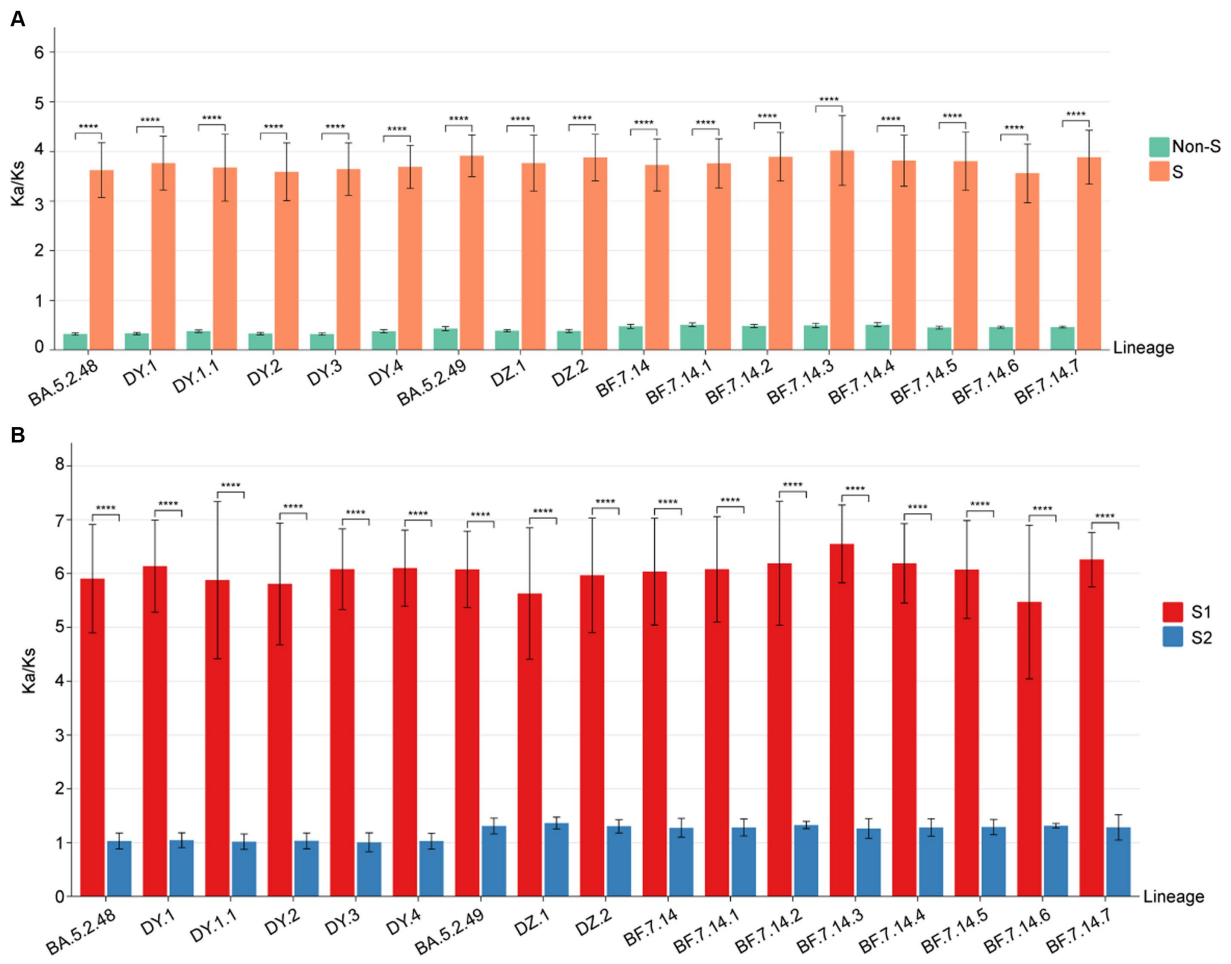

FIGURE 5
The Ka/Ks value comparison among all circulating lineages. **(A)** The Ka/Ks values of S and non-S genes. The Ka/Ks values are significantly higher for the S gene than non-S genes of all circulating lineages ($p < 0.05$). **(B)** The Ka/Ks values of S1 and S2 regions. The Ka/Ks values are significantly higher for the S1 region than S2 region of all circulating lineages ($p < 0.05$).

circulating lineages were primarily derived from non-synonymous substitutions in the S1 region. Additionally, the corresponding S2 regions were under slightly positive selections overall (Figure 5B).

4 Discussion

Our findings indicate that the prevailing variants were primarily derived from BA.5.2 and BF.7 lineages. The diversity of lineages varied among provinces and cities, suggesting regional differences in pandemic patterns. This variation in transmission advantage between BA.5.2 and BF.7 lineages is supported by different regions exhibiting distinct symptom profiles (27–29). Prior to December 2022, sporadic occurrence of BA.5.2 and its descendant lineages as well as BF.7 and its descendant lineages, was observed in China (30). This suggests that these occurrences reflected broader epidemiological patterns prevailing in China before this period. The number of new cases continued to rise rapidly throughout December 2022, peaking in January 2023, before declining from February 2023 onwards. BA.5.2- and BF.7-derived lineages showed fitness levels approximately 24 and 20 times higher than the prototype, which may explain their surge in this period (31).

The abundance of SARS-CoV-2 genomes presents a unique opportunity to infer the virus's evolutionary dynamics. The non-synonymous mutations made up the top viral mutations with most accumulated in the S and ORF1ab proteins, indicating local evolution and subsequent adaptation. The emergence date of these novel lineages was estimated based on the significant correlation between sampling dates and the root-to-tip distance, which provides information about the origin of SARS-CoV-2 lineages. Additionally, low-frequency mutations have been found throughout the entire genome of all circulating lineages, confirming the constant mutation of the virus and suggesting the potential emergence of new lineages.

The S protein binds to the host cell receptor angiotensin-converting enzyme 2 (ACE2), which determines viral cell entry. This process can be facilitated by the activation of the TM protease serine 2 (TMPRSS2) (32). Analysis of selection pressure has revealed a significant positive selection in the S protein, specifically in the S1 region. This implies that SARS-CoV-2 has undergone rapid evolution as a result of the ongoing evolutionary arms race between viruses and hosts (33). It is important to note that due to the variations in fitness and other factors, such as imported cases, new lineages of SARS-CoV-2 may emerge from existing ones through positive selection, rendering older lineages obsolete and potentially leading to new risks for human health.

In conclusion, the outbreak witnessed a rapid and substantial rise in SARS-CoV-2 infected cases as a result of the co-circulation of BF.7-derived and BA.5.2-derived lineages. It is imperative to consistently carry out extensive genomic monitoring to effectively track and understand the dynamics of SARS-CoV-2.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: [https://www.gisaid.org/, EPI_ISL_17805437 to EPI_ISL_17805525].

Ethics statement

The studies involving humans were approved by Ethics Committee of Sonya People's Hospital (NO: SYPH-2022-043). The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

WZ: Conceptualization, Data curation, Formal analysis, Methodology, Visualization, Writing – original draft. XW: Data curation, Software, Visualization, Writing – original draft. YL: Resources, Writing – original draft. LH: Resources, Writing – original draft. RZ: Writing – review & editing. CW: Methodology, Writing – review & editing. XZ: Writing – review & editing. TT: Writing – review & editing. LG: Conceptualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1273745/full#supplementary-material

References

1. Gussow AB, Auslander N, Faure G, Wolf YI, Zhang F, Koonin EV. Genomic determinants of pathogenicity in SARS-CoV-2 and other human coronaviruses. *Proc Natl Acad Sci U S A*. (2020) 117:15193–9. doi: 10.1073/pnas.2008176117

2. Sun P, Qie S, Liu Z, Ren J, Li K, Xi J. Clinical characteristics of hospitalized patients with SARS-CoV-2 infection: a single arm meta-analysis. *J Med Virol*. (2020) 92:612–7. doi: 10.1002/jmv.25735

3. Wang L, Zhou HY, Li JY, Cheng YX, Zhang S, Aliyari S, et al. Potential intervariant and intravariant recombination of Delta and omicron variants. *J Med Virol*. (2022) 94:4830–8. doi: 10.1002/jmv.27939

4. Mohammadi M, Shayestehpour M, Mirzaei H. The impact of spike mutated variants of SARS-CoV2 [alpha, Beta, gamma, Delta, and lambda] on the efficacy of subunit recombinant vaccines. *The Brazilian Journal of infectious diseases: an official publication of the Brazilian Society of Infectious Diseases*. (2021) 25:101606. doi: 10.1016/j.bjid.2021.101606

5. Umair M, Ikram A, Rehman Z, Haider SA, Ammar M, Badar N, et al. Genomic diversity of SARS-CoV-2 in Pakistan during the fourth wave of pandemic. *J Med Virol*. (2022) 94:4869–77. doi: 10.1002/jmv.27957

6. Moustafa AM. Planet PJ: emerging SARS-CoV-2 diversity revealed by rapid whole-genome sequence typing. *Genome Biol Evol*. (2021) 13. doi: 10.1093/gbe/evab197

7. Diseases NCfIaR: *SARS-CoV-2 variant classifications and definitions*. (2023). Available at: https://www.cdc.gov/coronavirus/2019-ncov/variants/variant-classifications.html

8. Sun Y, Wang M, Lin W, Dong W, Xu J. Evolutionary analysis of omicron variant BF.7 and BA.5.2 pandemic in China. *J biosafety and biosecurity*. (2023) 5:14–20. doi: 10.1016/j.jobb.2023.01.002

9. Lauring AS, Tenforde MW, Chappell JD, Gaglani M, Ginde AA, McNeal T, et al. Clinical severity of, and effectiveness of mRNA vaccines against, covid-19 from omicron, delta, and alpha SARS-CoV-2 variants in the United States: prospective observational study. *BMJ (Clinical research ed)*. (2022) 376:e069761. doi: 10.1136/bmj-2021-069761

10. Viana R, Moyo S, Amoako DG, Tegally H, Scheepers C, Althaus CL, et al. Rapid epidemic expansion of the SARS-CoV-2 omicron variant in southern Africa. *Nature*. (2022) 603:679–86. doi: 10.1038/s41586-022-04411-y

11. Butera Y, Mukantwari E, Artesi M, Umuringa JD, O'Toole ÁN, Hill V, et al. Genomic sequencing of SARS-CoV-2 in Rwanda reveals the importance of incoming travelers on lineage diversity. *Nat Commun*. (2021) 12:5705. doi: 10.1038/s41467-021-25985-7

12. Morang'a CM, Ngoi JM, Gyamfi J, Amuzu DSY, Nuertey BD, Soglo PM, et al. Genetic diversity of SARS-CoV-2 infections in Ghana from 2020-2021. *Nat Commun*. (2022) 13:2494. doi: 10.1038/s41467-022-30219-5

13. Grubaugh ND, Ladner JT, Lemey P, Pybus OG, Rambaut A, Holmes EC, et al. Tracking virus outbreaks in the twenty-first century. *Nat Microbiol*. (2019) 4:10–9. doi: 10.1038/s41564-018-0296-2

14. Xinhua; *China Focus: COVID-19 response further optimized with 10 new measures*. (2022). https://english.news.cn/20221207/ca014c043bf24728b8dcbc0198565fdf/c.html.

15. Xinhua: *Chinese vice premier stresses need to ensure smooth transition of COVID-19 response phases*. (2022). Available at: https://english.news.cn/20221214/3c1d5934a43d47d4a016194acea7bd1d/c.html.

16. Hirata Y, Katano H, Iida S, Mine S, Nagasawa S, Makino Y, et al. Genomic analysis of SARS-CoV-2 in forensic autopsy cases of COVID-19. *J Med Virol*. (2023) 95:e28990. doi: 10.1002/jmv.28990

17. Wang X, Zhu X, Lin Y, He L, Yang J, Wang C, et al. Tracking the first SARS-CoV-2 omicron BA.5.1.3 outbreak in China. *Front Microbiol*. (2023) 14:1183633. doi: 10.3389/fmicb.2023.1183633

18. Andrews S: *FastQC: A quality control tool for high throughput sequence data*. (2010). Available at: https://www.bioinformatics.babraham.ac.uk/projects/fastqc/.

19. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics*. (2014) 30:2114–20. doi: 10.1093/bioinformatics/btu170

20. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, et al. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J,comp bio: a J computational molecular cell biology*. (2012) 19:455–77. doi: 10.1089/cmb.2012.0021

21. Zhu W, Gu L. Clinical, epidemiological, and genomic characteristics of a seasonal influenza a virus outbreak in Beijing: a descriptive study. *J Med Virol*. (2023) 95:e29106. doi: 10.1002/jmv.29106

22. Hadfield J, Megill C, Bell SM, Huddleston J, Potter B, Callender C, et al. Nextstrain: real-time tracking of pathogen evolution. *Bioinformatics*. (2018) 34:4121–3. doi: 10.1093/bioinformatics/bty407

23. Zhang Z, Xiao J, Wu J, Zhang H, Liu G, Wang X, et al. ParaAT: a parallel tool for constructing multiple protein-coding DNA alignments. *Biochem Biophys Res Commun*. (2012) 419:779–81. doi: 10.1016/j.bbrc.2012.02.101

24. Lv S, Qiao X, Zhang W, Li Q, Wang P, Zhang S, et al. The origin and evolution of RNase T2 family and Gametophytic Self-incompatibility system in plants. *Genome Biol Evol*. (2022) 14. doi: 10.1093/gbe/evac093

25. Cori A, Ferguson NM, Fraser C, Cauchemez S. A new framework and software to estimate time-varying reproduction numbers during epidemics. *Am J Epidemiol*. (2013) 178:1505–12. doi: 10.1093/aje/kwt133

26. Park SW, Sun K, Abbott S, Sender R, Bar-On YM, Weitz JS, et al. Inferring the differences in incubation-period and generation-interval distributions of the Delta and omicron variants of SARS-CoV-2. *Proc Natl Acad Sci U S A*. (2023) 120:e2221887120. doi: 10.1073/pnas.2221887120

27. Zhang H, Lu Z, Yang F, Chen H, Zhang L, Zhao H, et al. Symptom profiles and vaccination status for COVID-19 after the adjustment of the dynamic zero-COVID policy in China: an observational study. *J Med Virol*. (2023) 95:e28893. doi: 10.1002/jmv.28893

28. Yu L, Wang C, Li X, Wang X, Kang Y, Ma X, et al. Clinical characteristics of abruptly increased paediatric patients with omicron BF.7 or BA.5.2 in Beijing. *Virol J*. (2023) 20. doi: 10.1186/s12985-023-02177-x

29. Huo D, Yu T, Shen Y, Pan Y, Li F, Cui S, et al. A comparison of clinical characteristics of infections with SARS-CoV-2 omicron subvariants BF.7.14 and BA.5.2.48 - China, October-December 2022. *China CDC Weekly*. (2023) 5:511–5. doi: 10.46234/ccdcw2023.096

30. Pan Y, Wang L, Feng Z, Xu H, Li F, Shen Y, et al. Characterisation of SARS-CoV-2 variants in Beijing during 2022: an epidemiological and phylogenetic analysis. *Lancet (London, England)*. (2023) 401:664–72. doi: 10.1016/S0140-6736(23)00129-0

31. Obermeyer F, Jankowiak M, Barkas N, Schaffner SF, Pyle JD, Yurkovetskiy L, et al. Analysis of 6.4 million SARS-CoV-2 genomes identifies mutations associated with fitness. *Science*. (2022) 376:1327–32. doi: 10.1126/science.abm1208

32. Huang Y, Yang C, Xu XF, Xu W, Liu SW. Structural and functional properties of SARS-CoV-2 spike protein: potential antivirus drug development for COVID-19. *Acta Pharmacol Sin*. (2020) 41:1141–9. doi: 10.1038/s41401-020-0485-4

33. Tang X, Qian Z, Lu X, Lu J. Adaptive evolution of the spike protein in coronaviruses. *Mol Biol Evol*. (2023) 40:msad089. doi: 10.1093/molbev/msad089

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"Low-risk groups" deserve more attention than "high-risk groups" in imported COVID-19 cases

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Objective: To estimate the optimal quarantine period for inbound travelers and identify key risk factors to provide scientific reference for emerging infectious diseases.

Methods: A parametric survival analysis model was used to calculate the time interval between entry and first positive nucleic acid test of imported cases in Guangzhou, to identify the influencing factors. And the COVID-19 epidemic risk prediction model based on multiple risk factors among inbound travelers was constructed.

Results: The approximate 95th percentile of the time interval was 14 days. Multivariate analysis found that the mean time interval for inbound travelers in entry/exit high-risk occupations was 29% shorter (OR 0.29, 95% CI 0.18–0.46, $p < 0.0001$) than that of low-risk occupations, those from Africa were 37% shorter (OR 0.37, 95% CI 0.17–0.78, $p = 0.01$) than those from Asia, those who were fully vaccinated were 1.88 times higher (OR 1.88, 95% CI 1.13–3.12, $p = 0.01$) than that of those who were unvaccinated, and those in other VOC periods were lower than in the Delta period. Decision tree analysis showed that a combined entry/exit low-risk occupation group with Delta period could create a high indigenous epidemic risk by 0.24.

Conclusion: Different strata of imported cases can result in varying degrees of risk of indigenous outbreaks. "low-risk groups" with entry/exit low-risk occupations, fully vaccinated, or from Asia deserve more attention than "high-risk groups."

KEYWORDS

COVID-19, imported case, quarantine period, time interval, risk factor

Introduction

On March 11, 2020, the World Health Organization (WHO) declared that the coronavirus disease 2019 (COVID-19) had developed into a global pandemic (1), and by August 9, 2023, approximately 769 million COVID-19 cases have been reported globally, with a cumulative total of 6.954 million deaths (2), and the majority of countries have reported COVID-19 case-fatality rates ranging from 0.5 to 5.0% (3). During the outbreak, inbound travelers are a high-risk group for potential infection. Therefore, it is crucial to defend externally against importation.

Guangzhou is a pivotal city in epidemic prevention and control of imported cases in China. Investigations showed that approximately 20,000 inbound travelers were under quarantine and observation in Guangzhou each day during the COVID-19 period. Even though China has

announced a roll-back of its strict anti-COVID-19 measures at this stage, against the background of a certain percentage of infected inbound travelers, it is still important for inbound travelers to take proper quarantine measures and self-monitoring for a while after entry. However, a study by Bai et al. published in JAMA found that it took 19 days for a close contact to test positive for nucleic acids and may have resulted in transmission including five people (4). Therefore, it is necessary to effectively manage quarantine of close contacts and monitor the risk groups of disease after the quarantine period has ended.

Model parameter estimation based on survival analysis (5) can better estimate the time interval between entry and the first positive nucleic acid test, incubation period (6), or latent period for infectious diseases, and also serve as a valuable validation and supplement to commonly known quarantine periods. This paper estimated the time distribution characteristics of imported cases from entry to the first nucleic acid positivity and identified the influencing factors through survival analysis. Additionally, a decision tree model was used to construct the influencing factors-based outbreak risk prediction model for inbound travelers (7), which can help to predict, evaluate and follow up the risk of an indigenous epidemic caused by inbound key travelers. It could explore effective methods of balancing healthcare costs and socio-economic benefits (8) while controlling the spread of epidemic outbreaks, and it can provide scientific references for the prevention and control of emerging infectious diseases.

Materials and methods

Data sources and study population

Data on the imported COVID-19 epidemics in Guangzhou were obtained from the official website of the Guangzhou Health Commission and the notification of imported epidemics published by the Guangzhou Center for Disease Control and Prevention as of twenty-four o'clock on August 31, 2022. The following were excluded from the study: (1) duplicate cases; (2) cases with excessive missing information; and (3) native cases. A total of 1,029 imported COVID-19 cases were collected in this study, and after cases with missing test data from entry to the first nucleic acid positivity were excluded, a total of 743 cases were used to calculate the survival analysis. This study was reviewed by the Institutional Review Board of the Guangzhou Center for Disease Control and Prevention.

Research method

Retrospective collection of information on reported inbound cases (confirmed cases and asymptomatic patients) in Guangzhou from March 1, 2020, to August 31, 2022, and extraction of demographic characteristics such as age, gender, occupation, and nationality of the patients, as well as information on their entry into China, history of sojourn, history of COVID-19 vaccination, and information on nucleic acid testing.

Measurements and definition

(1) Quarantine period: The quarantine period refers to the duration of the quarantine. Counting from the last day of contact with the patient, the quarantine period for the contact is established according to the longest incubation period of the disease. During the quarantine period the contact is placed under medical observation, retained for examination or other necessary measures, (2) Confirmed cases (9): patients who met the relevant epidemiologic history, clinical manifestations, and tested positive for COVID-19 nucleic acid by real-time fluorescence RT-PCR, (3) Asymptomatic patients: no clinical symptoms and respiratory specimens with positive COVID-19 pathogenicity test, (4) Imported cases (imported asymptomatic patients): cases with a history of residence in an outbreak country or region within 14 days before the positive nucleic acid test, and exclude infection in China, (5) The time interval between entry and the first positive nucleic acid test: the starting time of observation was the entry time of each study subject, with the first positive nucleic acid test result as the outcome event, and the survival time was defined as the interval from entry to the first positive nucleic acid test. Assuming that E and S are the entry time of a case and the time point at which a case's nucleic acids can be first detected as positive, respectively, the time interval between entry and the first positive nucleic acid test is T = S−E. When both E and S fall on limited intervals, the observation is called the doubly interval-censored data. Accordingly, when one of E and S is an exact value and the other falls on a limited interval, the observation is called the singly interval-censored data. When both E and S are exact values, the observed data is the fully data (10). The time of entry was determined for all cases in this study, and the time point at which nucleic acids could be first detected as positive fell on a limited interval, so the observations were singly interval-censored data, i.e., $T = (S_L - E, S_R - E)$, where the subscripts L and R denote the time of the last negative before a positive nucleic acid result, and the time of the first positive result (i.e., the lower and upper limits), respectively. When the interval between the positive result and the last negative result before the positive result was less than or equal to 1 day (performed with a time distance of 24 h) (11), both were considered to have occurred on the same day, i.e., L = R, then the time interval between entry and the first positive nucleic acid test (T) is fully data. In contrast, data were interval-censored if the time interval between the positive result and the last negative result was greater than 1 day because L did not equal R.

Statistical analysis

Statistical analysis was performed using R4.0.4 and SAS 9.4 software. Epidemiological characteristics of imported COVID-19 cases were analyzed using descriptive methods, count data were expressed as frequency, constitutive ratio, or proportion (%), and differences between groups were analyzed using the chi-square test or Fisher's exact probability method. Differences were considered statistically significant at $p < 0.05$.

Distributional and point estimates of the time interval between entry and the first positive nucleic acid test for imported cases were based on a parametric survival analysis model of the failure data (12, 13). The model is $\log(T) = \mu + \sigma \log(T_0)$ if there is no covariate, or $\log(T) = x'\beta + \sigma \log(T_0)$ if there is a covariate. Where T represents the failure time, $\propto$ represents the location parameter, σ represents the scale parameter, T_0 represents some sample that is in the baseline distribution, and x represents the value vector of the covariates. The model was fitted using the parametric method; the distributions commonly used for T are the Weibull distribution, the Log-normal

distribution, etc.; the maximum likelihood method was used for the fitting, and the log-likelihood function was:

$$L = \sum \log(f(\bar{\omega}_i)/\sigma + \sum \log\left(S(\bar{\omega}_i)\right) + \sum \log\left(F(\bar{\omega}_i)\right) + \sum \log\left(F(\bar{\omega}_i) - F(v_i)\right).$$

Where the first term of the formula sums over no censored data, the second term sums over right-censored data, the third term sums over left-censored data, and the last term sums over interval-censored data.

The covariate-free model was first used to fit the model using Log-normal, Log-logistic, Weibull, and Gamma distributions, respectively, to estimate the parameters of the time interval between entry and the first positive nucleic acid test and their distributional characteristics for each distribution, and to determine the fitted optimal distribution based on the maximum likelihood method and Bayesian Information Criterion; The distribution was then applied to a univariate covariate model analysis to analyze the individual effects of each factor on the time interval between entry and the first positive nucleic acid test, and those factors with $p < 0.05$ in the univariate analysis were entered into the multivariate analysis. Afterward, the factors that were statistically significant in the multivariate analysis were analyzed in subgroup analysis to estimate their percentile at some critical time of the time interval between entry and the first positive nucleic acid test.

The degree of risk of an indigenous outbreak due to imported cases was defined as follows: the time interval between entry and the first positive nucleic acid test ≤ P95 as low risk; > P95 as high risk. A decision tree model was used to combine statistically significant variables from the univariate analysis with the outbreak data to construct a dataset for the COVID-19 outbreak risk simulation model for Guangzhou City. Utilizing 70% of the data for training purposes and 30% for testing, a decision tree is built using the classification and regression trees (CART) algorithm (14), which can rank the importance of the influencing factors and construct a multi-risk factor-based simulation model of COVID-19 outbreak risk for inbound travelers in Guangzhou City.

Results

Baseline information for imported cases

In this study, 520 (50.53%) of the 1,029 imported cases were asymptomatic patients, and 509 (49.47%) were confirmed cases. The majority of imported cases were male (67.54%) and young adults (52.67%), and there were more imported cases from Asia (78.23%) and foreign nationalities (63.75%). Entry/exit high-risk occupational groups refer to those who work in key areas such as border crossings with a high risk of disease transmission, including crew members, seafarers, logistics personnel at airports and customs, and other occupational groups closely related to entry/exit. These groups accounted for 28.09% of the population. Imported cases from different VOC periods were dominated by the Omicron period, which accounted for 61.80% of the total. In addition, there were differences in the composition of asymptomatic patients and confirmed cases in terms of age, import source, nationality, and occupation, with Chinese nationals predominantly confirmed cases and foreigners predominantly asymptomatic patients (Table 1).

Covariate-free survival analysis results

The Log-normal, Log-logistic, Weibull, and Gamma distributions were employed to approximate the time interval between entry and the first positive nucleic acid test in Guangzhou. The optimal distribution model was determined through the Bayesian information criterion (BIC) or the maximum likelihood method (15). The log-likelihood ratio (LLR) and Bayes calculated from both the Log-normal and Log-logistic distributions were relatively close, and the location parameter estimates were generally similar. However, the Weibull and Gamma distributions estimate model parameters that were not statistically significant. Moreover, given that the Log-normal distribution has a lower likelihood ratio and BIC compared to the Log-logistic distribution, and since it is more commonly used in estimating latency distributions, it is considered to be the optimal choice for analyzing the time interval between the entry and the first positive nucleic acid test for COVID-19. The mean time interval between entry and the first positive nucleic acid test estimated by the Log-normal distribution was 0.4 days with a 95% confidence interval of (0.32, 0.51) days, which means that 50% of the imported cases tested positive within 1 day of entry. In addition, approximate 95% (94.51%) of the imported cases first tested positive within 14 days of entry (Table 2).

Univariate survival analysis results of influencing factors

Log-normal distribution has been employed in the univariate survival analysis. In total, various aspects such as sex, age, region, nationality, entry/exit risk occupation, international student status, background disease condition, vaccination history, and VOC period were investigated and analyzed. The results indicated that there was a statistically significant difference for the factors of entry/exit high-risk occupations ($p < 0.0001$), Oceania ($p = 0.02$), Africa ($p < 0.0001$), COVID-19 vaccination with 2 doses ($p < 0.0001$), COVID-19 vaccination with 3 or more doses ($p < 0.0001$), Alpha period ($p < 0.0001$), Beta period ($p < 0.0001$), and Omicron period ($p = 0.0001$) (Table S1).

Multivariate survival analysis results of influencing factors

Factors with $p < 0.05$ in the univariate survival analysis were calculated in the multivariate analysis, which found that the mean time interval between entry and the first positive nucleic acid test for inbound travelers in entry/exit high-risk occupations was 29% shorter (OR 0.29, 95% CI 0.18–0.46, $p < 0.0001$) than that of low-risk occupations, those from Africa were 37% shorter (OR 0.37, 95% CI 0.17–0.78, $p = 0.01$) than those from Asia, those who received 3 or more doses of the COVID-19 vaccinations were 1.88 times higher (OR 1.88, 95% CI 1.13–3.12, $p = 0.01$) than that of those who did not, and those in other VOC periods were lower than in the Delta period (Table 3).

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TABLE 1 Basic information on imported COVID-19 cases from abroad in Guangzhou.

Variable category	Aggregate *N* = 1,029 *n* (%)	Asymptomatic patients *N* = 520 *n* (%)	Confirmed cases *N* = 509 *n* (%)	*p* values
Sex				
Men	695 (67.54)	360 (51.80)	335 (48.20)	0.27
Women	334 (32.46)	160 (47.90)	174 (52.10)	
Age				
<18	64 (6.22)	34 (53.13)	30 (46.88)	<0.001
18~	542 (52.67)	300 (55.35)	242 (44.65)	
40~	381 (37.03)	176 (46.19)	205 (53.81)	
≥65	42 (4.08)	10 (23.81)	32 (76.19)	
Region				
Asia	805 (78.23)	376 (46.71)	429 (53.29)	<0.001
Africa	91 (8.84)	54 (59.34)	37 (40.66)	
Oceania	57 (5.54)	46 (80.70)	11 (19.30)	
North America	39 (3.79)	20 (51.28)	19 (48.72)	
Europe	34 (3.30)	22 (64.71)	12 (35.29)	
South America	3 (0.29)	2 (66.67)	1 (33.33)	
Nationality				
Chinese	361 (35.08)	92 (25.49)	269 (74.52)	<0.001
Hong Kong, Macau, and Taiwan	12 (1.17)	5 (41.67)	7 (58.33)	
Foreign	656 (63.75)	423 (64.48)	233 (35.52)	
Entry/exit risk occupations				
High	289 (28.09)	120 (41.52)	169 (58.48)	<0.001
Low	740 (71.91)	400 (54.05)	340 (45.95)	
International student				
Yes	85 (8.26)	61 (71.76)	24 (28.24)	<0.001
No	944 (91.74)	459 (48.62)	485 (51.38)	
Background diseases				
Yes	55 (5.34)	19 (34.55)	36 (65.46)	<0.001
No	784 (76.19)	426 (54.34)	358 (45.66)	
Unknown	190 (18.46)	75 (39.47)	115 (60.53)	
Vaccination doses				
Unvaccinated	322 (34.77)	196 (60.87)	126 (39.13)	<0.001
1 dose	64 (6.91)	40 (62.50)	24 (37.50)	
2 doses	256 (27.65)	102 (39.84)	154 (60/16)	
3 or more doses	284 (30.67)	144 (50.70)	140 (49.30)	
VOC periods				
Alpha	188 (18.41)	128 (68.09)	60 (31.91)	<0.001
Beta	34 (3.33)	19 (55.88)	15 (44.12)	
Gamma	32 (3.13)	15 (46.88)	17 (53.13)	
Delta	136 (13.32)	74 (54.41)	62 (45.59)	
Omicron	631 (61.80)	283 (44.85)	348 (55.15)	

Subgroup analysis

The study established the time interval between entry and the first positive nucleic acid test as 14 days, which can inform the quarantine period for inbound travelers. Therefore, we focused on the 14-day centile, which was defined as the proportion of imported cases with a positive first nucleic acid test within 14 days of entry. By calculating the 14-day centile for different factors, the proportion of

TABLE 2 Survival analysis results of four distribution parameter models.

Distribution form	LLR	BIC	Location parameter			The mean time interval between entry and the first positive nucleic acid test		95th percentile (day)	7-day centile	14-day centile
			Estimate	95% CI	*p* values	estimate	95%CI			
Log-normal	−1001.06	2015.33	−0.90	(−1.14, -0.67)	<0.0001	0.40	(0.32,0.51)	15.52	90.11%	94.51%
Log-logistic	−1018.26	2049.74	−0.82	(−1.04, -0.61)	<0.0001	0.44	(0.35,0.55)	20.35	89.36%	93.45%
Weibull	−989.56	1992.33	0.10	(−0.08, 0.29)	0.28	0.51	(0.41,0.63)	11.43	90.84%	96.31%
Gamma	−988.88	1997.59	−0.11	(−0.50, 0.29)	0.59	0.48	(0.34,0.67)	11.89	90.72%	96.01%

TABLE 3 Multivariate survival analysis of factors influencing the time interval between entry and the first positive nucleic acid test of COVID-19.

Variable category		References	B	SE	p values	OR	OR 95%CI
Entry/exit risk occupations	High	low	−1.23	0.23	<0.0001	0.29	(0.18, 0.46)
Region	Oceania	Asia	0.53	0.31	0.09	1.70	(0.93, 3.14)
	Africa		−1.00	0.38	0.01	0.37	(0.17, 0.78)
Vaccination doses	2 or more doses	unvaccinated	0.38	0.24	0.11	1.46	(0.92, 2.36)
	3 or more doses		0.63	0.26	0.01	1.88	(1.13, 3.12)
VOC periods	Alpha	Delta	−3.07	0.35	<0.0001	0.05	(0.02, 0.09)
	Beta		−4.73	1.05	<0.0001	0.01	(0.00, 0.07)
	Omicron		−1.30	0.24	<0.0001	0.27	(0.17, 0.44)

different types of inbound travelers who did not test positive until after the end of the quarantine period can be derived, thus identifying priority risk groups. The factors with statistical significance in the multivariate survival analysis were entered into subgroup analysis, and the 14-day centile of the time interval between entry and the first positive nucleic acid test was calculated. The results found that 6.52% of entry/exit low-risk occupational cases first tested positive 14 days after entry, which was higher than the proportion of high-risk occupational cases; 5.44% of Asian cases tested positive 14 days after entry, whereas basically all African cases could be detected as positive within 14 days of quarantine; and the proportion of cases who had received 3 or more doses of the COVID-19 vaccinations was 8.18%, which was far higher than the proportion of unvaccinated cases. As the Guangzhou COVID-19 Nucleic Acid Detection System was not fully standardized until May 2021, many of the nucleic acid positive detection times for imported cases in Guangzhou prior to May 2021 were missing. Consequently, the mean time interval between entry and the first positive nucleic acid test result in the Alpha and Beta periods was lower than that in the Delta period during multivariate analyses, possibly due to a lack of representative samples. As a result, subgroup analyses were only conducted for the Delta and Omicron periods, which mainly occurred after May 2021. The analysis found that 12.50% of imported cases in the Delta period first tested positive 14 days after entry, while 5.50% of imported cases in the Omicron period were tested 14 days after entry (Table 4).

Decision tree analysis

Cases detected within the quarantine period have a lower risk of causing an indigenous outbreak so the degree of risk of an indigenous outbreak due to imported cases was defined as follows: the time interval between entry and the first positive nucleic acid test ≤ P95 as low risk; > P95 as high risk. Factors with $p < 0.05$ at univariate survival analysis were calculated in the decision tree model, which had an average accuracy value of 92.83%. The decision tree model was constructed with four layers reflecting the importance of each factor in predicting the risk of causing an indigenous outbreak. The first layer was divided by VOC period, indicating that VOC period had the greatest influence, while the second layer of influencing factors was the entry/exit risk occupation, the third layer was the region, and the fourth layer was the COVID-19 vaccination doses.

We found that the probability that an imported case during the Delta period causes an indigenous outbreak to be a low risk was 0.84, which gave a high-risk probability of 0.16, which was higher than in other periods such as the Omicron period. Among Delta-period

TABLE 4 Subgroup analysis of the time interval between entry and the first positive nucleic acid test of COVID-19.

Variable category	14-day centile	Percentage of positive detections after 14 days of quarantine
Entry/Exit low-risk occupations	93.48%	6.52%
Entry/Exit high-risk occupations	98.12%	1.88%
Asia	94.56%	5.44%
Africa	99.26%	0.74%
Unvaccinated	97.73%	2.27%
3 or more doses of COVID-19 vaccinations	91.82%	8.18%
Delta	87.5%	12.50%
Omicron	94.5%	5.50%

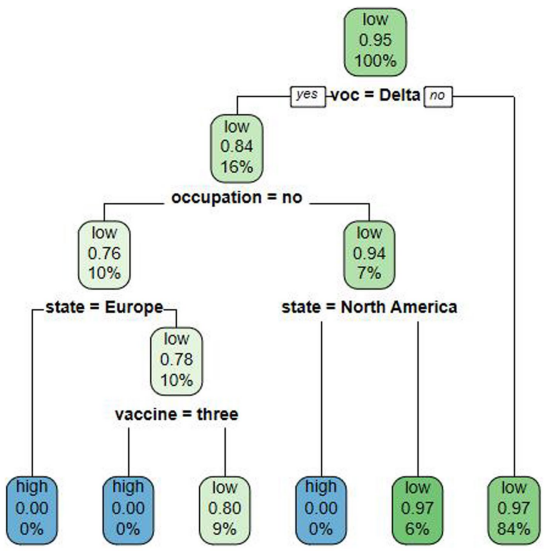

FIGURE 1
Multi-risk factor decision tree modeling of COVID-19 outbreak risk.

inbound travelers, the probability of an entry/exit low-risk occupational case causing a high risk of an indigenous outbreak was 0.24, which was higher than that of an entry/exit high-risk occupational case (0.06). As Europe and North America were nonsignificant factors in the univariate analysis, the model did not predict risk for these cases. The orientation of the factors influencing the risk of an indigenous outbreak resulting from imported cases, as revealed by the decision tree analysis, is in line with the orientation of the factors influencing the duration of the time interval between entry and the first positive nucleic acid test, as determined by the multivariate analysis (Figure 1).

Discussion

This paper provided a new estimation of the incubation period for COVID-19, focusing only on the period from entry to the time when the organism is infectious, expressed as the interval between the entry of an infected person and the time point when a positive test can be detected for the first time in a consecutive nucleic acid test. Moreover, the epidemiologic history of patients can be reproduced naturally based on interval-censored data (16).

The quarantine period of an infectious disease is generally the longest incubation period of the infectious disease (17). This paper estimated from a Log-normal distribution (18) that the approximate 95th percentile (94.51%) of the time interval between entry and the first positive nucleic acid test was 14 days, which is consistent with the conventional quarantine period of 14 days (19). Quarantine of 95% of the inbound travelers can effectively control the spread of the disease and prevent an epidemic on a large scale (20, 21). This study found that the time interval between entry and the first positive nucleic acid test for the total imported case was comparable to that of the Omicron period, so was the quarantine policy of the Omicron period utilized as a reference? Research had concluded that shortened quarantine period was attributed to the shorter average incubation period of the disease in the Omicron period (22, 23), milder clinical manifestations (24, 25), and better prognosis in most populations. As the epidemiological characteristics of the imported cases in this study varied, the quarantine policy during the Omicron period may not be suitable for the actual situation in this study. Therefore, selecting an appropriate quarantine period requires assessing the infectiousness and pathogenicity of the infectious disease at the time, as well as its societal risks.

Multivariate analysis found that the time interval between entry and the first positive nucleic acid test was shorter for people from Africa, entry/exit high-risk occupations, and people who were unvaccinated. The reason for this may be the low coverage of healthcare systems and low vaccination rates in Africa (26), consistent with the entry/exit high-risk occupations and those who have not been vaccinated with the COVID-19 vaccine, which have high viral loads in their bodies and are more likely to be detected during the quarantine period and immediately transferred for treatment, and therefore the risk of causing an indigenous outbreak is low. In contrast, those with longer positive detection intervals, such as those in entry/exit low-risk occupations, those who were fully vaccinated, and those who came from Asia, who appear to be "low-risk groups," have a relatively lower rate of positive detections during the quarantine period, and may become a potential infection source after quarantine period, which may result in a higher risk of causing an indigenous outbreak (27). Overall, it was found that the "low-risk group" should be given more attention than the "high-risk group." In addition, CART found that low-risk occupations only affected the imported cases of Delta strains, but had no significance on the imported cases of other strains. This suggested that there should

be more detailed records for imported Delta cases. The risk of causing an indigenous outbreak significantly increased when Delta and low-risk occupations were present in imported cases. Therefore, it is recommended to strengthen the degree of tracking of these persons after the end of the quarantine period.

Facing the potential emergence of various infectious diseases, it is imperative to construct a precise, cost-effective epidemic prevention and control system that promotes comprehensive economic and social development at the lowest possible cost, provided that there is a high probability of successfully identifying potentially infected individuals. This study proposes the development of a risk-graded management model for inbound travelers, which can effectively track the follow-up situation of inbound risky travelers with longer positive test times through big data after the end of the quarantine period. By improving the accuracy and efficiency of case detection, this approach can help minimize the risk of indigenous epidemic transmission caused by imported cases.

Some limitations of this study should be noted. First, the data collected in this study were partially derived from interviews with imported infected persons, which is subject to potential recall bias. Second, this study did not include all imported COVID-19 cases and therefore cannot represent the epidemiologic characteristics of all imported cases in Guangzhou. Third, the time range of the imported cases included in this study was relatively wide. If there is a sufficient sample size of inbound cases, a study should have chosen data from a short period of time to speculate on the quarantine period, which will be more accurate.

Conclusion

We found that those "low-risk groups" with low-risk occupations, fully vaccinated, and who came from Asia have a higher risk of developing an indigenous outbreak, and should be given more attention than the "high-risk groups." It is recommended to keep tracking these "low-risk groups" after the end of the quarantine period, to achieve precise prevention and control. This underscores the need to identify the risk groups through influencing factors, which can inform emerging infectious diseases.

Data availability statement

The datasets presented in this article are not readily available because due to contractual agreements with the data provider, the data used to produce this manuscript are not available for distribution. The analytic code is available upon request to the corresponding author. Requests to access the datasets should be directed to WZ, 1137164682@qq.com.

Ethics statement

The studies involving humans were approved by Institutional Review Board of the Guangzhou Center for Disease Control and Prevention. The studies were conducted in accordance with the local legislation and institutional requirements. The ethics committee/institutional review board waived the requirement of written informed consent for participation from the participants or the participants' legal guardians/next of kin because this trial is a retrospective study, only collecting patients' clinical data, does not affect any medical rights of patients, and does not additionally increase the risk of patients. We only analyze the sample data and all are implemented through sample coding to protect the privacy of the subjects fully.

Author contributions

WZ: Data curation, Writing – original draft. YT: Writing – original draft. ZZ: Writing – original draft. JC: Writing – original draft. XD: Writing – review & editing. XC: Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2023.1293747/full#supplementary-material

References

1. World Health Organization (2020). Director-General's opening remarks at the media briefing on COVID-19-11 march 2020[EB/OL]. Available at: https://www.who.int/director-general/

2. World Health Organization (2023). WHO coronavirus (COVID-19) dashboard [EB/OL]. Available at: https://covid19.who.int/

3. Medicine JHU (2023). Mortality in the most affected countries [EB/OL]. Available: https://coronavirus.jhu.edu/data/mortality

4. Bai Y, Yao L, Wei T, Tian F, Jin DY, Chen L, et al. Presumed asymptomatic carrier transmission of COVID-19. *JAMA*. (2020) 323:1406–7. doi: 10.1001/jama.2020.2565

5. Backer JA, Klinkenberg D, Wallinga J. Incubation period of 2019 novel coronavirus (2019-nCoV) infections among travellers from Wuhan, China, 20-28 January 2020. *Euro Surveill*. (2020) 25:2000062. doi: 10.2807/1560-7917.Es.2020.25.5.2000062

6. Huang S, Li J, Dai C, Tie Z, Xu J, Xiong X, et al. Incubation period of coronavirus disease 2019: new implications for intervention and control. *Int J Environ Health Res*. (2022) 32:1707–15. doi: 10.1080/09603123.2021.1905781

7. Huang ML, Hung YH, Lee WM, Li RK, Wang TH. Usage of case-based reasoning, neural network and adaptive neuro-fuzzy inference system classification techniques in breast cancer dataset classification diagnosis. *J Med Syst*. (2012) 36:407–14. doi: 10.1007/s10916-010-9485-0

8. Haas CN. On the quarantine period for ebola virus. *PLoS Curr*. (2014) 6:6. doi: 10.1371/currents.outbreaks.2ab4b76ba7263ff0f084766e43abbd89

9. National Health Commission of the People's Republic of China. Diagnosis and treatment plan for COVID-19 (trial version 6). *Clin Guid Compil Database*. (2020) 1:e2-e. doi: 10.3760/cma.j.issn.9999-998X.2020.01.002

10. Yin M-Z, Zhu Q-W, Lü X. Parameter estimation of the incubation period of COVID-19 based on the doubly interval-censored data model. *Nonlinear Dyn*. (2021) 106:1347–58. doi: 10.1007/s11071-021-06587-w

11. Benoni R, Campagna I, Panunzi S, Varalta MS, Salandini G, De Mattia G, et al. Estimating COVID-19 recovery time in a cohort of Italian healthcare workers who underwent surveillance swab testing. *Public Health*. (2021) 196:52–8. doi: 10.1016/j.puhe.2021.05.014

12. Mullahy J. Specification and testing of some modified count data models. *J Econ*. (1986) 33:341–65. doi: 10.1016/0304-4076(86)90002-3

13. Lambert D. Zero-inflated Poisson regression, with an application to defects in manufacturing. *Technometrics*. (1992) 34:1–14. doi: 10.2307/1269547

14. Lewis RJ. *An introduction to classification and regression tree (CART) analysis; proceedings of the annual meeting of the society for academic emergency medicine in San Francisco*. California: Citeseer (2000).

15. Virlogeux V, Li M, Tsang TK, Feng L, Fang VJ, Jiang H, et al. Estimating the distribution of the incubation periods of human avian influenza a (H7N9) virus infections. *Am J Epidemiol*. (2015) 182:723–9. doi: 10.1093/aje/kwv115

16. Geng Z, Li J, Niu Y, Wang X. Goodness-of-fit test for a parametric mixture cure model with partly interval-censored data. *Stat Med*. (2023) 42:407–21. doi: 10.1002/sim.9623

17. Yuying W, Yang Z, Xiaoliang W, Xiaoliang C, Tieqiang W, Dongsheng H, et al. Calculation method of incubation period of infectious diseases and its epidemiological significance. *Chin J Prev Med*. (2020) 54:1026–30. doi: 10.3760/cma.j.cn112150-20200629-00943

18. Li Q, Guan X, Wu P, Wang X, Zhou L, Tong Y, et al. Early transmission dynamics in Wuhan, China, of novel coronavirus-infected pneumonia. *N Engl J Med*. (2020) 382:1199–207. doi: 10.1056/NEJMoa2001316

19. Li ZY, Zhang Y, Peng LQ, Gao RR, Jing JR, Wang JL, et al. Demand for longer quarantine period among common and uncommon COVID-19 infections: a scoping review. *Infect Dis Poverty*. (2021) 10:56. doi: 10.1186/s40249-021-00847-y

20. Sun K, Wang W, Gao L, Wang Y, Luo K, Ren L, et al. Transmission heterogeneities, kinetics, and controllability of SARS-CoV-2. *Science*. (2021) 371:371. doi: 10.1126/science.abe2424

21. Kraemer MUG, Yang CH, Gutierrez B, Wu CH, Klein B, Pigott DM, et al. The effect of human mobility and control measures on the COVID-19 epidemic in China. *Science*. (2020) 368:493–7. doi: 10.1126/science.abb4218

22. Jansen L, Tegomoh B, Lange K, Showalter K, Figliomeni J, Abdalhamid B, et al. Investigation of a SARS-CoV-2 B.1.1.529 (omicron) variant cluster-Nebraska, November-December 2021. *MMWR Morb Mortal Wkly Rep*. (2021) 70:1782–4. doi: 10.15585/mmwr.mm705152e3

23. Backer JA, Eggink D, Andeweg SP, Veldhuijzen IK, van Maarseveen N, Vermaas K, et al. Shorter serial intervals in SARS-CoV-2 cases with omicron BA.1 variant compared with Delta variant, the Netherlands, 13 to 26 December 2021. *Euro Surveill*. (2022) 27:2200042. doi: 10.2807/1560-7917.ES.2022.27.6.2200042

24. Lee JJ, Choe YJ, Jeong H, Kim M, Kim S, Yoo H, et al. Importation and transmission of SARS-CoV-2 B.1.1.529 (omicron) variant of concern in Korea, November 2021. *J Korean Med Sci*. (2021) 36:e346. doi: 10.3346/jkms.2021.36.e346

25. Garrett N, Tapley A, Andriesen J, Seocharan I, Fisher LH, Bunts L, et al. High rate of asymptomatic carriage associated with variant strain omicron. *med Rxiv*. (2022). doi: 10.1101/2021.12.20.21268130

26. Petersen E, Ntoumi F, Hui DS, Abubakar A, Kramer LD, Obiero C, et al. Emergence of new SARS-CoV-2 variant of concern omicron (B.1.1.529) - highlights Africa's research capabilities, but exposes major knowledge gaps, inequities of vaccine distribution, inadequacies in global COVID-19 response and control efforts. *Int J Infect Dis*. (2022) 114:268–72. doi: 10.1016/j.ijid.2021.11.040

27. Shi Q, Hu Y, Peng B, Tang XJ, Wang W, Su K, et al. Effective control of SARS-CoV-2 transmission in Wanzhou. *China Nat Med*. (2021) 27:86–93. doi: 10.1038/s41591-020-01178-5

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Temporary impact on medical system and effectiveness of mitigation strategies after COVID-19 policy adjustment in China: a modeling study

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Background: As China amends its "zero COVID" strategy, a sudden increase in the number of infections may overwhelm medical resources and its impact has not been quantified. Specific mitigation strategies are needed to minimize disruption to the healthcare system and to prepare for the next possible epidemic in advance.

Method: We develop a stochastic compartmental model to project the burden on the medical system (that is, the number of fever clinic visits and admission beds) of China after adjustment to COVID-19 policy, which considers the epidemiological characteristics of the Omicron variant, age composition of the population, and vaccine effectiveness against infection and severe COVD-19. We also estimate the effect of four-dose vaccinations (heterologous and homologous), antipyretic drug supply, non-pharmacological interventions (NPIs), and triage treatment on mitigating the domestic infection peak.

Result: As to the impact on the medical system, this epidemic is projected to result in 398.02 million fever clinic visits and 16.58 million hospitalizations, and the disruption period on the healthcare system is 18 and 30 days, respectively. Antipyretic drug supply and booster vaccination could reduce the burden on emergency visits and hospitalization, respectively, while neither of them could not reduce to the current capacity. The synergy of several different strategies suggests that increasing the heterologous booster vaccination rate for older adult to over 90% is a key measure to alleviate the bed burden for respiratory diseases on the basis of expanded healthcare resource allocation.

Conclusion: The Omicron epidemic followed the adjustment to COVID-19 policy overloading many local health systems across the country at the end of 2022. The combined effect of vaccination, antipyretic drug supply, triage treatment, and PHSMs could prevent overwhelming medical resources.

KEYWORDS

epidemic control, infectious diseases, COVID-19, vaccine, medical rescue

Introduction

As one of the seven human coronaviruses (HCoVs) detected to date (1), the SARS-CoV-2 genome encodes seven accessory proteins that may contribute to immune evasion (2), and ORF9c and ORF10 play key roles in viral replication and immune evasion processes (3). In the past 3 years, a dynamic zero COVID-19 strategy has been adopted in China and the number of infections, morbidities, serious illnesses, and deaths in the country have remained at a low level (4). Based on the virulence and transmission characteristics of the Omicron variant, China issued adjustments to epidemic measures on 11 November and 7 December 2022, respectively, including canceling nationwide nucleic acid tests, no longer checking health codes in cross-regional floating personnel, and isolating asymptomatic and mild illness at home (5, 6). With the policy lifted, China may have several waves of peak infections, coupled with the massive population floating and the increased immune escape ability of the SARS-CoV-2 variants (7, 8).

It has been shown that a quarter of deaths could be attributed to a shortage of healthcare resources (9). Statistically, the first wave of the Omicron epidemic in both Taiwan and Hong Kong peaked within 2 months, during which the number of infections spiked, triggering severe medical challenges on medical sources and leading to a significant increase in mortality (10, 11). When the capacity of the healthcare system is exceeded, a sudden increase in the number of infections may result in unnecessary medical demands and run-on healthcare resources. Therefore, it is particularly important to take measures early to delay the peak of the pandemic and reduce the number of patients, avoiding catastrophic medical resource challenges.

In response to the peak in infections caused by COVID-19, the key to reducing medical crowding is to decrease the proportion of critically ill patients and then minimize the number of mild and general patients. Vaccination is currently an important means of preventing severe cases and death from COVID-19 (12). A case–control study pointed out that one or two booster doses of vaccination have increased VE against medically attended COVID-19 illness (13), and receiving anti-SARS-CoV-2 vaccines effectively protected HCWs from breakthrough infections (14). As of 13 December 2022, 90.37% of the Chinese population has completed vaccination, but those over 60 and 80 years old are only 86.6 and 66.4%, respectively (15). A real-world study in Hong Kong showed that three doses of homologous inactivated vaccine decreased hospitalization and mortality well, but the utility of protection against infection was less satisfactory (16). However, the heterologous booster vaccine could reduce susceptibility to Omicron infection by 39% within 60 days (17), and it may be more helpful in infection peak mitigation. At the same time, a hierarchical medical system plays a crucial role in triaging infection cases. For example, Singapore has implemented the Public Health Preparedness Clinic (PHPC) program to strengthen the surveillance and treatment of outbreaks in the primary care system, focusing efficiently on serious and critical illnesses in general hospitals (18). In addition, adequate rapid antigen testing (RAT) and drug stockpiles facilitate a reasonable assessment of the health status of patients, allow asymptomatic and mild patients to be isolated at home, and reduce the panic of the population at the peak of infection (19).

Therefore, we aimed to simulate the likely demand for fever clinic visits and hospitalization beds in 120 days after policy liberalization and match with realistic capacity in China to assess the risk of challenges on medical resources. To determine how China can safely transition from a zero-COVID approach, we estimate the effect of four-dose vaccinations, adequate antipyretic drug supply, non-pharmacological interventions (NPIs), and triage treatment on mitigating the infection peak.

Materials and methods

We conducted a modeling study to project the demand for medical sources (fever clinic visits and hospitalization beds, respectively) after adjustment to the COVID-19 policy in China. This modeling study relies on publicly available aggregated data only. As such, institutional review and informed consent are waived by the Institutional Review Board of the first affiliated hospital of Guangzhou Medical University (Guangzhou, China).

Data modeling

The compartmental model was chosen to simulate the transmission of COVID-19 between populations and to explore the impact of different interventions. Transitions between compartments are simulated through a stochastic chain binomial process. We developed an age-specific stochastic compartmental model (20–22) to describe the spread of the Omicron outbreak within a population that is stratified by different states. Considering the epidemiologic history of COVID-19, we divided the population into several compartments: susceptible (S), exposed (E), asymptomatic infected (A), symptomatic infected (I), recovered (R), critical (H), and dead (D). In this study, we model the dynamics of COVID-19 transmission with the Omicron BA.5 variant by considering virus-specific parameters such as the basic reproduction number (R0) and the incubation period (α). The

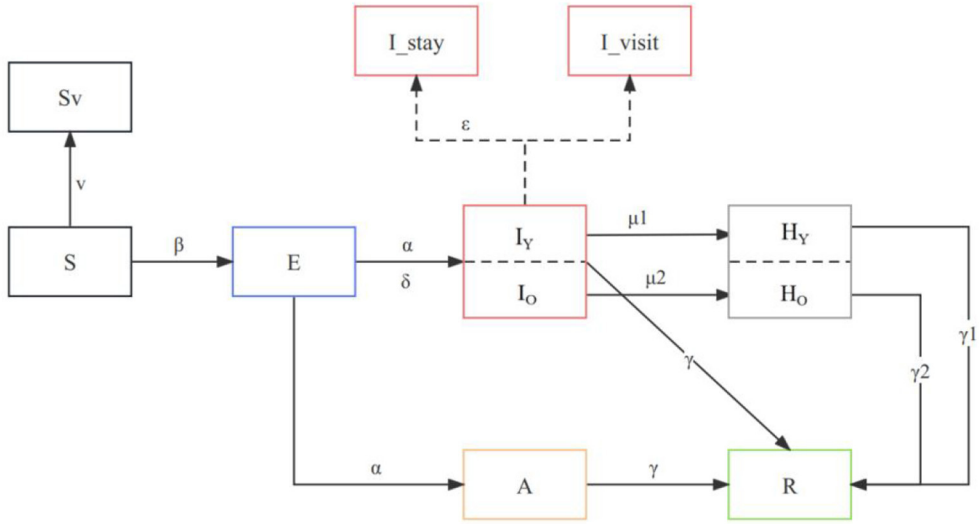

FIGURE 1
Model structure flow diagram with transition rates between states. The subscript "o" refers to the compartment of >60 years old and "y" to those who are <60 years old, and the parameters are age-specific. All compartments and parameters are defined in Supplementary Table 1.

TABLE 1 Vaccine effectiveness (VE) in preventing Omicron infection.

Vaccine (combination)	Vaccine effectiveness (VE) in preventing Omicron infection	Source	Vaccine effectiveness (VE) against hospitalization	Source
CoronaVac × 3 (<60 days after the 3rd dose)	8.6% (95% CI, 5.6–11.5)	Ranzani et al. (24)	86.3% (83.7–88.5)	Alejandro Jara, (25)
CoronaVac × 2 + BNT162b2	56.8% (95% CI, 56.3–57.3)		96.1% (95.3–96.9)	
CoronaVac × 2 + AZD1222	-		97.7% (97.3–98.0)	
BBIBP-CorV × 2 + V-01* (<60 days after the 3rd dose)	64% (95% CI, 23–83)	Wang et al. (17)		
CoronaVac × 2 + V-01* (<60 days after the 3rd dose)	39% (95% CI, 3–62)			

V-01* refers to the recombinant SARS-CoV-2 fusion protein vaccine.

incorporation of distinct parameters is shown in the flow diagram (Figure 1).

We accounted for age differences in severe rates, length of recovery, mortality rates, and vaccine protection rates to improve the projection of the reality. Age-specific parameters were included in the model, such as those vaccinated over 60 years old (V_O) and those vaccinated under 60 years old (V_Y), as well as vaccine efficacy in preventing severe illness (θ1, θ2, and θ3). We also consider the proportion of susceptible individuals aged 60 and above (ω) and the baseline severity rate of COVID-19 for young (ρ1) and older adult (ρ2) individuals. Our model incorporates the proportion of asymptomatic infections (δ) and the recovery periods for mild (γ), severe for young (γ1), and severe for older adult (γ2) cases. The description of the parameters and values is indicated in Supplementary Table 1.

As of 10 December 2022, the two- and three-dose vaccine uptake in China was 91% and 57%, respectively. Since more than 95% of vaccines administered are inactivated virus vaccines, we assume that all vaccines received are inactivated virus vaccines for their first two doses. It is reported that heterologous booster vaccination would enhance the protection against severe death (23), but the effects of different sequential vaccinations are different. Therefore, we simulate the vaccination effect by inputting parameters revealed by real-world studies to simulate the reality as possible (Table 1).

To account for the effects of vaccination on the transmission dynamics of the COVID-19 outbreak, we incorporated the vaccination rates of different age groups and the efficacy of vaccine protection against critical diseases. Specifically, we added the rate of protected critical disease θ for booster shots of three different types of vaccines [inactivated(θ_1), mRNA(θ_2), and adenovirus-vectorθ_3], based on the efficacy of three inactivated vaccines. Using this information, we calculated the rate of severe disease after vaccine protection μ as follows:

$$\mu = \rho * (1 - \text{vaccination proportion} * \theta),$$

where ρ represents the severe rate, and θ represents vaccine effectiveness against severe COVID-19. During the model development process, we incorporated the presence or absence of symptoms in exposed individuals and accounted for the potential

challenge of medical resources. Furthermore, we also considered the vaccination status of different age groups by stratifying the population accordingly. Finally, both infected and critical individuals gradually heal and turn into recovered individuals. Thus, our proposed model is the following:

Overall, our model provides a robust framework for analyzing the transmission dynamics of the COVID-19 outbreak, while considering important demographic and medical factors that may influence the spread of the disease.

$$\begin{cases} \frac{dS(t)}{dt} = -\frac{\beta * S(t) * (I_Y S(t) + I_O S(t) + I_Y V(t) + I_O V(t) + A(t))}{N} \\ \frac{dE(t)}{dt} = \frac{\beta * S(t) * (I_Y S(t) + I_O S(t) + I_Y V(t) + I_O V(t) + A(t))}{N} - \alpha * E(t) \\ \frac{dI_Y S(t)}{dt} = (1-\delta)*(1-\omega)*(1-\varepsilon)*\alpha * E(t) - (\mu_1 + \gamma) * I_Y S(t) \\ \frac{dI_O S(t)}{dt} = (1-\delta)*\omega*(1-\varepsilon)*\alpha * E(t) - (\mu_2 + \gamma) * I_O S(t) \\ \frac{dI_Y V(t)}{dt} = (1-\delta)*(1-\omega)*\varepsilon*\alpha * E(t) - (\mu_1 + \gamma) * I_Y V(t) \\ \frac{dI_O V(t)}{dt} = (1-\delta)*\omega*\varepsilon*\alpha * E(t) - (\mu_2 + \gamma) * I_O V(t) \\ \frac{dA(t)}{dt} = \delta * \alpha * E(t) - \gamma * A(t) \\ \frac{dH_Y(t)}{dt} = \mu_1 * (I_Y S(t) + I_Y V(t)) - \gamma_1 * H_Y(t) \\ \frac{dH_O(t)}{dt} = \mu_2 * (I_O S(t) + I_O V(t)) - \gamma_2 * H_O(t) \\ \frac{dR(t)}{dt} = \gamma * (I_Y S(t) + I_O S(t) + I_Y V(t) + I_O V(t) + A(t)) + \gamma_1 * H_Y(t) + \gamma_2 * H_O(t) \\ S = N - E_0 - I_0 - A_0 - H_0 - R_0 \\ \beta = R_t * \gamma \end{cases} \quad (1)$$

To calculate the transmission coefficient β and the effective reproduction number Rt in our model, we utilized the generation-interval-based method proposed by Wallinga J (26).

$$p_{ij} = w(t_i - t_j) / \sum_{i \neq k} w(t_i - t_k) \quad (2)$$

$$R_j = \sum_i p_{ij} \quad (3)$$

The relative probability of an individual i being infected by an individual j is determined by the generation interval, which accounts for differences in the time between the onset of symptoms in the two cases. This probability can be represented by a probability distribution, which is used to calculate the effective reproduction number Rt in equation (3). The resulting distribution of Rt can be used to estimate the number of future exposed, infected, and removed individuals by inputting it into the modified SEIR model described by equation (1). Furthermore, by utilizing a 95% confidence interval of Rt, we can obtain a distribution of the number of future cases with varying degrees of uncertainty.

Effectiveness of NPIs

Hong Kong has experienced the fifth and sixth COVID-19 wave (being Omicron) whereas Shanghai has had one dominated by Omicron BA.2 that led to 2 months of city-wide lockdown. Kathy Leung et al. have categorized NPIs implemented during these waves into four levels and estimated their effectiveness from the associated changes in reproductive number (27). Considering the epidemic during the relaxation of zero COVID-19 is also driven by Omicron, we refer to their experience to quantify the effectiveness of NPIs (Supplementary Table 2).

Scenario assumptions

We map China's combinations of NPIs and their intensity to the above-mentioned NPI levels. We assume that the NPIs after reopening (7 December 2022) are as effective as Level 1, which would reduce Rt by 15%. A dynamic adjustment of NPIs is anticipated. We assume that the majority of schools were

on winter holidays after a month (equivalent to level 2 NPI, which reduces Rt by 44%, Supplementary Table 2) and that 30% of the population had stockpiled drugs and self-isolated due to the government's health education campaign after reopening. The simulation considers the following conditions:

(1) 20,000 omicron-infected individuals were introduced into the Chinese population on 7 December 2022.
(2) The basic reproduction number (R_0) at the start of the simulation was set to 8.3, and the effective contact rate was 70%.
(3) As of 13 December 2022, the susceptible population in the baseline scenario was 1.4 billion, based on the vaccine efficacy (VE) against infection reported in Table 1.
(4) The VE is set according to the values in Table 1.
(5) The medical capacity at that time was taken into account.
(6) The model was simulated in 120 days.

Assessment of the overwhelming healthcare resource

According to a report by the National Health Commission of the People's Republic of China (28), there are 33,000 secondary or tertiary hospitals, 19,400 community health centers, and 22,000 fever sentinel sites that operate fever clinics nationwide. Assuming a maximum daily capacity of 150 visits in secondary and higher hospitals and 50 visits in community health and fever sentinel sites, the national maximum capacity for a single day is approximately 7.02 million visits. According to the 2016 National Healthcare Services and Quality Safety Report (29), the average number of respiratory beds in tertiary general hospitals in China is 61.15 and 41.46 in secondary general hospitals. Furthermore, there are 273,000 emergency transition beds, and the capacity of admission beds is 0.908 million.

Considering that COVID-19 patients may be asymptomatic, this group of patients does not pose a burden on the healthcare system. Moreover, mild COVID-19 are recommended to rest at home and take medication on their own, while there was a shortage of medication in the initial period of reopening. We assumed that mild COVID-19 without drug stockpiles and those severe would visit hospitals and take up resources for emergency and inpatient care, respectively. Therefore, we compared the projected number of hospital visits to the daily maximum admissible capacity to assess the overwhelming of healthcare resources.

Result

Baseline scenario

The baseline scenario simulated a situation where the majority of schools were on winter holidays (equivalent to level 2 NPI), and 30% of the symptomatic population had stockpiled drugs and therefore isolated themselves at home. The simulation involved the following conditions: (1) 20,000 Omicron-infected individuals were introduced into the Chinese population on 7 December 2022; (2) the basic reproduction number (R0) at the start of the simulation was set to 8.39, effective contact rate is 70% (considering NPIs reached level 2 at the time of reopening); (3) as of 13 December 2022, based on the vaccine efficacy (VE) against infection in Table 1, the current input susceptible population in the baseline scenario is 1.4 billion; (5) the VE against infection and developing severe is reported in Table 1; and (6) the simulation period is 120 days.

The simulated baseline scenario shows that the Omicron variant in China after the reopening on 7 December 2022, could trigger a tsunami of COVID-19 cases under the current epidemic control policy and vaccination rates. During the 120-day simulation period, the epidemic peaks on day 34, with an infection rate of over 1.1 billion (∼80% of the population in China) and a single-day peak of 152.13 million infected individuals (Figures 2A, D).

To evaluate the impact of this Omicron epidemic on the national healthcare system, we consider that symptomatic patients without drug stockpiling would attend the emergency department visit, and severe or critical COVID-19 patients would require hospital beds for respiratory illness.

The simulated baseline scenario shows that the Omicron variant in China after the reopening on 7 December 2022, could trigger a tsunami of COVID-19. During the 120-day simulation period, a single-day peak of 152.13 million infected individuals reached on day 34 (Figure 2A). Nationwide, as to the impact on the medical system, the estimated single-day peak of emergency department visits would reach 300 million visits on day 32, 4.4 times the current capacity, and the overwhelming period would last for 18 days (Figure 2B). The daily peak of hospitalization number (5.91 million, day 40) is 6.5 times of admission capacity for respiratory diseases in China, and the bed shortage period is estimated to last 36 days (Figure 2C).

Overall, this epidemic will raise an infection number of over 1.1 billion (80% of the population in China, Figure 2D), and result in 398.02 million emergency department visits (Figure 2E) and 16.58 million hospitalizations (Figure 2F).

Effectiveness of individual mitigation strategies

We study the impact of two strategies to alleviate the COVID-19 burden: (1) vaccine (promoting the fourth dose vaccination coverage with homologous and heterologous boosters) and (2) antipyretic drug stockpiling. In terms of vaccination strategies, increasing the coverage of homologous booster vaccinations (inactivated vaccines) did not significantly reduce the total number of emergency department visits. However, heterologous booster vaccinations (including mRNA and adenovirus-based boosters) significantly reduced the daily number of visits. Compared to the baseline scenario, increasing the fourth dose vaccine rate to 80–90% could reduce the daily visit by 1.4–1.5 times and shorten the peak visit period by 3 days, with similar effects observed in different age groups (Figures 3A, C, E).

Filling the vaccination coverage gaps (i.e., vaccinating all eligible individuals) could effectively reduce hospitalization rates, mainly impacting those over 60 years old. Improving homologous booster vaccinations (inactivated vaccines) at 80% and 90% coverage could reduce hospitalizations by 41.5% and 57.2%

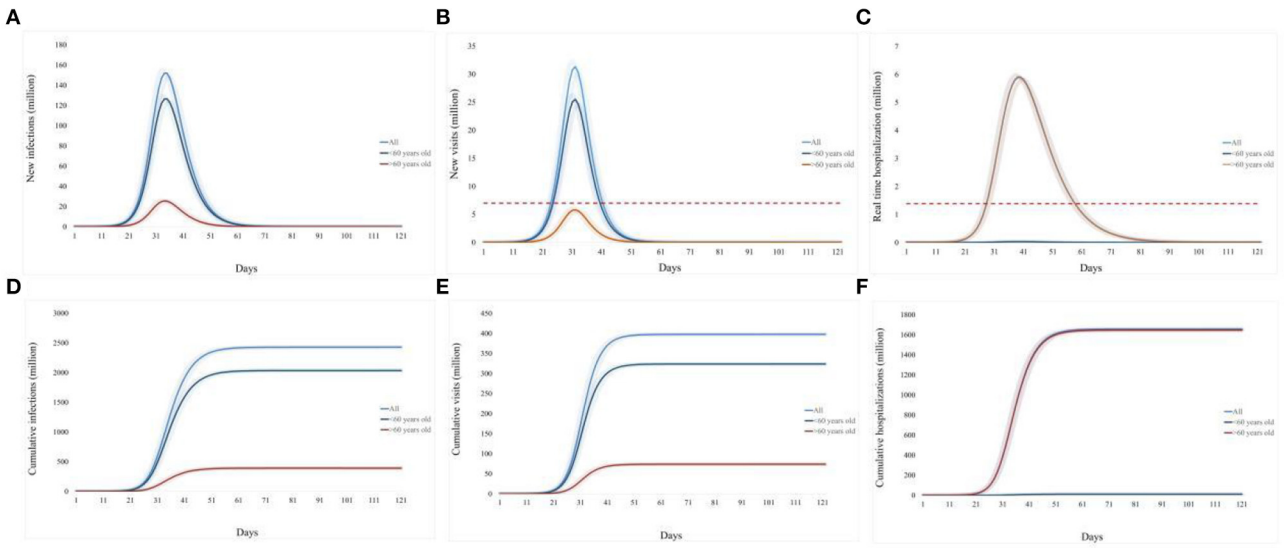

FIGURE 2
Prediction of the medical resource burden in China's Omicron outbreak under the baseline scenario from 7 December 2022 to 7 March 2023. **(A)**, Daily new infections; **(B)**, daily new emergency department visits; **(C)**, daily existing hospitalizations; **(D)**, cumulative infection over 120 days; **(E)**, cumulative emergency department visits over 120 days; **(F)**, cumulative hospitalizations over 120 days. The light blue curve represents all age groups, the dark blue line represents individuals under 60 years old, and the brown line represents individuals over 60 years old. The red dotted line refers to the existing medical capacity.

and shorten the bed shortage period by 7 and 11 days, respectively. Heterologous booster vaccinations could further reduce hospitalizations, with 80% coverage (mRNA or adenovirus) reducing hospitalizations by 67–68% and shortening the bed shortage period by more than half. With 90% heterologous booster coverage (mRNA or adenovirus), hospitalizations would be reduced by 82%, and there would be no bed shortage (Figures 3B, D, F).

Regarding antipyretic drug supply, we assumed that 50% and 70% of the population could access medications such as antipyretics or antiviral drugs and therefore facilitate at-home isolation when feasible. The results showed (Figure 3A) that this approach could reduce the total emergency department visit number by 1.4–2.3 times and shorten the peak visit period by 6 days. The impact of drug reserves was more concentrated in individuals under 60 years old, with 70% personal drug reserve supply greatly alleviating the pressure on emergency department visits (reducing the number of visits by up to 90%) (Figure 3C). This mitigation measure did not significantly impact the bed burden for respiratory diseases (Figures 3B, D, F).

Impact of combined mitigation strategies on healthcare resources in China after reopening

Our results showed that relying solely on individual mitigation strategies could not reduce emergency department visits and hospitalization to the current level of national healthcare resource allocation and admission capacity. Here, we evaluated the synergy of several different strategies: increasing vaccine coverage among unvaccinated individuals (including homologous or heterologous vaccination), personal epidemic material reserves, adopting different levels of NPI, and expanding healthcare resource allocation and service capacity by 1.5 times (daily emergency department visits of 0.908 million, converting general outpatient beds to respiratory specialty outpatient beds totaling 1.362 million). By combining different strategies for synergy, as shown in Figure 4, increasing the heterologous booster vaccination rate for older adult to over 90% is a key measure to alleviate the admission burden of emergency department admission (Supplementary Table 3). Furthermore, increasing personal anti-COVID drug reserves to over 70% could significantly reduce emergency department visits. The synergy between different strategies can effectively prevent overburdening the nation's healthcare resource allocation and service capacity, fundamentally alleviating the pressure to treat patients, and laying a solid foundation for the turning point in the COVID-19 epidemic.

Discussion

Our study projected the number of fever clinic visits and hospitalizations in China after releasing the zero COVID-19 policy. We estimated that the introduction of the Omicron variant would cause substantial surges in fever clinics and hospitalization, and the peak demand would overwhelm the healthcare system with an estimated burden of four times the available capacity. This epidemic is projected to result in 398.02 million fever clinic visits and 16.58 million hospitalizations, and the disruption period on the healthcare system is 18 and 30 days, respectively.

We found antipyretic drug supply and booster vaccination could reduce the burden on emergency visits and hospitalization, respectively. However, considering the current status, they cannot cope with the medical resources overwhelming raised by the

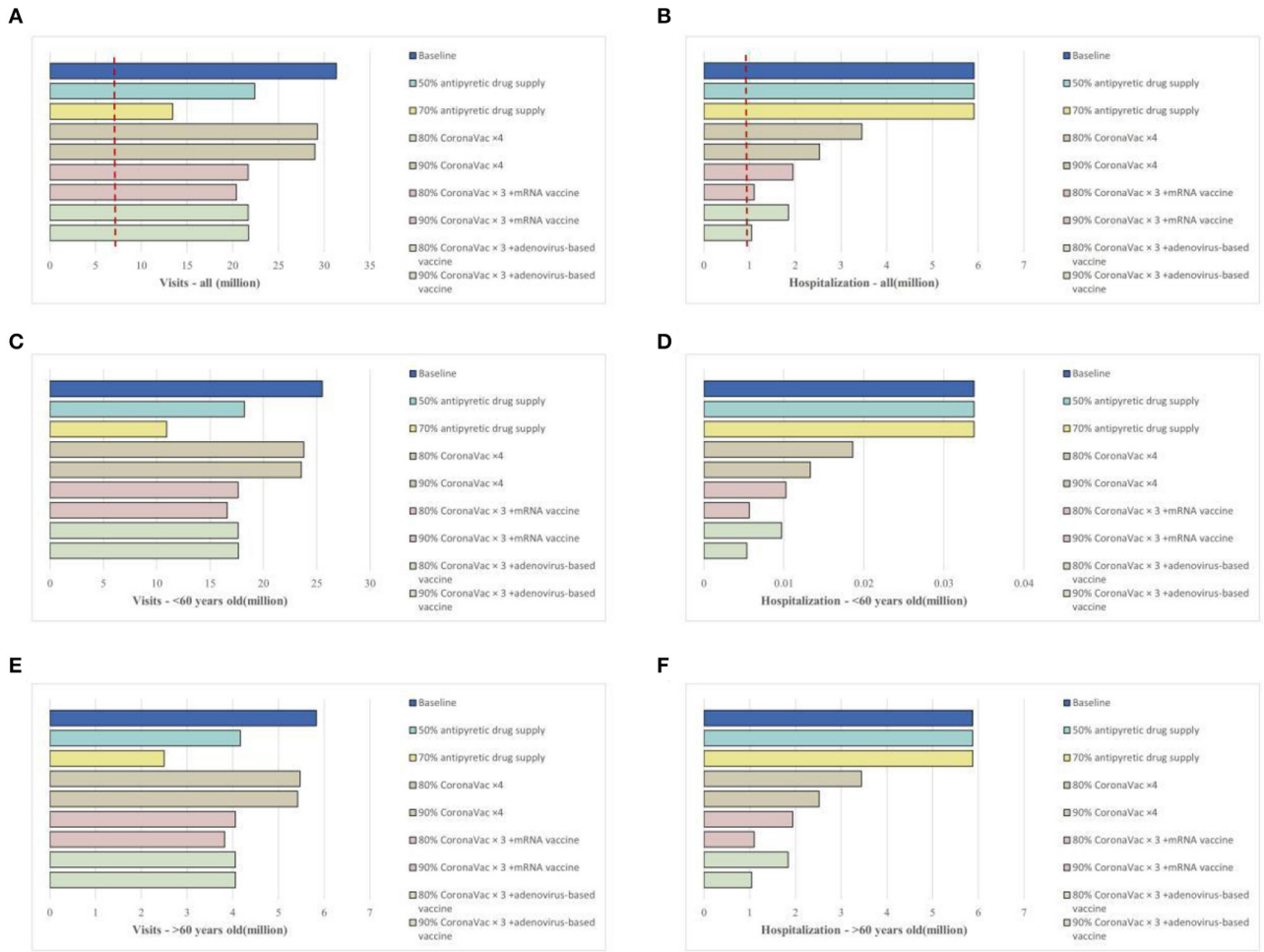

FIGURE 3
Impact of vaccination strategies or epidemic material reserves on the medical resource burden in China's Omicron outbreak under the baseline scenario. **(A)**, Daily new emergency department visits for all age groups; **(B)**, daily existing hospitalizations for all age groups; **(C)**, daily new emergency department visits for individuals over 60 years old; **(D)**, daily existing hospitalizations for individuals over 60 years old; **(E)**, daily new emergency department visits for individuals under 60 years old; **(F)**, daily existing hospitalizations for individuals under 60 years old.

existence of the zero COVID-19 policy. Lack of bed capacity, scarcity in drug supplies, and high occupancy rates further increase that burden.

Vaccination is effective in preventing COVID-19 patients from developing severe and death. As of November 2022, 0.89 billion have received three doses of inactivated vaccine in China (30). Our results reveal that the fourth dose of homologous inactivated vaccination and sequential mRNA vaccination introduced could reduce only a modest proportion of fever clinic visits. This indicates the limited effectiveness of 4-dose inactivated homologous vaccination and mRNA sequential vaccination against Omicron infection, which is consistent with the real-world studies in Hong Kong that the short-term effectiveness against Omicron infection of a third or fourth dose of either the mRNA or inactivated vaccine (31). Nevertheless, 4-dose vaccination significantly reduced the number of hospitalizations and the effects were similar between booster vaccine scenarios in inactivated vaccine and mRNA vaccine, which echoes with previous studies (32, 33). In addition, we found that the fourth dose of adenovirus vaccination was significantly reducing the peak both in fever clinic visits and hospitalization. This provides a new strategy for sequential vaccination in response to the co-prevalence of multiple SARS-CoV-2 variants, but monitoring of vaccine protective effects should be enhanced considering the lack of real-world study evidence.

In a questionnaire survey of Omicron infection in Macau after the lift of the zero COVID-19 policy, more than 60% of Omicron-infected patients had symptoms, such as fever (≥37.5C), dry or sore throat, blocked or runny nose, fatigue, headache, muscle soreness, that (32) may require antipyretic and other pharmacological interventions. An adequate supply of antipyretic medications at the onset of peak infection can safeguard the at-home health management of mild and asymptomatic COVID-19 patients, leaving valuable emergency resources for acute and critically ill patients.

We found that expanding the available capacity by 1.5 times would reduce the burden of public health decisions, and how to flexibly increase the admission capacity is the actual problem to be solved. In Singapore, the Public Health Preparedness

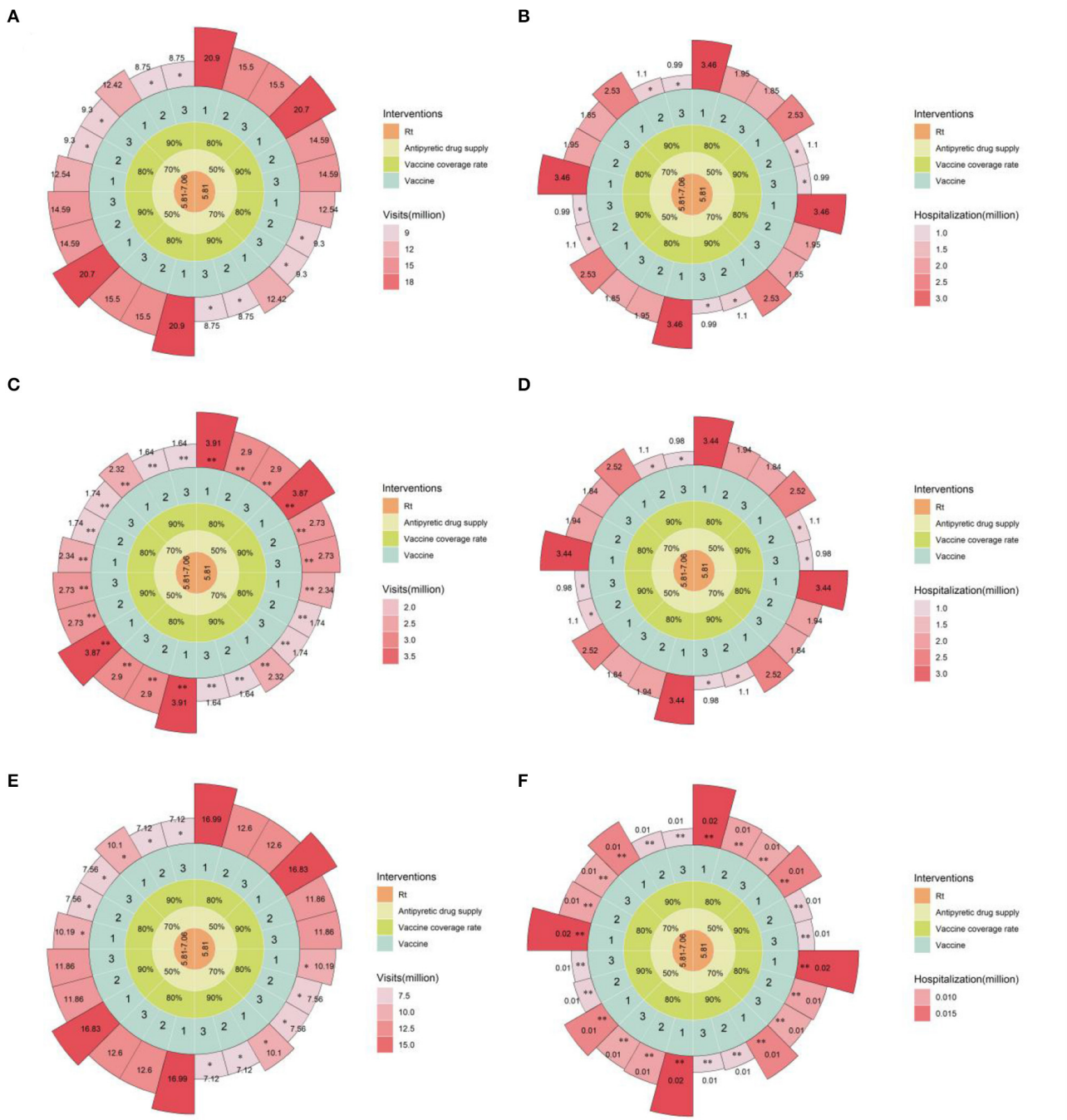

FIGURE 4
Estimated daily emergency department visits and hospitalizations under combined mitigation strategies in the baseline scenario. **(A)**, Daily new emergency department visits for all age groups; **(B)**, daily existing hospitalizations for all age groups; **(C)**, daily new emergency department visits for individuals over 60 years old; **(D)**, daily existing hospitalizations for individuals over 60 years old; **(E)**, daily new emergency department visits for individuals under 60 years old; **(F)**, daily existing hospitalizations for individuals under 60 years old. **indicates that the available single-day outpatient and inpatient capacity is lower than the existing available outpatient and inpatient capacity in China, *indicates that the available single-day outpatient and inpatient capacity is below 1.5 times the available outpatient and inpatient capacity in China. From the innermost to the outermost concentric circles, the circular Manhattan plot shows the combination of different intervention measures: 1 to 3 for CoronaVac x 4, CoronaVac x 3 + mRNA vaccine, CoronaVac x 3 + adenovirus vaccine booster schemes, respectively; 80 or 90% vaccination coverage in the population after policy liberalization; 50 or 70% personal epidemic material reserves; Rt represents different levels of NPI intensity. Rt = 5.81–7.06 corresponds to the situation where the first 60 days are under Level 2 NPI and the following 60 days under Level 1 NPI, while Rt = 5.81 corresponds to the entire 120-day simulation period being under Level 2 NPI.

Clinic (PHPC) system played a critical role in keeping general hospitals free of medical overcrowding after the outbreak. Such a triage treatment enables a large number of patients with mild-to-moderate COVID-19 to be treated in community clinics. In addition, telemedicine is recommended, to, reduce the face-to-face infection and to simplify the consultation, which was also undertaken in England, UK, in 2021 (34). With as many as 10,000 primary hospitals in China, improving and enhancing the level of care in secondary hospitals is a future endeavor that will facilitate the allocation of resources from tertiary hospitals to focus on critical patients and other non-neoconcentric emergencies.

This study has several limitations. First, large-scale nucleic acid screening is no longer centrally organized in China after reopening, so real infection status in the population is not available for validation. To fill this gap, the real-time reproductive number was inferred from the strength of current NPIs, and a 5% range was set to cover as many scenarios as possible. Moreover, we compared the prediction with nationwide monitoring data (Supplementary Figure 1) and found that the differences between the two data in the peak time of fever outpatient visits and hospitalizations were within a week. Second, we assumed that the vaccine effectiveness did not change over time during the projection because the decline of the neutralizing antibody of the fourth dose vaccination is not clear so far.

As antibodies decay and virus mutation, a second peak of SARS-CoV-2 infections may re-emerge in China. We compiled information on COVID-19 outbreaks in 22 developed countries or regions in 2022 (35), finding that the average period between two infection peaks was 149.35 days (median: 161 days), and the average number of new cases in the second peak of infection was 48.61% (median: 41.72%) of the first peak (Supplementary Table 4). As SARS-CoV-2 continues to mutate, ongoing clinical symptom surveillance and vaccine efficacy assessment will help us recognize the transmission risk (36–38). Our study will keep an eye on different durations of the symptoms in subsequent waves and focus on long-time immunogenicity and effectiveness of full vaccination and booster dose of COVID-19 vaccine (based on age and chronic conditions) so that the new round need on the medical system could be timely estimated. Our study provides several suggestions to alleviate the crowding of medical resources due to the above possible infection peak, which helps facilitate early layout and coordinated deployment of prevention and control resources.

Conclusion

The Omicron epidemic followed the adjustment of COVID-19 policy overload in many local health systems across the country, and the combined effect of vaccination, antipyretic drug supply, triage treatment, and PHSMs could prevent overwhelming medical resources.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

JL: Conceptualization, Formal analysis, Writing—original draft, Writing—review & editing. RC: Data curation, Writing—original draft, Writing—review & editing. ZL: Methodology, Software, Writing—review & editing. YW: Formal analysis, Writing—original draft. WH: Visualization, Writing—review & editing. RL: Supervision, Writing—review & editing. JS: Resources, Writing—review & editing. QL: Resources, Writing—review & editing. LL: Formal analysis, Writing—review & editing. MZ: Formal analysis, Writing—review & editing. ZC: Funding acquisition, Writing—review & editing. YG: Software, Writing—review & editing. WZ: Resources, Writing—review & editing. TL: Investigation, Writing—review & editing. AO: Data curation, Writing—review & editing. CH: Data curation, Investigation, Visualization, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1259084/full#supplementary-material

References

1. Kesheh MM, Hosseini P, Soltani S, Zandi M. An overview on the seven pathogenic human coronaviruses. *Rev Med Virol.* (2022) 32:e2282. doi: 10.1002/rmv.2282

2. Zandi M, Shafaati M, Kalantar-Neyestanaki D, Pourghadamyari H, Fani M, Soltani S, et al. The role of SARS-CoV-2 accessory proteins in immune evasion. *Biomed Pharmacother.* (2022) 156:113889. doi: 10.1016/j.biopha.2022.113889

3. Zandi M. ORF9c and ORF10 as accessory proteins of SARS-CoV-2 in immune evasion. *Nat Rev Immunol.* (2022) 22:331–331. doi: 10.1038/s41577-022-00715-2

4. Guan WJ, Zhong NS. Strategies for reopening in the forthcoming COVID-19 era in China. *Natl Sci Rev.* (2022) 9:nwac054. doi: 10.1093/nsr/nwac054

5. *Notice on Further Optimization of the Prevention and Control Measures of the New Crown Pneumonia Outbreak*. Available online at: http://www.gov.cn/xinwen/2022-11/11/content_5726122.htm (accessed October 30, 2023).

6. *Notice on Further Optimization of the Prevention and Control Measures of the COVID-19 Outbreak*. Available online at: http://www.gov.cn/xinwen/2022-12/07/content_5730475.htm (accessed October 30, 2023).

7. Uriu K, Ito J, Zahradnik J, Fujita S, Kosugi Y, Schreiber G, et al. Enhanced transmissibility, infectivity, and immune resistance of the SARS-CoV-2 omicron XBB15 variant. *Lancet Infect Dis.* (2023) 23:280–1. doi: 10.1016/S1473-3099(23)00051-8

8. Yamasoba D, Uriu K, Plianchaisuk A, Kosugi Y, Pan L, Zahradnik J, et al. Virological characteristics of the SARS-CoV-2 omicron XBB.1.16 variant. *Lancet Infect Dis.* (2023) 6:655–6. doi: 10.1101/2023.04.06.535883

9. Kadri SS, Sun J, Lawandi A, Strich JR, Busch LM, Keller M, et al. Association between caseload surge and COVID-19 survival in 558 U.S. Hospitals, March to August 2020. *Ann Intern Med.* (2021) 174:1240–51. doi: 10.7326/M21-1213

10. *Experience and Lessons From the "opening up" of Epidemic Prevention and Control in Hong Kong*. Available online at: https://chinanews.com.cn/dwq/2022/12-26/9921268.shtml (accessed October 30, 2023).

11. *The Epidemic in Taiwan is Heating up Rapidly, and the Medical Crisis is Causing a Tragedy*. Phoenix TV. Available online at: https://www.sohu.com/a/543229950_516458

12. *Monitoring COVID-19 Cases, Hospitalizations, and Deaths by Vaccination Status*. Centre for Disease Control and Prevention of America. Available online at: https://www.cdc.gov/coronavirus/2019ncov/vaccines/effectiveness/monitoring.html

13. Link-Gelles R, Levy ME, Natarajan K, Reese SE, Naleway AL, Grannis SJ, et al. Estimation of COVID-19 mRNA vaccine effectiveness and COVID-19 illness and severity by vaccination status during omicron BA4 and BA5 sublineage periods. *JAMA Netw Open.* (2023) 6:e232598. doi: 10.1001/jamanetworkopen.2023.2598

14. Moscara L, Venerito V, Martinelli A, Di Lorenzo A, Toro F, Violante F, et al. Safety profile and SARS-CoV-2 breakthrough infections among HCWs receiving anti-SARS-CoV-2 and influenza vaccines simultaneously: an Italian observational study. *Vaccine.* (2023) 41:5655–61. doi: 10.1016/j.vaccine.2023.07.043

15. *Press Conference on December 14.* (2022). Available online at: http://www.nhc.gov.cn/xcs/s3574/202212/b927d43bd0354ffeb5a8e1b91377302f.shtml (accessed October 30, 2023).

16. *Forward Planning After HK's Fifth Wave of Omicron BA.2*. Available online at: https://sph.hku.hk/zh-HK/News-And-Events/Press-Releases/2022/HKUMed-proposes-forward-planning-after-Hong-Kong (accessed October 30, 2023).

17. Wang X-Y, Mahmood SF, Jin F, Cheah WK, Ahmad M, Sohail MA, et al. Efficacy of heterologous boosting against SARS-CoV-2 using a recombinant interferon-armed fusion protein vaccine (V-01): a randomized, double-blind and placebo-controlled phase III trial. *Emerg Microbes Infect.* (2022) 11:1910–9. doi: 10.1080/22221751.2022.2088406

18. *I Am Showing Respiratory Symptoms (Like a Cough, Runny Nose or Fever), Where Should I go?* Available online at: https://www.gov.sg/article/i-am-showing-respiratory-symptoms-where-should-i-go (accessed October 30, 2023).

19. Bond KA, Smith B, Gardiner E, Liew KC, Williams E, Walsham N, et al. Utility of SARS-CoV-2 rapid antigen testing for patient triage in the emergency department: a clinical implementation study in Melbourne, Australia. *Lancet Reg Health West Pac.* (2022) 25:100486. doi: 10.1016/j.lanwpc.2022.100486

20. Chang S, Pierson E, Koh PW, Gerardin J, Redbird B, Grusky D, et al. Mobility network models of COVID-19 explain inequities and inform reopening. *Nature.* (2021) 589:82–7. doi: 10.1038/s41586-020-2923-3

21. Radulescu A, Williams C, Cavanagh K. Management strategies in a SEIR-type model of COVID 19 community spread. *Sci Rep.* (2020) 10:21256. doi: 10.1038/s41598-020-77628-4

22. Yang Z, Zeng Z, Wang K, Wong S-S, Liang W, Zanin M, et al. Modified SEIR and AI prediction of the epidemics trend of COVID-19 in China under public health interventions. *J Thorac Dis.* (2020) 12:165–74. doi: 10.21037/jtd.2020.02.64

23. González S, Olszevicki S, Gaiano A, Salazar M, Regairaz L, Baino ANV, et al. Protection of homologous and heterologous boosters after primary schemes of rAd26-rAd5, ChAdOx1 nCoV-19 and BBIBP-CorV during the omicron outbreak in adults of 50 years and older in Argentina: a test-negative case-control study. *Lancet Reg Health Am.* (2023) 27:100607. doi: 10.1016/j.lana.2023.100607

24. Ranzani OT, Hitchings MDT, Dorion M, D'Agostini TL, de Paula RC, de Paula OFP, et al. Effectiveness of the CoronaVac vaccine in older adults during a gamma variant associated epidemic of covid-19 in Brazil: test negative case-control study. *BMJ.* (2021) 374:n2015. doi: 10.1136/bmj.n2015

25. Jara A, Undurraga EA, Zubizarreta JR, González C, Pizarro A, Acevedo J, et al. Effectiveness of homologous and heterologous booster doses for an inactivated SARS-CoV-2 vaccine: a large-scale prospective cohort study. *Lancet Glob Health.* (2022) 10:e798–806. doi: 10.1016/S2214-109X(22)00112-7

26. Wallinga J. Teunis Different epidemic curves for severe acute respiratory syndrome reveal similar impacts of control measures. *Am J Epidemiol.* (2004) 160:509–16. doi: 10.1093/aje/kwh255

27. Leung K, Leung GM, Wu JT. Modelling the adjustment of COVID-19 response and exit from dynamic zero-COVID in China. *medRxiv.* (2022) 2022.12.14.22283460. doi: 10.1101/2022.12.14.22283460

28. *In Response to the Rapid Increase in Demand for Fever Clinic Treatment, Four Measures Were Taken*. National Health Commission of the People's Republic of China. Available online at: http://health.people.com.cn/n1/2022/1214/c14739-32587169.html (accessed May 6, 2023).

29. *National Healthcare Services and Quality Safety Report*. National Health Commission of the People's Republic of China. Available online at: https://www.sohu.com/a/377611355_389597

30. *27 Billion People Completed Full Vaccination for the New Crown*. National Health Commission of the People's Republic of China. Available online at: http://health.people.com.cn/n1/2022/1129/c14739-32576966.html (accessed May 6, 2023).

31. Lau JJ, Cheng SMS, Leung K, Lee CK, Hachim A, Tsang LCH, et al. Real-world COVID-19 vaccine effectiveness against the Omicron BA.2 variant in a SARS-CoV-2 infection-naive population. *Nat Med.* (2023) 29:348–57. doi: 10.1038/s41591-023-02648-2

32. Liang J, Liu R, He W, Zeng Z, Wang Y, Wang B, et al. Infection rates of 70% of the population observed within 3 weeks after release of COVID-19 restrictions in Macao, China. *J Infect.* (2023) 86:402–4. doi: 10.1016/j.jinf.2023.01.029

33. Wei Y, Jia KM, Zhao S, Hung CT, Mok CKP, Poon PKM, et al. Estimation of vaccine effectiveness of CoronaVac and BNT162b2 against severe outcomes over time among patients with SARS-CoV-2 omicron. *JAMA Netw Open.* (2023) 6:e2254777. doi: 10.1001/jamanetworkopen.2022.54777

34. Burgess R. Moving from face-to-face to telephone assessments with patients during the COVID-19 pandemic. *Nurs Stand.* (2021) 36:45–50. doi: 10.7748/ns.2021.e11690

35. Coronavirus (COVID-19) Cases.

36. Li Z, Liu S, Li F, Li Y, Li Y, Peng P, et al. Efficacy, immunogenicity and safety of COVID-19 vaccines in older adults: a systematic review and meta-analysis. *Front Immunol.* (2022) 13:965971. doi: 10.3389/fimmu.2022.965971

37. Tartof SY, Slezak JM, Puzniak L, Hong V, Frankland TB, Ackerson BK, et al. Effectiveness of a third dose of BNT162b2 mRNA COVID-19 vaccine in a large US health system: a retrospective cohort study. *Lancet Reg Health Am.* (2022) 9:100198. doi: 10.1016/j.lana.2022.100198

38. De Maria L, Sponselli S, Caputi A, Pipoli A, Giannelli G, Delvecchio G, et al. Comparison of three different waves in healthcare workers during the COVID-19 pandemic: a retrospective observational study in an Italian University Hospital. *J Clin Med.* (2022) 11:3074. doi: 10.3390/jcm11113074

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Beyond SARS-CoV-2: epidemiological surveillance of respiratory viruses in Jalisco, Mexico

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Introduction: Respiratory viral infections represent a significant global health burden. Historically, influenza, rhinovirus, respiratory syncytial virus, and adenovirus have been the prevalent viruses; however, the landscape shifted with the widespread emergence of SARS-CoV-2. The aim of this study is to present a comprehensive epidemiological analysis of viral respiratory infections in Jalisco, Mexico.

Methods: Data encompassing individuals with flu-like symptoms from July 2021 to February 2023 was scrutinized for viral diagnosis through PCR multiplex. The effect of social mobility on the increase in respiratory viral diagnosis infection was considered to estimate its impact. Additionally, sequences of respiratory viruses stored in public databases were retrieved to ascertain the phylogenetic classification of previously reported viruses in Mexico.

Results: SARS-CoV-2 was the most detected virus ($n = 5{,}703$; 92.2%), followed by influenza ($n = 479$; 7.78%). These viruses were also found as the most common co-infection ($n = 11$; 50%), and for those with influenza, a higher incidence of severe disease was reported ($n = 122$; 90.4%; $p < 0.001$). Regarding comorbidities and unhealthy habits, smoking was found to be a risk factor for influenza infection but a protective factor for SARS-CoV-2 (OR = 2.62; IC 95%: 1.66–4.13; OR = 0.65; IC 95%: 0.45–0.94), respectively. Furthermore, our findings revealed a direct correlation between mobility and the prevalence of influenza infection (0.214; $p < 0.001$).

Discussion: The study presents evidence of respiratory virus reemergence and prevalence during the social reactivation, facilitating future preventive measures.

KEYWORDS

SARS-CoV-2, respiratory viruses, epidemiological survey, clinical impact, viral coinfections

1 Introduction

Respiratory infections are considered nowadays a significant global health burden, causing a wide range of respiratory tract infections, mainly in the upper respiratory tract (1). Before the COVID-19 pandemic, influenza, rhinovirus, respiratory syncytial virus, and adenovirus were considered the most common etiological agents of respiratory disease (2). Except for influenza, these viruses were referred to only as "common colds," and only a few studies focused on their epidemiological and clinical characterization (2).

Since the emergence of SARS-CoV-2 in 2019, countries have conducted strategies for viral control; one of the most significant was the non-pharmaceutical interventions consisting of city lockdowns, physical distancing, use of personal protective equipment, and individual hygiene practices (3). For instance, there has been a decrease in reported cases of the syncytial virus, Influenza A and B virus, parainfluenza virus 1–3, adenovirus, and human metapneumovirus from 2011 to 2022 (4, 5). Overall, there was a significant decline in seasonal influenza reports during the COVID-19 pandemic in many countries, including the United States, Japan, England, Australia, Canada, South Africa, Singapore, Taiwan, South Korea, and Chile (4, 6, 7). Nevertheless, in 2021, the National Health Institute reported a reemergence of non-SARS-CoV-2 respiratory viruses in the respective countries (4, 8). The viral epidemiological fluctuation could be attributable to the elimination of the non-pharmaceutical interventions and a possible replication interference between viruses (4, 9).

The clinical presentation of respiratory viral infections can vary widely, ranging from asymptomatic or mild illness to severe respiratory distress and organ failure. Coinfections with multiple respiratory viruses are uncommon, and whether they can complicate the clinical course and management of affected individuals is not well described. Moreover, underlying comorbidities, such as diabetes and hypertension, or unhealthy habits, such as smoking, can increase the risk of severe illness and complications (2, 10).

Surveillance systems play a critical role in monitoring the circulation and genetic evolution of respiratory viruses, providing essential data for public health responses, and aiding in the development of targeted interventions. According to the PAHO report, respiratory viruses followed a common epidemiological pattern in Latin America until 2020, with the emergence of the SARS-CoV-2 pandemic, in which there was a decline in the number of cases detected; but, in 2022, an increase in the diagnosis of non-SARS-CoV-2 respiratory viruses was reported (11). In Mexico, the thoroughness of the epidemiological description of SARS-CoV-2 differs from other respiratory viruses; even for the influenza virus, diagnosis and reports are uncommon. The national health institution in Mexico reported more than 10 million acute respiratory infections in 2022 (12), yet these were categorized collectively as acute respiratory infections without viral classification; Jalisco state fits in this same scenario, with no viral respiratory characterization carried despite the augment of the diagnosis of non-SARS-CoV-2 infection (12). Moreover, a previous study described the circulation of a high diversity of respiratory viruses (13). This work aims to provide an overview of the diversity of respiratory viruses and discuss the epidemiology, clinical manifestations, and social impact on the transmission dynamics, as well as its implications on disease severity.

2 Materials and methods

2.1 Study population and sample processing

As part of a comprehensive strategy to study and monitor the epidemiology of respiratory viruses in the Jalisco state, the Universidad de Guadalajara established a diagnosis laboratory during the COVID-19 pandemic for outpatient SARS-CoV-2 epidemiological surveillance. In this context, nasopharyngeal samples and clinical-demographic data from patients with flu-like illness (ILI) and severe acute respiratory infections (SARI) were retrospectively recovered. The eligibility criteria for the patients enrolled in the present study were as follows: (i) outpatient of any age or sex group requiring molecular diagnosis at the Laboratorio de Diagnóstico de Enfermedades Emergentes y Reemergentes (LaDEER; laboratory validated by the Mexico National Health Institute for respiratory viral diagnosis for epidemiological surveillance); (ii) people who had three or more ILI or SARI symptoms, according to the WHO surveillance case definitions, or other symptoms related to respiratory infection such as fever ≥37.0°C, anosmia, dysgeusia, cough, nasal congestion, chest pain, headache, among others. For the purpose of this study, severity degree was defined according to the number of symptoms; (iii) individuals previously in contact with people with a respiratory infection. All the clinical and epidemiological information, such as comorbidities, symptoms, and demographics, was retrieved by implementing a telephone survey, as mentioned previously (14).

A total of 6,184 nasopharyngeal samples were processed by RT-qPCR. The viral RNA was extracted with the Viral RNA Auto Extraction & Purification Kit (Cat. 3103010059, 3DMed) using the ANDiS 350 Automated Nucleic Acid Extraction System (3DMed). Initially, all samples were examined the same day they were sampled for the most common respiratory viruses, influenza, and SARS-CoV-2, employing the COVIFLU Kit Multiplex (Cat. G2LCoFM-04, Genes2life SAPI de CV, Irapuato, Mexico), which identifies the N gene of SARS-CoV-2, and the coding region of matrix protein (M) for influenza A and B using a Quant Studio 5 (Applied Biosystems); after this analysis, RNA and oro-nasopharyngeal tube were stored at −80°C for subsequent examinations. The samples that were negative for SARS-CoV-2 were screened for other viral infections using Bluefinder 22 (Cat. G2LBF22–01, Genes2Life, Mexico) in the IntelliQube automated PCR instrument (BioSearch Technologies), according to the manufacturer's instructions; this kit is designed for the diagnosis of 22 respiratory pathogens, such as Rhinovirus, Enterovirus, Bocavirus, Metapneumovirus, Adenovirus, Influenza H1N1 (09 pdm), Influenza H3N2, Influenza B (Victoria and Yamagata lineages), Syncytial A/B, SARS-CoV-2, Parainfluenza (1, 2, 3, and 4), and Human coronavirus (OC43, 229E, NL63, and HKU1). A sample was considered positive when Ct-values were below 35 and when a clear sigmoid curve was observed for the corresponding marker; additionally, a coinfection was defined when an individual tested positive for two or more viruses.

2.2 Mobility data report retrieval

In order to evaluate the relationship between the epidemic behavior of respiratory pathogens and the mobility rate in the Jalisco state, we used the database from Google, LLC website (15). With this

information and the epidemiological data, we analyzed the shifting patterns of respiratory virus distribution according to the impact of the local population mobility in the Jalisco state.

2.3 Phylogenetic analysis from Mexico

A comprehensive search through the Nucleotide section in the public database from the National Center for Biotechnological Information (NCBI), using the keywords "(Virus)," "and," "Jalisco," "complete genome" of the main respiratory virus circulating in West Mexico was performed; however, since no results were obtained, we amplified the search to include the entirety of the country. A search for Influenza, Respiratory Syncytial Virus, Human Coronavirus, Adenovirus, Parainfluenza, and Rhinovirus was carried out. Using only the downloaded sequence data, we performed a multiple alignment using MAFFT v.7. A phylogenetic tree was constructed using the Neighbor-Joining algorithm, employing Tamura-Nei as the substitution model of DNA evolution. Phylogenetic tree statistical reliability was evaluated by bootstrap analysis of 10,000 replicates.

2.4 Statistical analysis

All qualitative data was summarized as frequencies and percentages, while quantitative data as median and standard deviation. The chi-square test or Fisher's exact test was performed for comparative analyses. Kruskal-Wallis and Nemenyi *post hoc* tests were used to determine the most common combinations of symptoms and the most prevalent comorbidities. In addition, the correlation between the mobility rate from July 2021 to February 2023 with viral incidence was evaluated by the Kendall-Tau test. All data was analyzed with R Studio software (RStudio Team, 2020) and graphics in GraphPad Prism version 9. Statistically significant differences were considered with a cut-off value of $p < 0.05$.

3 Results

3.1 Demographic and clinical data

A total of 6,184 individuals were included in the present study, corresponding to 24.1, 75.8, and 0.1% recruited in 2021, 2022, and 2023, respectively. The overall mean age of the population was 37.29 ± 15.98 (males 37.79 ± 16.33; females 36.91 ± 15.69), with a predominant representation of female individuals ($n = 3{,}533$, 57.1%). Data stratification involved categorizing participants into age groups (<18, 18–60, and > 60 years) to compare the distribution between males and females. The main prevalence was observed in the 18–60 age group, consisting of 4,947 individuals (80%), with a female predominance. 3,591 individuals (70.2%) of the population manifested symptoms; headache was reported by 46.6% ($n = 2{,}421$; from 5,190 with clinical data) of the individuals, followed by cough and sore throat with 42.1% ($n = 2{,}183$) and 39.2% ($n = 2{,}032$), respectively. Moreover, the comparative analysis revealed a higher proportion of females experiencing symptomatology compared to males and, excluding fever, fatigue, myalgia, and arthralgia, common respiratory symptoms were more common in women.

Furthermore, the survey revealed comorbidities or unhealthy habits in 26.4% ($n = 1{,}372$) of the patients, with obesity as the most prevalent condition (10.8%; $n = 559$), followed by smoking and hypertension (9.8 and 6.3%, respectively). Additionally, males presented the highest proportion of any comorbidity. When evaluating comorbidity information independently, the proportion of males reporting a previous disease was the largest (Table 1).

3.2 Prevalence of respiratory viruses

From the analyzed samples, mono-infections were represented by 99.58% ($n = 6{,}158$), while the rest, 0.42% ($n = 26$), were coinfections (Figure 1A). SARS-CoV-2 was the most prevalent viral agent found as a mono-infection, with 5,703 individuals identified and a total of 481 cases of non-SARS-CoV-2 respiratory virus, representing 7.78% (Figure 1B). From this last, 479 participants were infected with influenza (477 corresponded to lineage A, from which 263 cases, 54.9%, were subsequently identified as H3N2 lineage), two of Influenza B (from the Victoria lineage), two human coronaviruses, five adenoviruses, one Bocavirus, three rhinoviruses, one Syncytial virus, and one Enterovirus (Figure 1B). The main coinfection observed was SARS-CoV-2 and Influenza A virus, detected in 13 samples. Besides SARS-CoV-2 and Influenza virus, HKU was the only one identified as a mono-infection. It is important to point out that influenza was present in most of the coinfection states. Likewise, coinfection cases involving SARS-CoV-2, Rhinovirus, and Enterovirus were identified (Figure 1C).

3.3 Impact of the social mobility in Jalisco and the reappearance of non-SARS-CoV-2 respiratory virus

According to the local public health institute database, no respiratory viruses were detected in West Mexico during the pandemic lockdown in 2020. Similarly, our epidemiological surveillance system has not detected the circulation of influenza in the region; nonetheless, influenza cases were reported at the end of 2021 (8). As so, we focused on evaluating if the reappearance of this virus was associated with the mobility change in Jalisco (Mexico) due to the removal of lockdown restrictions. The analysis showed that since December 2021, a re-emergence of Influenza infection cases was observed, in parallel with the trend of increased mobility observed at the end of October 2021 (Figure 2). The maximum peak of detected cases of respiratory viruses was registered during the fourth SARS-CoV-2 wave caused by the Omicron variant in January 2022, corresponding to the second epidemiological week. Additionally, we used the Kendall-tau correlation to quantify the relationship between the Influenza cases and the mobility data; mobility showed a positive correlation with infection cases of 0.214 ($p < 0.001$). No data further than October 2022 was retrieved due to the end of the aforementioned Google project.

3.4 Clinical impact of respiratory viruses

Illness produced by respiratory viruses presents a similar clinical behavior that can hardly be differentiated without a molecular

TABLE 1 Clinical and demographic information of the included population.

Variable	Total n (%)	Male n (%)	Female n (%)	P-significance
	6,184	2,651 (42.9)	3,533 (57.1)	<0.001
Age	37.29 ± 15.98	37.79 ± 16.33	36.91 ± 15.69	0.046
Age group (years)				0.007
<18	591 (9.6)	278 (10.5)	313 (8.9)	NS
18–60	4,947 (80)	2072 (78.2)	2,875 (81.4)	<0.001
>60	646 (10.4)	301 (11.4)	345 (9.8)	NS
Comorbidities (n)		n (%)		
Populations manifesting comorbidities	1,372 (26.4)	635 (28.8)	737 (24.7)	<0.001
Diabetes	221 (4.3)	107 (4.9)	114 (3.8)	NS
Obesity	559 (10.8)	247 (11.2)	312 (10.4)	NS
Hypertension	326 (6.3)	147 (6.7)	179 (6.0)	NS
Chronic kidney disease	13 (0.3)	7 (0.3)	6 (0.2)	NS
Cancer	11 (0.2)	5 (0.2)	6 (0.2)	NS
Hepatic disease	5 (0.1)	3 (0.1)	2 (0.1)	NS
Smoking	510 (9.8)	271 (12.3)	239 (8.0)	<0.001
Immunosuppression	10 (0.2)	2 (0.1)	8 (0.3)	NS
Asthma	184 (3.5)	65 (2.9)	119 (4.0)	0.046
HIV	6 (0.1)	6 (0.3)	0 (0.0)	0.006
Symptoms (n)		n (%)		
Populations manifesting symptoms (5190)	3,591 (70.2)	1,459 (66.3)	2,132 (71.5)	<0.001
Anosmia (4000)	266 (6.7)	92 (5.5)	174 (7.5)	0.01
Dysgeusia (4000)	291 (7.3)	106 (6.3)	185 (8.0)	0.041
Fever (5190)	1770 (34.1)	755 (34.3)	1,015 (34.0)	NS
Cough (5190)	2,183 (42.1)	853 (38.7)	1,330 (44.5)	<0.001
Nasal Congestion (5190)	1,018 (19.6)	402 (18.2)	616 (20.6)	0.034
Chest pain (5190)	595 (11.5)	218 (9.9)	377 (12.6)	0.002
Fatigue (5190)	1,359 (26.2)	549 (24.9)	810 (27.1)	NS
Difficulty breathing (5190)	403 (7.8)	151 (6.9)	252 (8.4)	0.035
Headache (5190)	2,421 (46.6)	951 (43.1)	1,470 (49.2)	<0.001
Chill (5190)	737 (14.2)	282 (12.8)	455 (15.2)	0.013
Myalgia (5190)	1,247 (24)	501 (22.7)	746 (25.0)	NS
Arthralgias (5190)	961 (18.5)	410 (18.6)	551 (18.5)	NS
Rhinorrhea (5190)	1,242 (23.9)	472 (21.4)	770 (25.8)	<0.001
Sore throat (5190)	2032 (39.2)	791 (35.9)	1,241 (41.6)	<0.001
Diarrhea (5190)	344 (6.6)	156 (7.1)	188 (6.3)	NS
Abdominal pain (5190)	184 (1.9)	67 (3.0)	117 (3.9)	NS

NS, Non-significant ($p > 0.05$). The chi-square test and U de Mann–Whitney were employed for statistical analysis.

diagnosis. In this regard, we recovered information such as symptoms and comorbidities ($n = 5{,}841$; missing clinical data of $n = 343$). Briefly, 3,591 individuals (69.3%) reported any symptoms; the female population exhibited a statistically significant increase compared to males (female = 59.4% vs. males = 40.6%; <0.001), with the highest proportion of people reporting 4–6 symptoms ($n = 1{,}654$; 46.06%). The people infected with the Influenza virus were prone to manifest illness-like symptoms at 90.37%; while Bocavirus, Human Coronavirus, and Rhinovirus had 100%, respectively; however, the information from Bocavirus, Human Coronavirus, and Rhinovirus should be interpreted cautiously since only a few cases were detected and mainly as a coinfection. In this sense, Influenza stands as an infection that causes an increased severity. On the other hand, 60.9% of subjects with SARS-CoV-2 presented symptoms (Figure 3A).

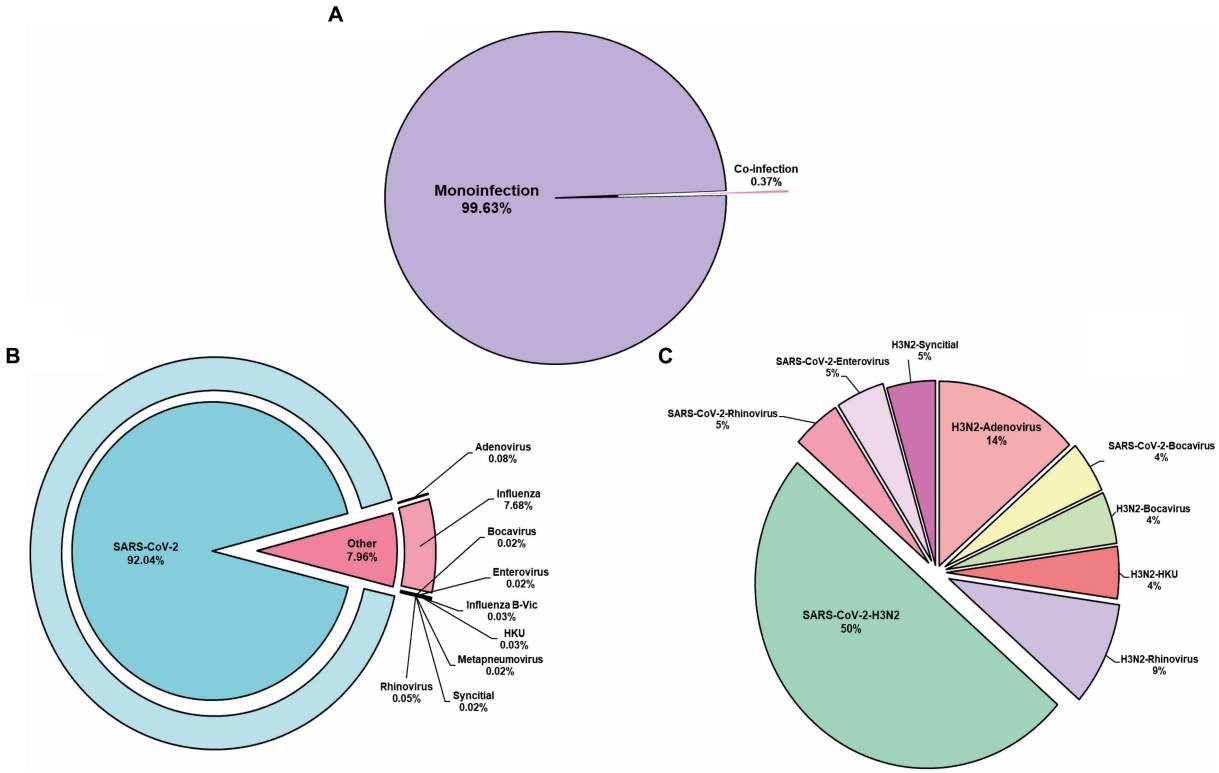

FIGURE 1
Respiratory viruses determined. **(A)** Mono-infection represents the major cause of disease found in our population. **(B)** From the mono-infection, SARS-CoV-2 was the prevalent virus identified. **(C)** Represents the co-infection identified. HKU, Human coronavirus.

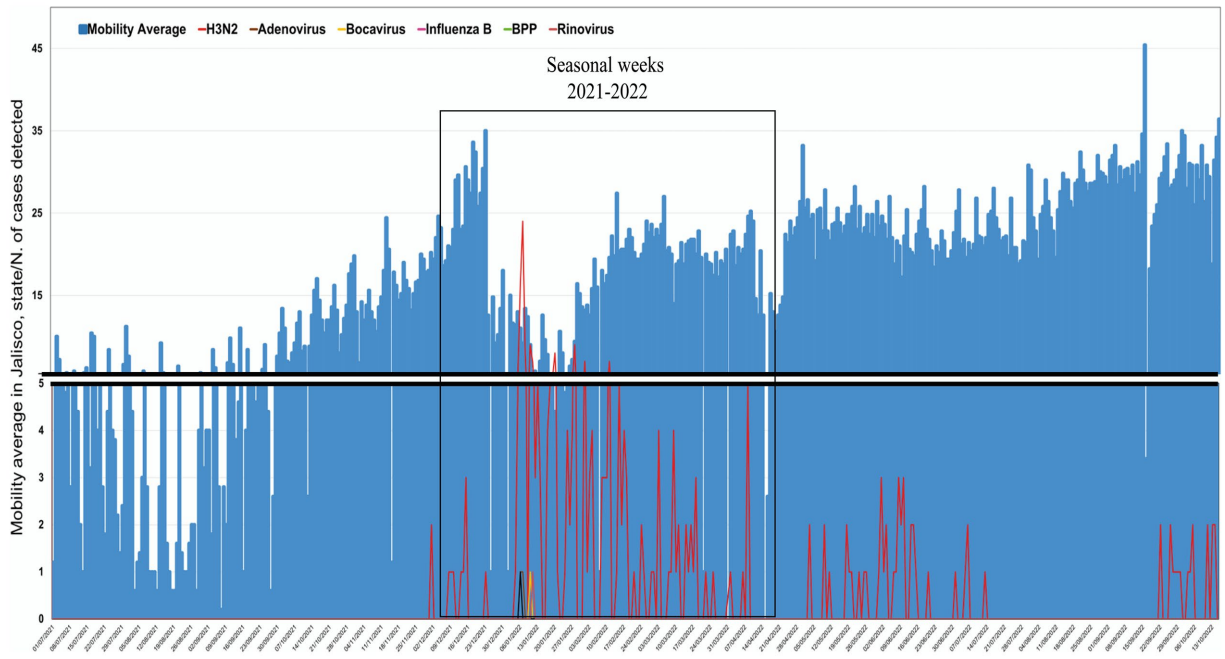

FIGURE 2
Impact of social mobility on viral respiratory detection. An increase in social mobility was observed in the second half of 2021, which correlates with the new viral respiratory detection.

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Headaches ($n = 2{,}421$; 46.6%), cough ($n = 2{,}183$; 42.1%), sore throat ($n = 2{,}032$; 39.2%), and fever ($n = 1{,}770$; 34.1%) were the most frequent symptoms reported, independently of the viral infection.

Concerning viral infection and the related age (<18, 18–60, and > 60), we grouped the participants according to the number of symptoms reported (1–3, 4–6, and > 7) as is shown in Figure 3B; individuals infected with SARS-CoV-2 or Influenza in the age rank groups from 18 to 40 and 40 to 61 years old presented a significant highest proportion of symptoms (SARS-CoV-2: $n = 2{,}905$, 50.8%; 1,733, 30.3%; Influenza: 300, 12.2%, 92, 19.2%, respectively; $p < 0.001$): these population exhibited mostly 4–6 symptoms. This same age group showed the highest proportion of individuals with more than 7 symptoms (61.23 and 33.84, respectively). People from the 18–60 age group exhibited a higher proportion of symptomatology of different severity (80.6%), see Supplementary Table S1.

Regarding SARS-CoV-2 and Influenza coinfections, 100% ($n = 9$) manifested symptomatology; yet, when we evaluated this viral infection individually, influenza showed a significant difference (90.37%) compared to SARS-CoV-2 (60.9%; $p < 0.001$). Once that is re-grouped according to the number of symptoms, those infected with influenza tend to have a higher proportion of severe disease than SARS-CoV-2 since 26.4% of people had >7 symptoms. Notably, those co-infected with SARS-CoV-2 and Influenza were prone to have an augmented disease severity (33.3%; $p < 0.001$), represented by a higher number of reported symptoms. With respect to sex influence over the disease severity, females showed statistical differences versus males in symptomatology reported (females 59.4% vs. males 40.6%; <0.001); these differences were maintained in people with SARS-CoV-2 mono-infection. Regarding the population with Influenza infection, we only observed differences between sex group in those with >7 (females 33.8% vs. males 19.61%); see Table 2.

In order to evaluate the impact of the comorbidities or unhealthy habits on respiratory viral infection, a univariate analysis was carried out. Firstly, we observed that individuals with rhinovirus have the largest proportion of comorbidities (50%), but since only two subjects responded to the survey, the data could not be representative. Patients monoinfected with Influenza and SARS-CoV-2 have 35 and 26% of previous comorbidities, respectively; the most reported was smoking (18 and 10%, respectively), followed by obesity (12 and 11%, respectively) and hypertension (7 and 6%, respectively; Figure 4A). Remarkably, in the context of Influenza infection, comorbidity represents a risk factor for the infection, with an OR = 1.86; IC 95%: 1.29–2.67, as smoking (OR = 2.62; IC 95%: 1.66–4.13). While these comorbidities are shown as a protective factor for SARS-CoV-2 infection (OR = 0.65; IC 95%: 0.45–0.94); in this sense, no risk factors were detected in SARS-CoV-2 diagnosed people, quite the opposite, cancer, hepatic disease, smoking, immunosuppression, and HIV were shown as a protective factor (Figure 4B). No risk factors were observed for adenovirus, but the number of cases was scarce.

3.5 Phylogenetic analysis of common respiratory viruses isolated in Mexico

A total of 334 respiratory viral sequences were found in Mexico (Table 3); 120 were partial sequences, and 214 were complete genomes; the most representative were influenza and rhinovirus, with a total of 123 sequences each, representing 36.82% of all viral respiratory infections reported from 2009 to 2018, (for influenza, mainly H1N1 were reported with a total of 114 sequences, and 9 were H3N2; while for rhinovirus serotype A was prevalent with 76 sequences, serotype B with 4, and serotype C a total of 43), followed by Bocavirus with a total of 37 sequences, with an 11.07% reported from 2001 to 2016, (all the sequences retrieved were HBoV1), Parainfluenza obtained a total of 27 sequences in the years 1995 to 2019, which represents 8.08% (the most significant were serotype 3 with 21 sequences and serotype 1 with six sequences). For the human syncytial virus, 21 sequences (6.28%) from 1980 to 2020 were recovered, mainly represented by serotype A with a total of 16 sequences and serotype B with 5. The metapneumovirus was less common, having only three genotype A

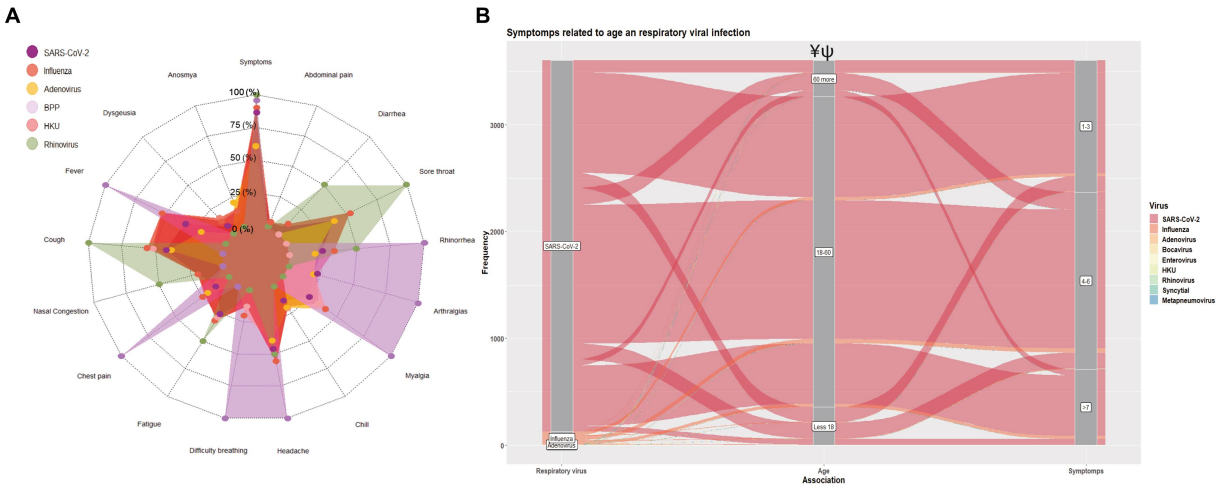

FIGURE 3
Impact of respiratory virus infection on the clinical presentation. **(A)** Prevalence of symptoms description according to the viral infection. **(B)** Sankey plot showing the distribution of the number of symptoms (0, 1–3, 4–6, and > 7) according to the age group and viral infection. Significant differences between age groups ¥: Influenza vs. SARS-CoV-2; ψ: Influenza/SARS-CoV-2 vs. Influenza. The chi-square test was used to compare proportions, and a value of p <0.05 was considered significant.

TABLE 2 The number of symptoms among respiratory viral infections as mono-infection and poli-infection.

Viral infection	Sex group	Symptomatic	Number of symptoms reported		
			1–3 n (%)	4–6 n (%)	>7 n (%)
Total (n, 5,841)	Total	3,591 (69.3)	1,230 (34.25)	1,654 (46.06)	707 (19.69)
	Male	1,459 (40.6)	532 (43.3)	633 (40.1)	264 (37.3)
	Female	2,132 (59.4)	698 (56.7)	991 (59.9)	443 (62.7)
	p-value	<0.001	<0.001	<0.001	<0.001
SARS-CoV-2 (n, 5,711)	Total	3,477 (60.9)	1,193 (34.3)	1,608 (46.3)	676 (19.4)
	Male	1,411 (40.6)	510 (36.2)	647 (45.8)	254 (18)
	Female	2066 (59.4)	683 (33.1)	961 (46.5)	422 (20.4)
	p-value	<0.001	<0.001	<0.001	<0.001
Influenza (n, 135)	Total	122 (90.4)	41 (33.6)	47 (38.5)	34 (27.9)
	Male	51 (41.8)	24 (47.06)	17 (33.33)	10 (19.61)
	Female	71 (58.2)	17 (23.9)	30 (42.3)	24 (33.8)
	p-value	NS	NS	NS	0.016
SARS-CoV-2 and Influenza (n, 9)	Total	9 (100)	4 (44.4)	2 (22.3)	3 (33.3)
	Male	3 (33.3)	2 (66.7)	1 (33.3)	0 (0)
	Female	6 (66.7)	2 (33.3)	1 (16.7)	3 (50)
	p-value	NS	NS	NS	NS
Influenza and Adenovirus (n, 3)	Total	3 (60)	1 (33.3)	1 (33.3)	1 (33.3)
	Male	1 (33.33)	0 (0)	0 (0)	1 (100)
	Female	2 (66.7)	1 (100)	1 (100)	0 (0)
	p-value	NS	NS	NS	NA
Influenza and Bocavirus (n, 1)	Total	1 (100)	0 ()	0 ()	1 (100)
	Male	1 (100)	0 (0)	0 (0)	1 (100)
	Female	0 (0)	0 (0)	0 (0)	0 (0)
	p-value	NA	NA	NA	NA
Influenza and Rhinovirus (n, 2)	Total	2 (100)	0 (0)	2 (100)	0 (0)
	Male	2 (100)	0 (0)	2 (100)	0 (0)
	Female	0 (0)	0 (0)	0 (0)	0 (0)
	p-value	NA	NA	NA	NA
Influenza B victoria (n, 1)	Total	1 (100)	0 (0)	0 (0)	1 (100)
	Male	0 (0)	0 (0)	0 (0)	0 (0)
	Female	1 (100)	0 (0)	0 (0)	1 (100)
	p-value	NA	NA	NA	NA
p-value on virus comparison		<0.001 ¥,ψ	<0.001 ¥	<0.001 ¥	<0.001 ¥

NS, non-significant; NA, non-aplicable. *P*-value < 0.001; ¥: Influenza vs SARS-CoV-2; ψ: Influenza/SARS-CoV-2 vs Influenza. The chi-square test and U de Mann–Whitney were employed for statistical analysis.

sequences between 1983 and 2018 (0.89%) (Table 3). It is important to remark that for HKU, only one sequence has been sequenced and reported on public databases in Mexico, the OC63; no further sequences have been found. As for adenovirus, no sequences obtained for respiratory infections were reported (Supplementary Figure S1).

4 Discussion

In the last year, the world experienced a return to social activity, resulting from the removal of non-pharmacological interventions, which had been reported to have an impact on the reemergence of respiratory viruses since the WHO surveillance data reflected a substantial increase in global viral circulation, after the decrease during COVID-19 pandemic (16). Respiratory infections represent one of the leading causes of morbidity and mortality worldwide (17, 18). In México, the national health authorities reported 10 million respiratory infections in 2021, representing a decrease compared to 2019, which presented with more than 20 million infections (12). However, since the end of 2021 and the beginning of 2022, an increase in the number of cases of infection with non-SARS-CoV-2 respiratory virus was reported (9, 12), but the information is scarce; additionally,

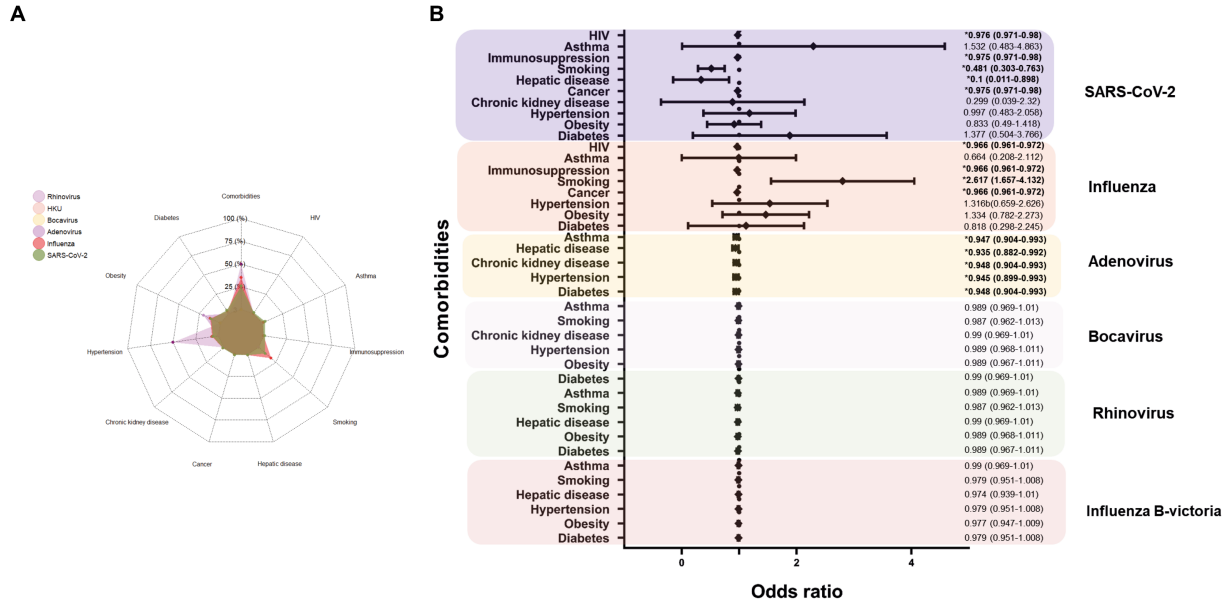

FIGURE 4
Association among respiratory viruses and comorbidities. **(A)** Prevalence of comorbidities description according to the viral infection. **(B)** Forest plot showing the risk for the viral infection according to the comorbidities; smoking showed a contrasting result as a risk factor for influenza and protection for SARS-CoV-2 infection.

TABLE 3 Respiratory viruses reported in a public database from Mexico.

Respiratory virus	Sequences n (%)	Year
6	334	1980–2020
Influenza	123 (36.82)	2009–2018
H1N1	114 (92.86)	
H3N2	9 (7.31)	
Rhinovirus	123 (36.82)	2009–2018
Serotype A	76 (61.7)	
Serotype B	4 (3.25)	
Serotype C	43 (34.92)	
Bocavirus	37 (11.07)	2001–2016
Subtype HBoV1	37 (100)	
Parainfluenza	27 (8.08)	1995–2019
Serotype 1	6 (22.2)	
Serotype 3	21 (77.77)	
Syncytial	21 (6.28)	1980–2020
Serotype A	16 (76.19)	
Serotype B	5 (23.8)	
Metapneumovirus	3 (0.89)	1983–2018
Genotype A	3 (100)	

most of the efforts on respiratory virus diagnostics are focused solely on influenza (13), which affect the public health since the circulation of respiratory viruses different from Influenza and SARS-CoV-2 goes largely unnoticed. In the present document, we intend to describe the epidemiological and clinical characteristics of the respiratory viruses circulating in Jalisco, Mexico, and how social mobility might be involved in viral recirculation.

On average, the adult population experiences around two or five episodes of respiratory infection annually, with certain age groups, such as children and the older adult, more likely to be affected by more than seven events per year (7) demonstrated by numerous studies that prove pediatric populations are the most susceptible to respiratory infections (13, 19, 20), while the older adult are prone to demise (18). However, our study population was mostly 18 to 40 years old; this is in line with previous studies in the region of Jalisco, where people with SARS-CoV-2 were mainly in this age group (14, 21), probably due to the burden of economic activity placed on young adults. (22).

Throughout history, numerous infectious agents, including bacteria, viruses, and fungi, have been identified as causes of respiratory tract infections, with viral agents being the most prominent. Rhinovirus stands out as responsible for 50% of all respiratory infections worldwide, followed by human Coronaviruses (23). This information is consistent with Fernandes-Matano et al., which found rhinovirus as the most prevalent non-influenza respiratory virus circulating in Mexico, followed by syncytial and metapneumovirus (13). Historically, the influenza virus has been the main etiological agent of respiratory disease; because of this, the national health system has focused efforts on the diagnosis of this virus, ignoring the epidemiological distribution of other viral respiratory different to Influenza or SARS-CoV-2; therefore, the information regarding it needs to be improved. Herein, we reported that, in order, SARS-CoV-2, Influenza (lineage H3N2 and few cases of B Victoria), and adenovirus are the most prevalent, which is consistent with the epidemiological reports from the national health authority (12), with the exception of adenovirus, from which, the information in adults is limited and has been reported mainly in the pediatric

population (24). Lately, public health institutions have been reporting a national prevalence of influenza H3N2 and Omicron subvariants (25).

Our data shows that social mobility was associated with the reemergence of the influenza virus at the end of 2021 and the beginning of 2022; additionally, new cases of other respiratory viruses were diagnosed during this period, although no correlation was observed between the incidence of these respiratory viruses and mobility. It has been well established that non-pharmacological interventions impact the number of infections reported; one of the most important measures is social mobility, which previously showed utility for this analysis (26–28). For the present study, no residential mobility data were included since we were looking for the impact of public spaces; Kishore K et al. demonstrated a negative correlation between the epidemiological data and the epidemiological indicators (28). Yet, it is important to remark that the correlation that we observed can be classified as weak; however, since LaDEER provides outpatient care for diagnosing respiratory viruses, the information may not reflect what occurs at the hospital level, where the correlation and number of infections may be even higher. Although mobility information from Google is no longer available, this was a handy tool that might have helped the national health authorities in the decision-making process during the COVID-19 pandemic; moreover, this and other mobility tools might be used to control future outbreaks, especially in Mexico, where monitoring of the epidemiological distribution of respiratory viruses is insufficient.

It has been published that respiratory disease symptoms share similar clinical characteristics with some clinical variations in the presence of certain symptoms (18). In order to differentiate the clinical presentation, a meta-analysis evaluating the clinical outcomes of the principal respiratory etiologies showed that respiratory diseases mainly present fever, sore throat, rhinorrhea, headache, myalgia, and cough; for COVID-19 and the common cold, fever was predominant, while influenza was characterized by myalgia and cold (29). A study published earlier in the COVID-19 pandemic reported that compared to influenza cases, runny nose, dyspnea, sore throat, and rhinorrhea were uncommon in patients with COVID-19 (30). These studies concord with our data since we reported that cough, sore throat, and fever were the most frequent symptoms manifested by our population; however, we observed that headache was the most recurrently described by the studied individuals but uncommon in the meta-analysis.

Recent studies focused on the population with COVID-19 from Jalisco showed that headaches were one of the most recurrent symptoms, which was in line with our findings (14, 21). Considering the coinfection, no statistical differences with mono-infections were found in our study; the information in this regard is inconsistent. A study published by Crotty et al. concluded that multiple viral infections were high-risk factors for patient mortality (31–33). Otherwise, several studies have shown that multiple viral infections do not increase the disease severity (31, 34–37); in fact, some studies have shown an inverse association among pediatric patients coinfected with respiratory viruses compared with sole infection (38, 39). This data is contradictory, but a possible explanation for a reduced risk of the worst clinical outcome could be due to secondary viral interference by the generation of interferon in infected patients because of a first viral entrance (40). It is difficult to solve this problem with our analysis since our study population is not significant. Future studies and meta-analyses that include SARS-CoV-2 and new influenza lineages should be conducted.

Our research shows a high risk of infection with influenza and smoking, contrary to the data from SARS-CoV-2 infected people, in which a low risk for viral infection was found. The unhealthy smoking habit and the risk for influenza infection association are well studied, and the data demonstrates that people who consume tobacco have more infections than those who do not (41). Nevertheless, regarding SARS-CoV-2 and smoking, the information is unclear. A similar result was found in California, where a significantly low-risk level for SARS-CoV-2 positivity was found (42, 43); Kashyap et al. suggest that a possible explanation could be a weak immune response and large and deep deliberate exhalations, which expel large quantities and concentrations of viral particles (44). Other possible mechanisms have been reviewed by Shariq-Usman et al., which include low production of pro-inflammatory cytokines, local vasodilatation, low expression of ACE2, and high production of nitric oxide; however, the authors recommend that this information should be taken with caution since several biases and knowledge gaps were identified (45), and it was demonstrated that smokers have a worst diseases prognosis at the time of infection (46, 47). Furthermore, when the data was evaluated according to the existence of any comorbidity, statistical analysis showed that people have lower odds of infection with SARS-CoV-2; this should be interpreted carefully since our data is from a local population, of which 80% present a comorbidity (48), leading to a bias in the calculation.

One of the principal limitations of this study is the lack of consideration for vaccination status against SARS-CoV-2 or other viruses. This is noteworthy because previous vaccination has been demonstrated to impact infection rates with various respiratory viruses, particularly the influence of the Influenza vaccine on SARS-CoV-2 infection. It's crucial to acknowledge that there might be a statistical bias due to the small population size associated with viruses other than Influenza and SARS-CoV-2. Furthermore, this study provides data at the local level, and it's essential to recognize that analyzing information at the local level can influence epidemiological statistics. Therefore, caution is advised when extrapolating these findings to estimate data at the state or national level. Lastly, it's important to highlight that the diagnostic evaluation for respiratory viruses, aside from SARS-CoV-2 and Influenza, was limited. Consequently, the epidemiological burden information presented in this study may be underrepresented.

5 Conclusion

In conclusion, we present evidence regarding the social activation and the endemic respiratory virus reemergence as well the description of their prevalence in Jalisco, Mexico, which may lay the groundwork for follow-up in future studies that provide information in the establishment of prevention measures for epidemiological control. Nevertheless, many unanswered questions remain regarding the impact of reduced antigenic exposure to viral agents on the severity of respiratory infections in the coming years and its implications for rearranging the genotypic distribution of various respiratory viruses in the post-pandemic period.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Comité de ética en Investigación, CUCS, Universidad de Guadalajara (registry number CONBIOÉTICA-14-CEI-002-20191003; protocol code: CI-04821). The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from tacit consent through a privacy notice in the diagnosis results. This notice informed how patients could object to the processing of their data. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

IP-U: Data curation, Methodology, Writing – original draft. NV: Funding acquisition, Project administration, Writing – review & editing. JM-V: Funding acquisition, Project administration, Writing – review & editing. MP-R: Methodology, Validation, Writing – review & editing. AC-A: Methodology, Validation, Writing – review & editing. RS-S: Methodology, Supervision, Writing – review & editing. AV-L: Methodology, Validation, Writing – review & editing. OG-G: Methodology, Validation, Writing – review & editing. JZ-M: Formal analysis, Writing – review & editing. MR-S: Methodology, Validation, Writing – review & editing. OV-S: Conceptualization, Project administration, Supervision, Writing – review & editing. MG-C: Conceptualization, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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References

1. Respiratory Infections. CDC Yellow Book 2024. (2023). Available at: https://wwwnc.cdc.gov/travel/yellowbook/2024/posttravel-evaluation/respiratory-infections

2. Thomas M, Bomar PA. *Upper respiratory tract infection*. Florida: Statpearls Publishing (2023).

3. Zhang W, Wu Y, Wen B, Zhang Y, Wang Y, Yin W, et al. Nonpharmaceutical interventions for COVID-19 reduced the incidence of infectious diseases: a controlled interrupted time-series study. *Infect Dis Poverty*. (2023) 12:15. doi: 10.1186/s40249-023-01066-3

4. Chow EJ, Uyeki TM, Chu HY. The effects of the COVID-19 pandemic on community respiratory virus activity. *Nat Rev Microbiol*. (2023) 21:195–210. doi: 10.1038/s41579-022-00807-9

5. Li Y, Wang X, Blau DM, Caballero MT, Feikin DR, Gill CJ, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in children younger than 5 years in 2019: a systematic analysis. *Lancet*. (2022) 399:2047–64. doi: 10.1016/S0140-6736(22)00478-0

6. Tang JW, Lam TT, Zaraket H, Lipkin WI, Drews SJ, Hatchette TF, et al. Global epidemiology of non-influenza RNA respiratory viruses: data gaps and a growing need for surveillance. *Lancet Infect Dis*. (2017) 17:e320–6. doi: 10.1016/S1473-3099(17)30238-4

7. Lai YH, Lin KY, Chang CH, Chang SC. Collateral benefit of non-pharmacological interventions against COVID-19 to prevent community-acquired pneumonia in Jin-Shan, new Taipei, April to December 2020. *J Formos Med Assoc*. (2021) 120:2195–6. doi: 10.1016/j.jfma.2021.08.004

8. Comunicación Social Del Gobierno Del Estado De Jalisco. (2023). Se reportan en Jalisco los primeros dos casos de influenza estacional Gobierno del Estado de Jalisco. Available at: https://www.jalisco.gob.mx/es/prensa/noticias/136552

9. Principi N, Autore G, Ramundo G, Esposito S. Epidemiology of Respiratory Infections during the COVID-19 pandemic. *Viruses*. (2023) 15:1160. doi: 10.3390/v15051160

10. Zhu L, Luo T, Yuan Y, Yang S, Niu C, Gong T, et al. Epidemiological characteristics of respiratory viruses in hospitalized children during the COVID-19 pandemic in southwestern China. *Front Cell Infect Microbiol*. (2023) 13:1142199. doi: 10.3389/fcimb.2023.1142199

11. Epidemiological Alert. (2023). Influenza, respiratory syncytial virus and SARS-CoV-2. Available at: https://www.paho.org/en/documents/epidemiological-alert-influenza-respiratory-syncytial-virus-and-sars-cov-2-6-june-2023

12. Dirección General de Epidemiología. (2023). Dirección General de Epidemiología Anuario de Morbilidad 1984 -2022. Available on: https://epidemiologia.salud.gob.mx/anuario/html/index.html

13. Fernandes-Matano L, Monroy-Muñoz IE, Angeles-Martínez J, Sarquiz-Martinez B, Palomec-Nava ID, Pardavé-Alejandre HD, et al. Prevalence of non-influenza respiratory viruses in acute respiratory infection cases in Mexico. *PLoS One*. (2017) 12:e0176298. doi: 10.1371/journal.pone.0176298

14. Peña Rodríguez M, Hernández Bello J, Vega Magaña N, Viera Segura O, García Chagollán M, Ceja Gálvez HR, et al. Prevalence of symptoms, comorbidities, and reinfections in individuals infected with wild-type SARS-CoV-2, Delta, or omicron variants: a comparative study in western Mexico. *Front Public Health*. (2023) 11:1149795. doi: 10.3389/fpubh.2023.1149795

15. COVID-19 Community Mobility Report. (2023). COVID-19 community mobility report. Available at: https://www.google.com/covid19/mobility?hl=es

16. Huang QS, Wood T, Jelley L, Jennings T, Jefferies S, Daniells K, et al. Impact of the COVID-19 nonpharmaceutical interventions on influenza and other respiratory viral infections in New Zealand. *Nat Commun*. (2021) 12:1001. doi: 10.1038/s41467-021-21157-9

17. GBD 2016 Lower Respiratory Infections Collaborators. Estimates of the global, regional, and national morbidity, mortality, and aetiologies of lower respiratory infections in 195 countries, 1990-2016: a systematic analysis for the global burden of disease study 2016. *Lancet Infect Dis*. (2018) 18:1191–210. doi: 10.1016/S1473-3099(18)30310-4

18. Respiratory infections. (2023). Deaths by sex and age group for a selected country or area and year. Available at: https://platform.who.int/mortality/themes/theme-details/topics/topic-details/MDB/respiratory-infections

19. Agca H, Akalin H, Saglik I, Hacimustafaoglu M, Celebi S, Ener B. Changing epidemiology of influenza and other respiratory viruses in the first year of COVID-19 pandemic. *J Infect Public Health*. (2021) 14:1186–90. doi: 10.1016/j.jiph.2021.08.004

20. Huang XB, Yuan L, Ye CX, Zhu X, Lin CJ, Zhang DM, et al. Epidemiological characteristics of respiratory viruses in patients with acute respiratory infections during 2009-2018 in southern China. *Int J Infect Dis*. (2020) 98:21–32. doi: 10.1016/j.ijid.2020.06.051

21. Viera-Segura O, Vega-Magaña N, García-Chagollán M, Peña-Rodríguez M, Muñoz-Sánchez G, Carranza-Aranda AS, et al. A comprehensive descriptive epidemiological and clinical analysis of SARS-CoV-2 in West-Mexico during COVID-19 pandemic 2020. *Int J Environ Res Public Health*. (2021) 18:10644. doi: 10.3390/ijerph182010644

22. INEGI. (2023). México en cifras Instituto Nacional de Estadística y Geografía. INEGI. Available at: https://www.inegi.org.mx/app/areasgeograficas/

23. Kardos P, Malek FA. Common cold -an umbrella term for acute Infections of nose, throat, Larynx and Bronchi. *Pneumologie*. (2017) 71:221–6. doi: 10.1055/s-0042-116112

24. Wong-Chew RM, Farfán-Quiroz R, Sánchez-Huerta JL, Nava-Frías M, Casasola-Flores J, Santos-Preciado JI. *Frecuencia de virus respiratorios y características clínicas de niños que acuden a un hospital en México*. Mexico: Salud Pública de Mexico (2010).

25. CONACYT. (2023). Vigilancia de variantes del virus SARS-CoV-2. Available at: https://salud.conacyt.mx/coronavirus/variantes/

26. Sadique MZ, Edmunds WJ, Smith RD, Meerding WJ, de Zwart O, Brug J, et al. Precautionary behavior in response to perceived threat of pandemic influenza. *Emerg Infect Dis*. (2007) 13:1307–13. doi: 10.3201/eid1309.070372

27. Saha J, Barman B, Chouhan P. Lockdown for COVID-19 and its impact on community mobility in India: an analysis of the COVID-19 community mobility reports, 2020. *Child Youth Serv Rev*. (2020) 116:105160. doi: 10.1016/j.childyouth.2020.105160

28. Kishore K, Jaswal V, Verma M, Koushal V. Exploring the utility of Google mobility data during the COVID-19 pandemic in India: digital epidemiological analysis. *JMIR Public Health Surveill*. (2021) 7:e29957. doi: 10.2196/29957

29. Czubak J, Stolarczyk K, Orzeł A, Frączek M, Zatoński T. Comparison of the clinical differences between COVID-19, SARS, influenza, and the common cold: a systematic literature review. *Adv Clin Exp Med*. (2021) 30:109–14. doi: 10.17219/acem/129573

30. Pormohammad A, Ghorbani S, Khatami A, Razizadeh MH, Alborzi E, Zarei M, et al. Comparison of influenza type a and B with COVID-19: a global systematic review and meta-analysis on clinical, laboratory and radiographic findings. *Rev Med Virol*. (2021) 31:e2179. doi: 10.1002/rmv.2179

31. Crotty MP, Meyers S, Hampton N, Bledsoe S, Ritchie DJ, Buller RS, et al. Epidemiology, co-Infections, and outcomes of viral pneumonia in adults: an observational cohort study. *Medicine*. (2015) 94:e2332. doi: 10.1097/MD.0000000000002332

32. Harada Y, Kinoshita F, Yoshida LM, Minh LN, Suzuki M, Morimoto K, et al. Does respiratory virus coinfection increases the clinical severity of acute respiratory infection among children infected with respiratory syncytial virus? *Pediatr Infect Dis J*. (2013) 32:441–5. doi: 10.1097/INF.0b013e31828ba08c

33. Tran QL, Benitez G, Shehadeh F, Kaczynski M, Mylonakis E. Clinical outcomes associated with SARS-CoV-2 co-infection with rhinovirus and adenovirus in adults-a retrospective matched cohort study. *Int J Environ Res Public Health*. (2022) 20:646. doi: 10.3390/ijerph20010646

34. Li Y, Pillai P, Miyake F, Nair H. The role of viral coinfections in the severity of acute respiratory infections among children infected with respiratory syncytial virus (RSV): a systematic review and meta-analysis. *J Glob Health*. (2020) 10:010426. doi: 10.7189/jogh.10.010426

35. Asner SA, Rose W, Petrich A, Richardson S, Tran DJ. Is virus coinfection a predictor of severity in children with viral respiratory infections? *Clin Microbiol Infect*. (2015) 21:264.e1–6. doi: 10.1016/j.cmi.2014.08.024

36. Lim FJ, de Klerk N, Blyth CC, Fathima P, Moore HC. Systematic review and meta-analysis of respiratory viral coinfections in children. *Respirology*. (2016) 21:648–55. doi: 10.1111/resp.12741

37. Asner SA, Science ME, Tran D, Smieja M, Merglen A, Mertz D. Clinical disease severity of respiratory viral coinfection versus single viral infection: a systematic review and meta-analysis. *PLoS One*. (2014) 9:e99392. doi: 10.1371/journal.pone.0099392

38. Martínez-Roig A, Salvadó M, Caballero-Rabasco MA, Sánchez-Buenavida A, López-Segura N, Bonet-Alcaina M. Viral coinfection in childhood respiratory tract infections. *Arch Bronconeumol*. (2015) 51:5–9. doi: 10.1016/j.arbres.2014.01.018

39. Canducci F, Debiaggi M, Sampaolo M, Marinozzi MC, Berrè S, Terulla C, et al. Two-year prospective study of single infections and coinfections by respiratory syncytial virus and viruses identified recently in infants with acute respiratory disease. *J Med Virol*. (2008) 80:716–23. doi: 10.1002/jmv.21108

40. Piret J, Boivin G. Viral interference between Respiratory viruses. *Emerg Infect Dis*. (2022) 28:273–81. doi: 10.3201/eid2802.211727

41. Lawrence H, Hunter A, Murray R, Lim WS, McKeever T. Cigarette smoking and the occurrence of influenza -systematic review. *J Infect*. (2019) 79:401–6. doi: 10.1016/j.jinf.2019.08.014

42. Young-Wolff KC, Slama N, Alexeeff SE, Sakoda LC, Fogelberg R, Myers LC, et al. Tobacco smoking and risk of SARS-CoV-2 infection and disease severity among adults in an integrated healthcare system in California. *Nicotine Tob Res*. (2023) 25:211–20. doi: 10.1093/ntr/ntac090

43. Li J, Martinez MC, Frosch DL, Matt GE. Effects of smoking on SARS-CoV-2 positivity: a study of a large health system in northern and Central California. *Tob Use Insights*. (2022) 15:1179173X2211147. doi: 10.1177/1179173X221114799

44. Kashyap VK, Dhasmana A, Massey A, Kotnala S, Zafar N, Jaggi M, et al. Smoking and COVID-19: adding fuel to the flame. *Int J Mol Sci*. (2020) 21:6581. doi: 10.3390/ijms21186581

45. Usman MS, Siddiqi TJ, Khan MS, Patel UK, Shahid I, Ahmed J, et al. Is there a smoker's paradox in COVID-19? *BMJ Evid Based Med*. (2021) 26:279–84. doi: 10.1136/bmjebm-2020-111492

46. Chen R, Hui KPY, Liang Y, Ng KC, Nicholls JM, Ip MSM, et al. SARS-CoV-2 infection aggravates cigarette smoke-exposed cell damage in primary human airway epithelia. *Virol J*. (2023) 20:65. doi: 10.1186/s12985-023-02008-z

47. Chavez J, Hai R. Effects of cigarette smoking on influenza virus/host interplay. *Pathogens*. (2021) 10:1636. doi: 10.3390/pathogens10121636

48. INEGI. (2023). Estadística de Defunciones Registradas de Enero a Junio de 2022 (Preliminar). Available at: https://www.inegi.org.mx/contenidos/saladeprensa/boletines/2023/DR/DR-Ene-jun2022.pdf

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Enabling countries to manage outbreaks: statistical, operational, and contextual analysis of the early warning and response system (EWARS-csd) for dengue outbreaks

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Introduction: Dengue is currently the fastest-spreading mosquito-borne viral illness in the world, with over half of the world's population living in areas at risk of dengue. As dengue continues to spread and become more of a health burden, it is essential to have tools that can predict when and where outbreaks might occur to better prepare vector control operations and communities' responses. One such predictive tool, the Early Warning and Response System for climate-sensitive diseases (EWARS-csd), primarily uses climatic data to alert health systems of outbreaks weeks before they occur. EWARS-csd uses the robust Distribution Lag Non-linear Model in combination with the INLA Bayesian regression framework to predict outbreaks, utilizing historical data. This study seeks to validate the tool's performance in two states of Colombia, evaluating how well the tool performed in 11 municipalities of varying dengue endemicity levels.

Methods: The validation study used retrospective data with alarm indicators (mean temperature and rain sum) and an outbreak indicator (weekly hospitalizations) from 11 municipalities spanning two states in Colombia from 2015 to 2020. Calibrations of different variables were performed to find the optimal sensitivity and positive predictive value for each municipality.

Results: The study demonstrated that the tool produced overall reliable early outbreak alarms. The median of the most optimal calibration for each municipality was very high: sensitivity (97%), specificity (94%), positive predictive value (75%), and negative predictive value (99%; 95% CI).

Discussion: The tool worked well across all population sizes and all endemicity levels but had slightly poorer results in the highly endemic municipality at predicting non-outbreak weeks. Migration and/or socioeconomic status are factors that might

impact predictive performance and should be further evaluated. Overall EWARS-csd performed very well, providing evidence that it should continue to be implemented in Colombia and other countries for outbreak prediction.

KEYWORDS

outbreak prediction, outbreak response, dengue, Colombia, climate-sensitive diseases, vector-borne disease

1 Introduction

Dengue, an infectious disease transmitted by *Aedes* mosquitoes (mainly *Aedes aegypti* and *Aedes albopictus*) is currently considered the fastest-spreading mosquito-borne disease in the world, with the incidence increasing 30-fold in the last 50 years (1, 2). Over half of the world's population live in areas at risk of dengue (3). Annually, dengue infects over 390 million people, kills over 10,000 people, and is responsible for 1.14 million disability-adjusted life years (DALYs) (2, 4). Dengue has seen a rise in cases due to climate change, human mobility, trade, and unplanned urbanization (5). Dengue's increasing transmission rate has created a large health burden on many communities, especially when outbreaks occur. There is currently no effective cure for dengue and the best way to minimize the dengue health burden is vector control measures of the *Aedes* mosquito (6).

The updated EWARS-csd tool (Early Warning and Response System for climate-sensitive diseases tool developed under the auspices of the Special Program for Research and Training in Tropical Diseases at the World Health Organization, TDR-WHO) was developed to predict dengue outbreaks before they occur to prevent potential outbreaks. The tool can utilize epidemiological, meteorological, social, and entomological variables to predict possible future dengue outbreaks (1). EWARS-csd includes interactive graphical features to improve results interpretation for users at the national (central) dashboard 1 and local (municipality) dashboard 2 levels. The EWARS-csd tool predicts disease outbreaks in time and space, allowing it to trigger vector control activities in areas of high transmission risk. In addition, it quantifies the magnitude (outbreak rate) and its certainty interval, which will have significant vector control and response implications. It employs the robust Distribution Lag Non-linear Model in combination with the integrated nested Laplace approximation (INLA) Bayesian regression framework (7).

The tool is operated through the open-access software "R" to make it accessible to users in Low- and Middle-Income Countries (LMICs). It does not require skilled users to operate it effectively. The tool was updated from EWARS to EWARS-csd (formerly EWARS+) in 2019 to improve the mathematical approach, provide descriptive data for users, predict the magnitude of disease incidence, provide confidence intervals, model all municipalities in a country together, and have fewer calibration features (8). Currently, EWARS-csd is being implemented in 17 countries (8). This includes Colombia, which is hyperendemic for dengue and experiences the highest mean dengue case fatality rate in the Americas (19 deaths per 10,000 symptomatic cases) (9). The TDR-WHO sponsored training, installation, and technical support in the implementation of EWARS-csd in certain municipalities of Colombia. Overall, Colombia's mandatory reporting of dengue cases and available case data, made it an optimal place to perform a validation study of EWARS-csd. A validation study is necessary because the previous version of EWARS was unable to generate results in Colombia due to inconsistency of disease trends caused by the seasonal effect (as in many Latin American countries) and because the tool did not perform well for municipalities of low endemicity.

The overall aim of this study was to validate whether the modernized EWARS-csd model provides reliable and operational alarm signals for dengue outbreaks in Colombia and elsewhere. Essentially, this study intended to assess the sensitivity and positive predictive value metrics for EWARS-csd for municipalities of different endemicity levels.

2 Materials and methods

2.1 Study area

The EWARS-csd validation study was conducted in partnership with WHO, the Universities of Gothenburg and Freiburg as well the Colombian National Institute of Health's (Instituto Nacional de Salud, INS), surveillance team. For the validation study, data was used from 2 of Colombia's 32 states ("departamentos"): Bolívar and Cesar (Figure 1). Both states were part of Colombia's pilot study of EWARS-csd. The two states border each other. Cesar also shares a border with Venezuela. Bolívar has a population of 2 million, and Cesar has a population of 1.2 million (10). In total 11 municipalities of varying endemicity levels were used in this study: 4 from Bolívar and 7 from Cesar.

2.2 Data

Secondary data for this project was aggregated from Colombia's National Institute of Health (INS) in coordination with their national vector-control team. INS provided data on the 11 municipalities listed above, including data from 2015 to 2020 covering the mean relative humidity (%), the number of hospitalized cases, the population of the municipality, the mean temperature (°C), and the sum of rain per week (mm). The hospitalized cases data came from hospital records and were all lab-confirmed cases of dengue which required hospitalization, and these were included using the case definition according to the Ministry of Health (MoH) and INS, which was set out in the public health surveillance protocols (12). The meteorological data, which were the potential alarm indicators, was provided by the Institute of Hydrology, Meteorology and Environmental Studies (IDEAM) of Colombia. The temporal data was measured as an epidemiological

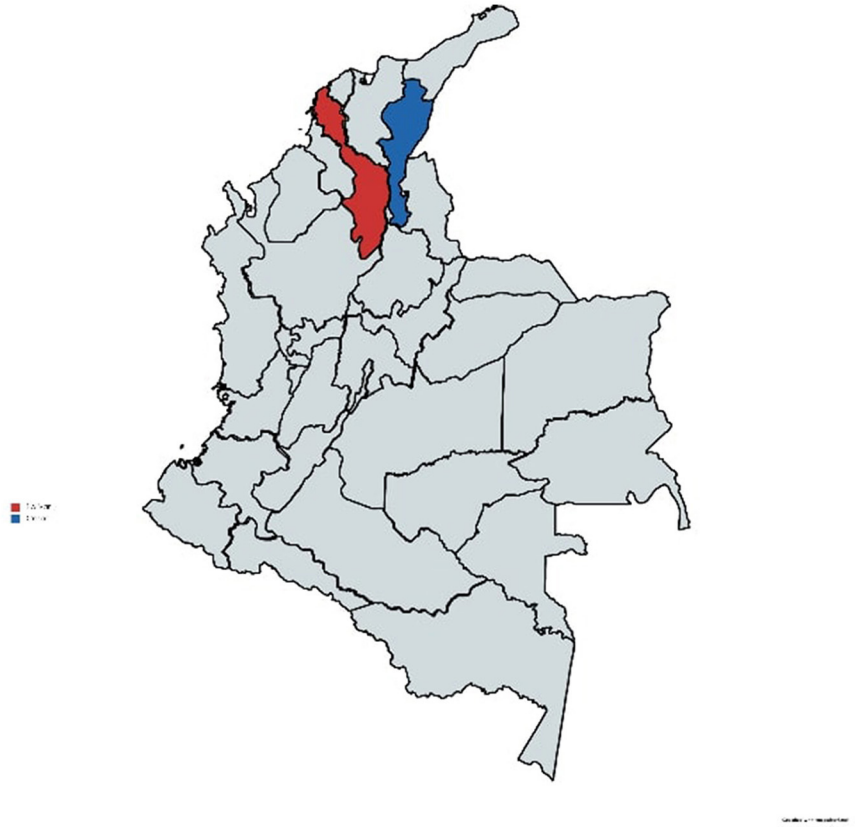

FIGURE 1
Map of the two states analyzed in the study. Bolívar (in red) and Cesar (in blue). Reprinted with MapChart.net's permission (11).

week (from Sunday to Saturday) and the spatial unit was based on pre-existing administrative units ("municipios" or municipalities).

2.3 EWARS-csd

The EWARS-csd toolkit on the open software R was used to validate the data. Dashboard 1, which is used by the national health system level, was utilized for the validation. The tool uses spatiotemporal covariance to provide robust estimates through a distributed lag non-linear Bayesian framework (13). It uses a baseline model and non-linear function of incidence-week in order to capture seasonality or the unknown variability annually (13). The model produces out-of-sample predicted probabilities of exceeding the outbreak threshold from alarm indicator parameters. It is compared with the endemic channel, which represents the historical pattern of disease incidence or dengue hospitalization incidence.

2.4 Validation

The model can be tested at different calibrations to see which tool settings, such as run-in year, *z*-value (see below), and time-lag or prediction period, provide the optimal model measured by statistical metrics. In a retrospective cross-evaluation tool process, the run-in year is the year that the data would be cut between either being part of the historic data to build the model or to be part of the future data, which is used to predict outbreaks (model evaluation). The run-in years available were 2016–2020.

The *z*-value is a multiplier of the weekly standard deviation of hospitalized cases (or other outbreak indicators) (14). The importance of this calibration is to change the outbreak threshold, or the upper line of the endemic channel, which is useful to account for different endemic settings (15). *Z*-values were calibrated between 1 and 4 in this study.

The time-lag is the period between exposure (e.g., change in the climate condition) and the disease outbreak manifestation (8). This is measured in weeks. For this validation, time-lags between 8 and 14 weeks were evaluated as this range is generally supported in the literature; though, it could be expanded as the time-lag is not fully understood, especially with different variables interacting with one another (16, 17).

To predict outbreaks, there must be defined alarm indicators, or variables which indicate that an outbreak is coming. These variables can be meteorological, entomological, or potentially social and logistic alarm indicators (15). The validation study in Colombia used the following variables as alarm indicators: rain sum (i.e., weekly rainfall) and mean weekly temperature. These variables would be used to predict the outbreak indicator. This validation

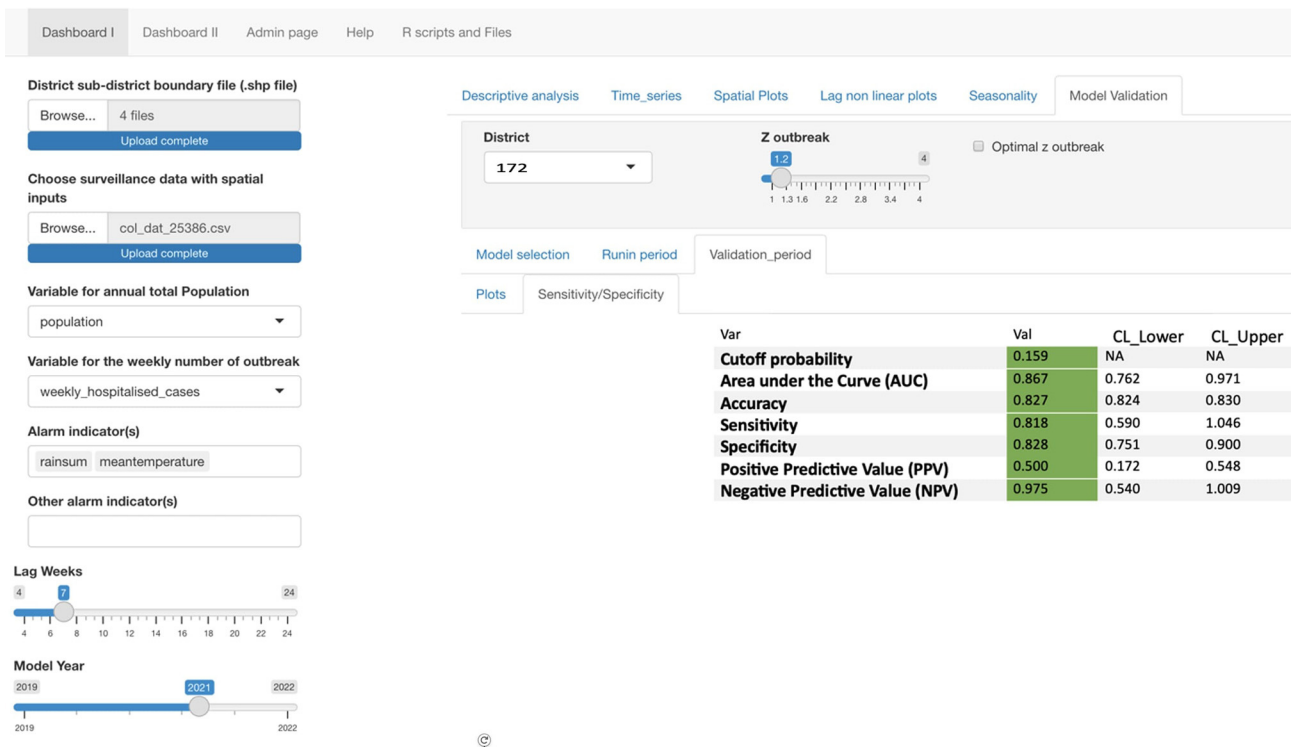

FIGURE 2
Screenshot displaying EWARS-csd dashboard 1 with different calibration variables: lag weeks, model year, and Z outbreak. It also demonstrates the outbreak variable: weekly_hospitalized_cases and alarm indicators mean temperature and rain sum. The top left indicates that it is working on the Dashboard 1 level, and the four shape files must be uploaded and the specific data from the municipalities.

study used the outbreak indicator of weekly hospitalized cases (see Figure 2 for depiction of EWARS-csd dashboard).

For the validation, optimization measurements and receiver operating characteristics (ROC) were calculated to determine the optimal calibrations of sending alarm signals. The ROC includes cutoff probability, area under the curve (AUC), accuracy, sensitivity, specificity, PPV, and NPV.

Sensitivity-the proportion of events that occurred (i.e., outbreaks) that were correctly predicted (14).

Specificity-the proportion of events that were predicted not to occur and did not occur.

PPV-the probability of following an outbreak signal by EWARS-csd that the period will truly have a disease outbreak. The proportion of true alarm signals.

NPV-the probability of following non-outbreak signals by EWARS-csd that the period will truly not have a disease outbreak. The proportion of true non-alarm signals.

2.5 Data calibration in EWARS-csd

For this validation study, the optimal (highest) sum of sensitivity and PPV was recorded for each municipality. To achieve this, different calibrations of the tool were evaluated, so each municipality was calculated at each unique cut-off year (2016–2020) while varying the time-lag (8–14 weeks) and then adjusting the z-value (1–4) to find the optimal level of sensitivity and PPV. This repetitive process allowed for an in-depth understanding of how the calibration variables interact with each other. The highest sensitivity and PPV sum for each municipality each year was then recorded as well as the other information relating to that calibration such as: the endemicity level, the cutoff probability, the AUC, accuracy, sensitivity (95% CI), specificity (95% CI), PPV (95% CI), NPV (95% CI), run-in year, z-value, lag-time/lag non-linear, and the sensitivity and PPV total. If multiple calibration measures resulted in the same sum of sensitivity and PPV, then the median calibration values were taken.

2.6 Endemicity levels

A municipality's outbreak threshold depends on the endemic channel, or the number of cases a community usually (e.g., during the past 5 years) experiences. To generate endemicity levels, interquartile ranges of the hospitalized cases were taken for all the municipalities together using Stata. Category cut-offs without "zero" cases were used to avoid the lowest range being 0. Overall, there were 966 weeks with hospitalization values of 0. Quartile <25% was considered low endemicity, quartile 25–50% was considered moderate endemicity, and quartiles >50% was considered high endemicity. The low

endemicity municipalities are classified as having a median of weekly hospitalized dengue cases of 1 or less, moderate endemicity as 2 or 3, and high as over 3 hospitalizations per week.

2.7 Analysis

Numerical and graphical statistical descriptions were sought for each municipality and stratified by different categories. The data was used from the most optimal sensitivity and PPV per municipality. The median and range were calculated for sensitivity, specificity, PPV, NPV, run-in year, *z*-value, and the time-lag. The median was chosen because the data is not normally distributed, and the median is less sensitive to skewed data. The results were then divided by endemicity levels, provinces, and population sizes to allow for further analysis. While there are no universally agreed predefined cut-off points, this study considers optimization measurements below 50% as poor, 50–70% as fair, and above 70% as good performance results.

2.8 Disease incidence rates

For further analysis, disease incidence rate graphs, computed from the corresponding municipalities, were produced to visualize how well the tool predicted outbreaks (see Figure 3). The tool predicts the magnitude of the outbreak incidence. When the exceedance probability, predicted from the alarm indicators, crosses the cutoff threshold, then it will be considered an alarm signal. The cutoff threshold is based on the endemicity of the municipality plus the standard deviation multiplied by the *z*-value. The alarm signals can be compared to when outbreaks occurred according to the disease incidence data provided.

2.9 Ethical considerations

This project made ethical considerations throughout the entire process. Ethical endorsement was obtained from the Ethics Committee of the University of Freiburg (N°-145/18) which was approved by local health authorities. The data validated was in agreement with and obtained from Colombia's INS. All data was taken at the aggregated level with no personal information recorded for EWARS-csd. Specific ethical approval related to the validation study was not required. The results of this study are being shared with the INS to better implement the tool for the evaluated municipalities and to prevent dengue outbreaks throughout the whole country to benefit the affected communities.

3 Results

3.1 Endemicity levels

Among the 11 municipalities studied between the years 2015 to 2020, there were 20,154 hospitalized cases of dengue. Overall, there were five low endemicity municipalities, five moderate endemicity municipalities, and one high endemicity municipality, which allows for evaluation of the tool's performance at different levels of dengue endemicity (Table 1).

3.2 Summary statistics of the tool's performance

For each municipality, the highest sensitivity and PPV value is recorded in Table 2, along with the other measurements at the calibrations that resulted in the most optimal value. Municipality 172, 178, 190, 446, and 464 all had multiple calibration combinations that resulted in the same optimal sensitivity and PPV, so for these municipalities, the median value of optimal calibrations was recorded. The optimal combined sensitivity and PPV value was 2.00 in municipality 178, which was of low endemicity. The least optimal sensitivity and PPV value was 1.46 in municipality 466, also of low endemicity.

3.3 Outbreak prediction in high, middle, and low endemicity municipalities of Colombia

The tool provided disease incidence graphs of the outbreak prediction scenarios. As seen in Figure 3, the tool was fairly accurate in low endemicity municipalities as outbreak alarms were often before the outbreak points. This is indicated with the blue alarm dots that are produced when the green exceedance probability line extends beyond the red cutoff probability line. If the tool is predicting well then, the blue dots should be followed around 12 weeks later with an orange outbreak dot showing that an outbreak occurred. This graph can be summarized quantitatively.

In municipality 449 (low endemicity), a good sensitivity (92%), specificity (94%), and NPV (99%) were found with a fair level of PPV (60%) (Table 2). The predicted (purple line) and observed incidence rate of hospitalized dengue cases (dark blue) lines also run quite closely to each other, indicating the tool can accurately forecast dengue incidence rates (Figure 4).

For the moderate endemic municipality 170, it also provided strong predictions as indicated by the graph (Figure 5) and Table 2, with good scores across all measurements: sensitivity (97%), specificity (94%), PPV (77%), and NPV (99%). The probability cutoff i.e., alarm threshold for municipality 170 is quite high for a moderate municipality. This is most likely because the calibration was set with years of higher dengue hospitalization incidence.

For the high endemic municipality 468, the disease incidence rate graphs and results provided fair predictions for specificity (57%) and PPV (67%) and good predictions for sensitivity (92%) and NPV (88%) (Figure 6 and Table 2).

3.4 Optimal values

When analyzing all municipalities' optimal calibrations, there were good results for the median sensitivity (0.97) with a tight range (0.80–1.00) (Table 3). It means that the model has managed

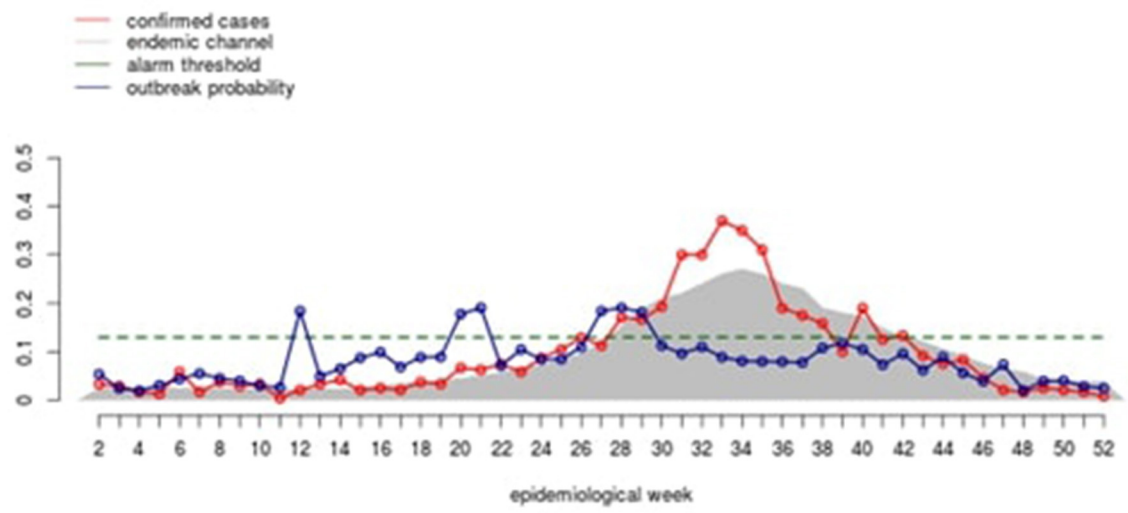

FIGURE 3
Diagram from the EWARS+ program that the municipality medical officer. The red line indicates predicted incidence, in this case hospitalized cases (the tool is currently being updated to match this heading). The blue line indicates the outbreak probability.

TABLE 1 Median weekly dengue hospitalizations by municipality and endemicity level.

Bolívar municipality	Municipality name	Median weekly hospitalizations	Endemicity level	Population in 2020
170	Cartagena	3	Moderate	1,028,736
172	El Carmen De Bolívar	2	Moderate	72,595
178	Santa Cruz de Mompós	1	Low	46,408
190	Santa Rosa del Sur	1	Low	34,568
Cesar municipality	**Municipality name**	**Median weekly hospitalizations**	**Endemicity level**	**Population in 2020**
445	Aguachica	3	Moderate	118,652
446	Agustin Codazzi	2	Moderate	64,676
449	Bosconia	1	Low	43,326
452	Curumani	2	Moderate	39,667
464	San Alberto	1	Low	28,453
466	San Martin	1	Low	28,769
468	Valledupar	9	High	454,906

to predict 97% of all outbreaks that happened that year. Specificity also had good median results (0.94) but a wider range (0.57–1.00). This means that the model managed to predict 94% of all non-outbreaks. The PPV was a lower median value (0.75) compared to the NPV (0.99), but both were still in the good range. For PPV, 75% of alarm signals for outbreaks were correctly predicting outbreaks (true positive). For NPV, 99% of the lack of alarm signals were correct in predicting an outbreak would not occur (true negative). The optimal median run-in year was 2019 and median optimal z-value was 2.45. The optimal median lag time was 12 (Table 3).

3.4.1 Endemicity levels

When endemicity was accounted for, the tool appeared optimal for moderate endemicity municipalities with sensitivity, specificity, PPV, and NPV all being highest in this level (Table 4). The tool appeared to be least optimal in the high endemicity municipality. 2019 as the cut-off year was the median value across all three municipality levels. A longer lag of 13 weeks was the median in moderate municipalities, but it was 11 and 10 weeks in low and high endemic municipalities, respectively.

3.4.2 Differences between states

When comparing between the two states, the tool appeared to be more optimal in Bolívar with higher values of sensitivity, specificity, PPV, and NPV (Table 5). The lag-time median was the same for both at 12 weeks. The median run-in year was similar between both. The Z-value had a median of 2.68 in Bolívar and 1.25 in Cesar but the same range throughout.

TABLE 2 Summary table of most optimal calibrations for each municipality.

Municipality	Endemicity	Sensitivity (95% CI)	Specificity (95% CI)	PPV (95% CI)	NPV (95% CI)	Run-in year cut-off	*Z*-value	Lag-time (weeks)	Sensitivity and PPV total
178	Low	1.00	1.00	1.00	1.00	2020	2.45	13	2.00
190	Low	1.00	0.88	0.75	1.00	2019	3.10	10.5	1.75
449	Low	0.92	0.94	0.60	0.99	2018	4.00	11	1.52
464	Low	0.92	0.72	0.68	0.93	2018	1.25	13	1.59
466	Low	0.83	0.94	0.63	0.98	2020	3.30	9	1.46
170	Moderate	0.97	0.94	0.77	0.99	2017	2.90	11	1.74
172	Moderate	1.00	0.96	0.75	1.00	2020	1.60	13	1.75
445	Moderate	1.00	0.84	0.81	1.00	2019	1.20	12	1.81
446	Moderate	0.80	0.98	0.80	0.98	2019	3.55	13	1.60
452	Moderate	1.00	0.90	0.85	1.00	2019	1.00	13	1.85
468	High	0.92	0.57	0.67	0.88	2019	1.10	10	1.59

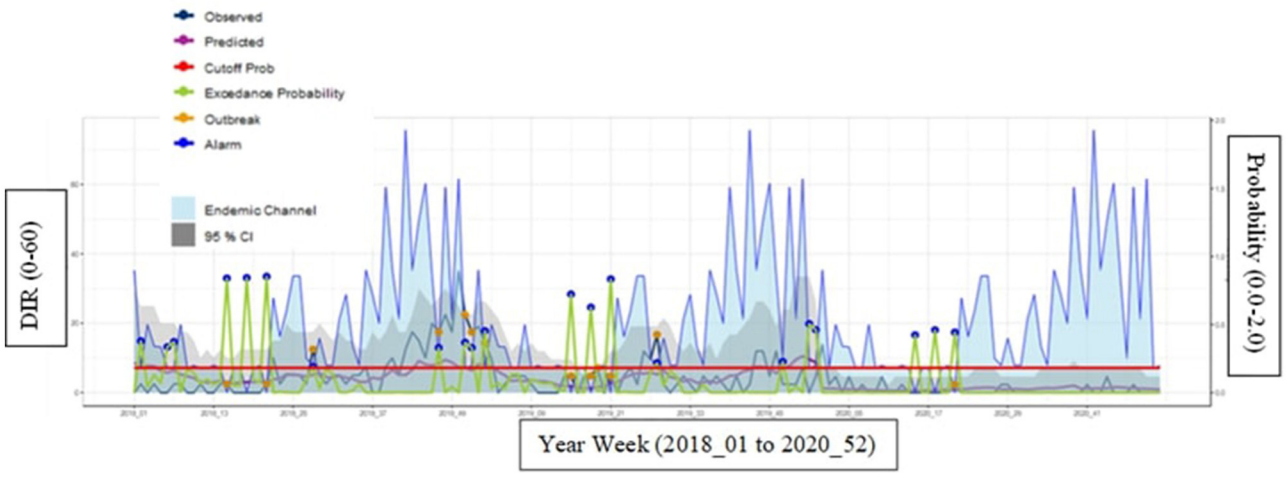

FIGURE 4
Municipality 449 (low endemicity) at most optimal calibrations with a cutoff year of 2018 and *Z*-score of 4.0. Description of how to interpret the graph. Endemic channel in light blue with 95%. Confidence intervals (gray area) representing the "normal" incidence rate of hospitalized dengue cases (upper limit = z*SD of incidence in each week). Observed incidence: Notified incidence of dengue hospitalizations. Predicted incidence: Incidence predicted by the EWARS+ tool. Cut-off for outbreak indicator (outbreak probability). When the alarm indicator (exceedance probability) crosses this line the alarm indicator turns into an alarm signal. Exceedance probability (i.e., outbreak probability): predicted weekly number of cases or incidence above the expected, i.e., above the endemic channel.

3.4.3 Population effect

When separating the municipalities by population size, there were quite similar values for the performance variables between municipalities with over 100,000 people and under (Table 6). The run-in year was the same for both in 2019. The lag-time was longer at 13 weeks for smaller populations compared to 11 weeks for bigger populations.

4 Discussion

4.1 Performance of EWARS-csd

This prediction tool provided reliable results, which helped to validate its performance. The highest sensitivity (i.e., the proportion of correctly predicted outbreaks) and PPV value (i.e., the proportion of true positive alarms) were the determining factors for which calibrations to use in each municipality. When analyzing all the municipalities, the minimum value in the validity tests was 0.57 for specificity. PPV's minimum value was 0.60. For sensitivity, the minimum value was 0.80 and for NPV, it was 0.88. All values are within the predefined "fair" to "good" results category. The high sensitivity and specificity value demonstrate the tool has good predictive performance, which is important from a global health perspective to not miss outbreaks. The results also indicated that the tool showed some deficiencies regarding the proportion of true positive alarms (PPV value) but still provided fair scores of 60 and more percent. Overall, these results demonstrate the tool had lower scores in operations, compared to performance. PPV

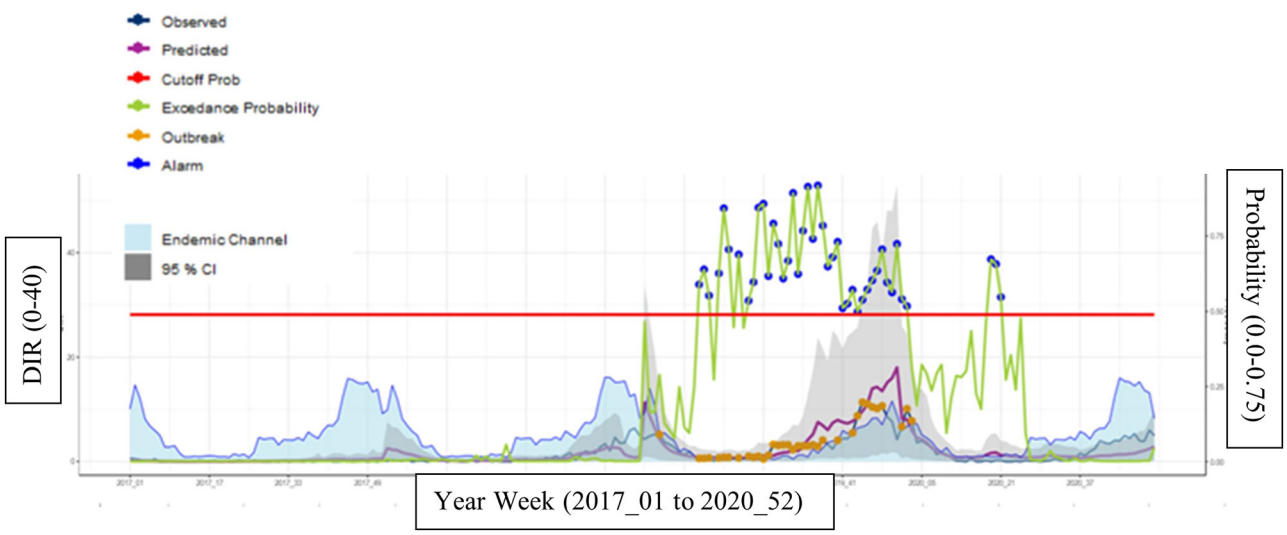

FIGURE 5
Municipality 170 (moderate endemicity) at most optimal calibrations with a cutoff year of 2017 and a *Z*-score of 2.9.

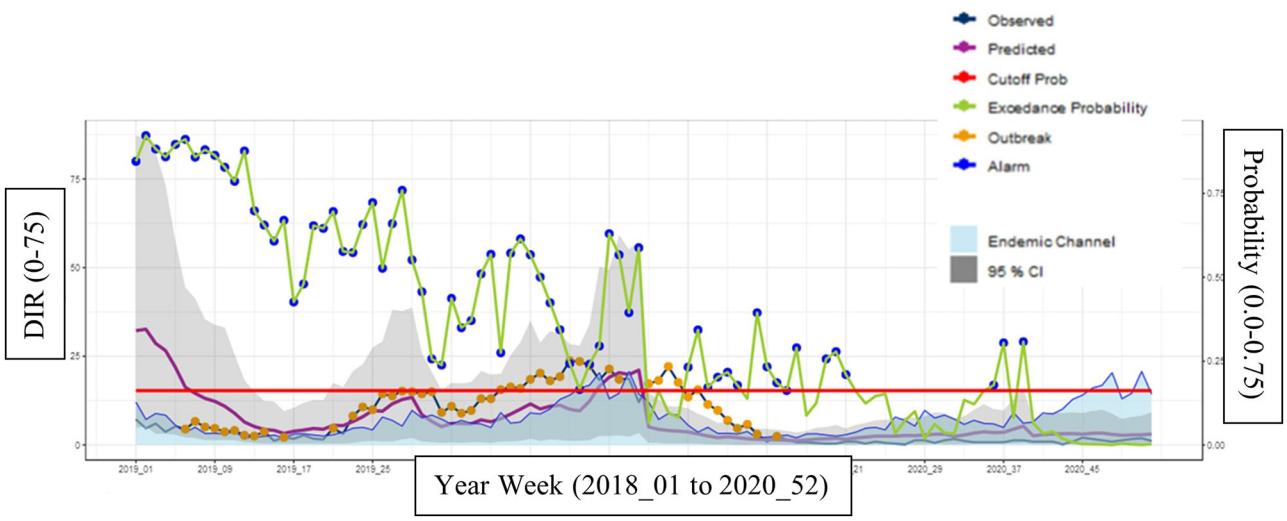

FIGURE 6
Municipality 468 (high endemicity) at most optimal calibrations with a cutoff year of 2019 and a *Z*-score of 1.1.

and NPV are important in the operation, or usage, of the tool as it is not effective to have an overprediction of outbreak alarms as it will not help health systems identify true outbreaks. This means that some extra resources may be deployed for outbreaks that are not likely to occur. However, because EWARS+ proposes different levels of alarm that trigger scaled-up responses, few vector control resources will be engaged if there is a false alarm that is not sustained. The tool proposes a stepwise response based on initial, early, and late alarms to balance how much action should be taken at different alarm signals; this could help to catch any false alarm signals. It is important to note that the data received did not provide municipality specific meteorological data but rather state-level data. Municipality meteorological data would grossly improve the correct prediction with a high PPV, as shown elsewhere (1). Overall, the tool performed well in both prediction and operation. The findings of this validation study are important to reconfirming outbreak prediction with a simple tool as users at the municipality level receive a simplified graph showing the alarm level (18).

The findings of this study also demonstrate successful modeling based on mean temperature and rain sum as alarm indicators (19). While other models also find associations between relative humidity and fractional cloud cover, the results of this study and its high validation scores with only two variables suggest that temperature and precipitation might be the most influential in predictions. Entomological indices like the Ovitrap index will also be particularly helpful in indicating the effort by the vector control services (20). For example, the Ovitrap index can decrease (due to vector control) the outbreak risk, despite

TABLE 3 Median and range of the most optimal sensitivity and PPV after calibration for all municipalities combined.

Variable	Median (range)
Sensitivity	0.97 (0.80–1.00)
Specificity	0.94 (0.57–1.00)
PPV	0.75 (0.60–1.00)
NPV	0.99 (0.88–1.00)
Run-in year	2019 (2017–2020)
Z-value	2.45 (1.00–4.00)
Lag-time (weeks)	12 (9–13)

TABLE 4 Median and range of the most optimal sensitivity and PPV value after calibration, separated by endemicity levels.

Variable	Low endemicity	Moderate endemicity	High endemicity
	Median (range)	Median (range)	Median (only value)
Sensitivity	0.92 (0.83–1.00)	1.00 (0.80–1.00)	0.92
Specificity	0.94 (0.72–1.00)	0.94 (0.84–0.98)	0.57
PPV	0.68 (0.60–1.00)	0.80 (0.75–0.85)	0.67
NPV	0.99 (0.93–1.00)	1.00 (0.98–1.00)	0.88
Run-in year	2019 (2018–2020)	2019 (2017–2020)	2019
Z-value	3.10 (1.25–4.00)	1.60 (1.00–3.55)	1.10
Lag-time (weeks)	11 (9–13)	13 (11–13)	10

TABLE 5 Median and range of the most optimal sensitivity and PPV value after calibration, separated by the Bolívar and Cesar provinces.

Variable	Bolívar	Cesar
	Median (range)	Median (range)
Sensitivity	1.00 (0.97–1.00)	0.92 (0.80–1.00)
Specificity	0.95 (0.88–1.00)	0.89 (0.57–0.98)
PPV	0.76 (0.75–1.00)	0.68 (0.60–1.00)
NPV	1.00 (0.99–1.00)	0.98 (0.88–1.00)
Run-in year	2019.5 (2017–2020)	2019 (2018–2020)
Z-value	2.68 (1.60–3.10)	1.25 (1.60–3.10)
Lag-time (weeks)	12 (10.5–13)	12 (9-13)

TABLE 6 Median and range of the most optimal sensitivity and PPV value after calibration, separated by populations over and under 100,000 people per municipality.

Variable	Population <100,000	Population >100,000
	Median (range)	Median (range)
Sensitivity	0.96 (0.80–1.00)	0.97 (0.92–1.00)
Specificity	0.94 (0.72–1.00)	0.84 (0.57–0.94)
PPV	0.75 (0.60–1.00)	0.77 (0.67–0.81)
NPV	0.997 (0.932–1.0)	0.99 (0.88–1.00)
Run-in year	2019 (2018–2020)	2019 (2017–2019)
Z-value	2.78 (1.00–4.00)	1.20 (1.10–2.90)
Lag-time (weeks)	13 (9-13)	11 (10–12)

continued high temperature or rainfall. However, a study by Ong et al. testing machine learning algorithms for dengue prediction found that meteorological variables had better predictor capabilities than vector indices, possibly because the indices measure immature mosquitoes, which cannot transmit disease (21). In addition, oftentimes vector indices data is collected inconsistently resulting in less predictive value; however, if collected consistently, entomological data has been found to have powerful predictive abilities (12). Dengue's rapid spread to new areas has caused a variety of predictive models to be developed to test a broader set of predictors in unique combinations to see if more optimal results can be obtained (see Supplementary Table 1) (22). In addition, the EWARS-csd study supports the time-lag/lag non-linear model, describing the time needed between ideal climatic conditions and dengue outbreaks, with 12 weeks being the median time with the best results (23).

4.2 Endemicity levels

Across all three levels of endemicity, the tool provided strong predictor signals. While there was only one highly endemic municipality to analyze (a limitation of this study), this study provided insights into how the tool works at different endemicity levels. It also supported the assumption that the tool is independent of endemicity levels. For example, the tool yielded the highest validity in moderate municipalities, though municipalities with low endemic levels also had the tool perform well. The high-endemic municipality, municipality 468-Valledupar, also performed well, but it had the lowest ROC scores across all four categories. For example, there was a noticeable low specificity value of 0.57, compared to the low and moderate municipalities having a value around 0.90. The initial hypothesis was that the tool performs better in highly endemic areas, due to more cases for run-in years and more equipped municipalities for case reporting etc. Furthermore, the vector control activities may be different in the study municipalities.

One possible reason the tool may have performed more poorly in the highly endemic municipality is because there may be more routine vector control activity already here which would have prevented the outbreaks from occurring. Retrospective data was used, and it did not contain information on vector control activities, which would be important information for future studies. Some other possible reasons for this are that Valledupar is highly populated as the capital of the Cesar municipality with over 450,000 residents. Dengue has been found to spread quicker in more populated areas (24). However, when looking at other

municipalities with high populations, it did not seem to have an effect on the tool's performance. It is also possible that municipality 468's close location to the Venezuelan border and its ongoing refugee crisis impacts the dengue burden in this region in ways the tool cannot predict. Another factor could have been that the health system was overwhelmed because of the high caseloads and may have led to errors in case reporting as the low specificity indicates that it did not predict non-outbreak windows as well.

The high endemic municipality also had a shorter lag time of 10 weeks compared to the other municipalities, which would mean that vector control responses would need to be faster. Overall, all median values across the highly endemic municipality were still over the 0.50 mark, indicating the tool still performed well. Future studies should further examine how the tool works in highly endemic municipalities by employing a larger sample of municipalities and including other alarm indicators. Overall, it is promising that the tool performance is independent across endemicity levels, and it supports the idea that it can be used in places of all different endemicity, being data driven.

4.3 Differences between states

The 11 municipalities used in this study came from two states: Cesar and Bolívar. The two border each other and are part of the greater Atlantic Coast region of Colombia (9). They also should receive equal health funding from centralized, national resources. Though geographically close to each other, the tool performed differently between the two states. It performed better across all four measurements in Bolívar compared to Cesar. There are a variety of possible reasons why this may have occurred, and it is possible it was outside of the tool's predicting capabilities.

4.3.1 Migration effect

Cesar, which is directly on the border with Venezuela, had a higher percentage of Venezuelan migrants per total population (4.3 vs. 3.8%) (25). The greater influx of migrants and refugees both living and passing through Cesar compared to Bolívar could impact Cesar's poorer performance on the EWARS-csd tool. Venezuelan migration has spread and increased arboviruses throughout Latin America and this could increase dengue's impact in Colombia (26). In 2021, 77% of Venezuelans living in Colombia lacked access to healthcare and many also suffered from food insecurity (25). These make people more prone to suffering from dengue and could increase the municipality's overall risk of dengue outbreaks. Human mobility, such as migration, is an issue that might encourage the development of a human mobility variable for EWARS-csd.

4.3.2 Socioeconomic status effect

The literature on poverty's relation to dengue is mixed as poor housing infrastructure and inadequate water storage both could increase a community's risk of an outbreak (27). However, increased mobility, which may also affect those of higher SES has also been associated with outbreaks (3). In 2021, Bolívar had higher scores in human development index (0.74), health index (0.82), educational index (0.69), and income index (0.72) (28). Cesar comparatively had lower scores across all categories: human development index (0.72), health index (0.79), educational index (0.65), and income index (0.71) (28). When comparing the tool's performance between Bolívar and Cesar, Bolívar performed better across all measurements. This could suggest that the tool performs better in communities that have higher development levels as the tool does not consider sociological factors, which could also drive dengue outbreaks and response, and lead to the tool's discrepancies. While climate conditions are especially important for the tool prediction, societal influences may also impact the probability of dengue outbreaks by better informing the model of most-disadvantaged hot-spots of disease transmission. This relation should be evaluated further, and future research could explore if socioeconomic status could be a predictive measure in the tool.

4.3.3 Population effect

The tool performed quite similarly for municipalities with populations over 100,000 and below. The literature often supports that mosquitoes have adapted well to urban environments (5). Some have even considered dengue an urban disease (20). However, studies still find that rural communities play an increasingly important role in dengue transmission, and studies have found that urban and rural transmission rates are similar (20). The results of this study support that the tool plays similar roles for rural and urban areas. However, when looking at the operationalization, the lag time is shorter at 11 weeks for bigger populations compared to smaller populations, which would mean that local vector control teams would have less time to respond to outbreaks. This study demonstrates that the tool works for both environments and that the population of a municipality is less important to include in the tool's optimization.

4.4 Limitations

One of the major limitations of this study is that the meteorological data collected from Colombia was not specific to each municipality. While Colombia has installed many local municipality meteorological stations to better analyze local conditions and support the knowledge about local climate conditions, there were administrative complications, which meant that this data was not received. Instead, the data received was homogeneous for each municipality in the same province. This resulted in the tool predicting mainly from the overall province seasonality, instead of being specific to the specific climatic conditions of the municipality. In addition, the municipalities examined within each department did not have selection criteria based on representativeness, so this is important to note for department-level analyses. Another limitation of the study is that for Bolívar's four municipalities evaluated, there was 0 mm of rainfall for each week in 2020. Although drought is a natural phenomenon which reflects a real-life scenario in Colombia during some years, the human behaviors associated with drought including how people may store water, which create hotspots for mosquitoes, may have impacted the evaluation by the model (7). For two of

the four municipalities in Bolívar, 2020 as the cut-off year was most optimal, which is interesting because it has only considered temperature for the prospective data.

Another limitation of the study is that it did not consider relative humidity due to technical issues. The success of the tool's optimization without this third indicator is very promising and future studies are warranted to see if adding relative humidity could increase optimization. Also, the true number of dengue cases is unknown as many cases are not reported, so only weekly hospitalized cases were used as an outbreak indicator, which could also be underestimated due to the COVID-19 pandemic, which overlaps with the study period. This is only a small proportion of all cases, and it means less data was available to monitor dengue levels.

5 Conclusion

With dengue spreading around the world and its burden being felt in more communities, it is crucial that community control services are equipped with the right resources and knowledge to combat the disease. This study provides important validation of the EWARS-csd tool and specifically how it predicts dengue outbreaks in Colombia. The tool performed well across all 11 Colombian municipalities measured, across various endemicity levels and population sizes. The tool did perform slightly better in Bolívar municipalities compared to Cesar which could be due to Cesar having lower human development indexes and/or having higher migration rates from Venezuela. Colombia and the 16 other countries currently implementing the EWARS-csd tool are working to fully integrate the tool within their national surveillance program to better focus their dengue efforts on the communities most impacted. However, 128 countries are affected by dengue. This is an unfinished regional, national, and global agenda, and this study provides crucial assurance to these countries of the tool's validity. WHO has promoted EWARS-csd in predicting dengue outbreaks, and this study should provide confidence on their decision and allow them to continue expanding this tool to better prepare other communities (29).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Author contributions

MSc: Software, Writing–original draft, Writing–review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Project administration, Resources. FP: Data curation, Investigation, Methodology, Validation, Writing–review & editing, Software. MB: Investigation, Data curation, Methodology, Software, Validation, Writing–review & editing. MSe: Investigation, Data curation, Methodology, Software, Validation, Writing–review & editing. CM: Software, Methodology, Writing–original draft, Conceptualization, Funding acquisition, Supervision, Writing–review & editing, Data curation, Formal analysis, Investigation, Project administration, Resources, Validation, Visualization. AK: Funding acquisition, Supervision, Writing–original draft, Writing–review & editing, Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Project administration, Resources, Software, Validation, Visualization. LH-A: Funding acquisition, Methodology, Supervision, Writing–original draft, Writing–review & editing, Conceptualization, Data curation, Formal analysis, Investigation, Project administration, Software, Validation, Resources, Visualization.

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Conflict of interest

CM is currently a staff member of the World Health Organization; the author alone is responsible for the views expressed in this publication and they do not necessarily represent the decisions, policies, or views of the WHO.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1323618/full#supplementary-material

References

1. Hussain-Alkhateeb L, Kroeger A, Olliaro P, Rocklöv J, Sewe MO, Tejeda G, et al. Early warning and response system (EWARS) for dengue outbreaks: recent advancements towards widespread applications in critical settings. *PLoS ONE.* (2018) 13:e0196811. doi: 10.1371/journal.pone.0196811

2. Soneja S, Tsarouchi G, Lumbroso D, Tung DK. A review of Dengue's historical and future health risk from a changing climate. *Curr Environ Health Rep.* (2021) 8:245–65. doi: 10.1007/s40572-021-00322-8

3. Gubler DJ. Dengue, urbanization and globalization: the unholy trinity of the 21st century. *Trop Med Health.* (2011) 39(4Suppl.):S3–11. doi: 10.2149/tmh.2011-S05

4. Rocklöv J, Tozan Y. Climate change and the rising infectiousness of dengue. *Emerg Top Life Sci.* (2019) 3:133–42. doi: 10.1042/ETLS20180123

5. Naish S, Dale P, Mackenzie JS, McBride J, Mengersen K, Tong S. Climate change and dengue: a critical and systematic review of quantitative modelling approaches. *BMC Infect Dis.* (2014) 14:167. doi: 10.1186/1471-2334-14-167

6. Guzman MG, Gubler DJ, Izquierdo A, Martinez E, Halstead SB. Dengue infection. *Nat Rev Dis Primer.* (2016) 2:16055. doi: 10.1038/nrdp.2016.55

7. Lowe R, Gasparrini A, Van Meerbeeck CJ, Lippi CA, Mahon R, Trotman AR, et al. Nonlinear and delayed impacts of climate on dengue risk in Barbados: a modelling study. *PLOS Med.* (2018) 15:e1002613. doi: 10.1371/journal.pmed.1002613

8. Hussain-Alkhateeb L, Kroeger A. *Innovation and Collaboration: Latest From the WHO-Spatio-temporal EWARS Framework. Global Arborvirus Initiative.* Geneva: WHO.

9. Gutierrez-Barbosa H, Medina-Moreno S, Zapata JC, Chua JV. Dengue infections in colombia: epidemiological trends of a hyperendemic country. *Trop Med Infect Dis.* (2020) 5:156. doi: 10.3390/tropicalmed5040156

10. Colombia: Administrative Division. *City Population.* (2023). Available online at: https://citypopulation.de/en/colombia/admin/ (accessed April 3, 2023).

11. MapChart. *The Americas - Detailed Map.* MapChart.net.

12. Cardenas R, Hussain-Alkhateeb L, Benitez-Valladares D, Sánchez-Tejeda G, Kroeger A. The Early Warning and Response System (EWARS-TDR) for dengue outbreaks: can it also be applied to chikungunya and Zika outbreak warning? *BMC Infect Dis.* (2022) 22:235. doi: 10.1186/s12879-022-07197-6

13. Hussain-Alkhateeb L. *The WHO Spatio temporal EWARS Framework.* Gothenburg: University of Gothenburg (2022).

14. World Health Organization, UNICEF/UNDP/World Bank/WHO Special Programme for Research and Training in Tropical Diseases. *Operational Guide Using the Web-Based Dashboard: Early Warning and Response System (?EWARS)? for Dengue Outbreaks, Report No.: 2.* World Health Organization (2020). p. 50. Available online at: https://apps.who.int/iris/handle/10665/332323 (accessed March 3, 2023).

15. Kroeger A. *Forecasting Outbreaks.* Gothenburg: University of Gothenburg (2022)

16. Hii YL, Rocklöv J, Wall S, Ng LC, Tang CS, Ng N. Optimal lead time for dengue forecast. *PLoS Negl Trop Dis.* (2012) 6:e1848. doi: 10.1371/journal.pntd.0001848

17. Wang P, Zhang X, Hashizume M, Goggins WB, Luo C. A systematic review on lagged associations in climate–health studies. *Int J Epidemiol.* (2021) 50:1199–212. doi: 10.1093/ije/dyaa286

18. Meckawy R, Stuckler D, Mehta A, Al-Ahdal T, Doebbeling BN. Effectiveness of early warning systems in the detection of infectious diseases outbreaks: a systematic review. *BMC Public Health.* (2022) 22:2216. doi: 10.1186/s12889-022-14625-4

19. Bowman LR, Tejeda GS, Coelho GE, Sulaiman LH, Gill BS, McCall PJ, et al. Alarm variables for dengue outbreaks: a multi-centre study in Asia and Latin America. *PLoS ONE.* (2016) 11:e0157971. doi: 10.1371/journal.pone.0157971

20. Wright E, Carrillo MA, Matamoros D, Sanchez RC, Yañez J, Di Lorenzo G, et al. Applicability of the Mexican ovitrap system for Aedes vector surveillance in Colombia. *Pathog Glob Health.* (2023) 117:554–64. doi: 10.1080/20477724.2022.2146049

21. Ong SQ, Isawasan P, Ngesom AMM, Shahar H, Lasim AM, Nair G. Predicting dengue transmission rates by comparing different machine learning models with vector indices and meteorological data. *Sci Rep.* (2023) 13:19129. doi: 10.1038/s41598-023-46342-2

22. Hopp M, Foley J. Worldwide fluctuations in dengue fever cases related to climate variability. *Clim Res.* (2003) 25:85–94. doi: 10.3354/cr025085

23. Lowe R. *Climate Change, Dengue and Global Health Resilience.* ISNTD Symposium: The International Society for Neglected Tropical Diseases (2022). Available online at: https://www.youtube.com/watch?v=iUJ_dMkFs5M (accessed March 15, 2023).

24. Márquez S, Lee G, Gutiérrez B, Bennett S, Coloma J, Eisenberg JNS, et al. Phylogenetic analysis of transmission dynamics of dengue in large and small population centers, Northern Ecuador. *Emerg Infect Dis.* (2023) 29:221226. doi: 10.3201/eid2905.221226

25. ACAPS. *Country Analysis: Colombia.* (2022). Available online at: https://www.acaps.org/country/colombia/crisis/venezuelan-refugees- (accessed April 5, 2023).

26. Maljkovic Berry I, Rutvisuttinunt W, Sippy R, Beltran-Ayala E, Figueroa K, Ryan S, et al. The origins of dengue and chikungunya viruses in Ecuador following increased migration from Venezuela and Colombia. *BMC Evol Biol.* (2020) 20:31. doi: 10.1186/s12862-020-1596-8

27. Nature. *Controlling Dengue Outbreaks.* (2022). Available online at: https://www.nature.com/scitable/topicpage/controlling-dengue-outbreaks-22403714/ (accessed March 5, 2023).

28. Global Data Lab. *Subnational HDI.* (2021). Available online at: https://globaldatalab.org/shdi/table/2021/shdi$+$healthindex$+$edindex$+$incindex/COL/?levels=1$+$4&interpolation=0&extrapolation=0 (accessed April 5, 2023).

29. Hussain-Alkhateeb L, Pelull S, Kroeger A, Frieler N, Merle CSC. *Policy Brief-Innovation and Collaboration: the EWARS Framework for Infectious Diseases.* Berlin: Global Health Hub Germany (2022). p. 12. Available online at: https://www.globalhealthhub.de/fileadmin/general_documents/GHHG_PolicyBrief_2_ENG_310122_kl.pdf~ (accessed March 22, 2023).

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Assessing the impact of COVID-19 on epidemiological changes of severe pediatric respiratory syncytial virus infections in Malaysia

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Introduction: Respiratory syncytial virus (RSV) is one of the leading causes of hospitalization and mortality among children with respiratory tract infections. The non-pharmaceutical preventive measures against severe acute respiratory syndrome coronavirus (COVID-19) may have reduced the transmission of RSV, altering its tropical epidemiological seasonality. Thus, this study represents the first attempt to evaluate changes in RSV epidemiology in the context of COVID-19 pandemic in Malaysia.

Methods: Conducted at a tertiary hospital in Kuala Lumpur, Malaysia, this retrospective study analyzed collated data of children aged <12 years who were admitted for severe respiratory infections from 2017 to 2022. Time series models were used to predict the differences between actual and forecasted RSV cases, while logistic regression assessed the statistical association between RSV and COVID-19.

Results: Among the 4,084 children analyzed, we reported a significant inverse relationship between RSV and COVID-19 infections during the pandemic (2020–2021) ($p < 0.05$). In 2020, the RSV positivity rate sharply declined to 8.3 and 5.9%, respectively, in the two prominent seasons. Time series analysis showed a tremendous decrease in cases compared to the expected values, with reductions of 98.3% in the first season and 95.7% in the second season. However, following the lifting of the restriction order in 2022, RSV infections rose sharply with a positivity rate of 36.3%, higher than pre-COVID-19 pandemic levels.

Conclusion: This study provides evidence of increasing RSV cases post-COVID-19 pandemic, due to immunity debt. Hence, the healthcare system must be prepared to address future RSV outbreaks with the appropriate implementation of prophylaxis and public health measures.

KEYWORDS

RSV infection, COVID-19, children, immunity debt, preventive restriction

1 Introduction

Globally, seasonal epidemics of respiratory syncytial virus (RSV) is one of the leading causes of hospitalization and mortality among children, particularly those under the age of 5 (1). The severe manifestations of RSV disease include pneumonia and bronchiolitis, with the latter typically being self-limiting (2). This is supported by the Pediatric Etiology Research for Child Health (PERCH), which identified RSV as the most common pathogen isolated from hospitalized children with severe pneumonia in Africa and Asia, accounting for 31% of all cases (3). In 2015, an estimated 33 million episodes of lower respiratory tract infections (LRTIs) were attributed to RSV infections in children under the age of 5, resulting in 3.2 million hospitalizations and 120, 000 deaths worldwide (4). Despite the known morbidity and mortality associated with RSV, there is currently no approved vaccine to prevent RSV infections (1). Thus, preventive measures aimed at reducing the spread of RSV remain the most promising intervention in controlling these seasonal epidemics.

Respiratory diseases reached a catastrophic milestone with the emergence of severe acute respiratory syndrome coronavirus (COVID-19) in December 2019 in Wuhan, China, impacting millions of adults and children (5). Within 1 year after its emergence, global reported deaths due to this pandemic had reached 5.94 million by 31st December 2021. Interestingly, this figure was hypothesized to be underestimated by a factor of 3.07 based on excess mortality rates, which is the net difference between the actual number of deaths during the pandemic and the expected number based on past trends in all-cause mortality (6). In Malaysia, COVID-19 was first detected in January 2020 and surged in March 2020, prompting the government to implement Movement Control Order (MCO) to restrict mass movements and gatherings in combating the pandemic.

Importantly, COVID-19 shares a similar air-borne transmission mechanism with RSV (1). Therefore, the preventive recommendations implemented during the MCO to mitigate COVID-19 transmission may have also helped to preventing local transmission of RSV. Notably, the United States reported historically low weekly percentages of positive RSV rates early in the pandemic (<1.0% per week compared to approximately 12–16% during the pre-COVID era) while an Australian study also observed decreased RSV activity due to COVID-19 restrictions (7, 8). Regionally in Asia, China also experienced two sharp declines in RSV infections during the two national outbreaks of the COVID-19 pandemic (9). In our previous epidemiological study, we observed a sharp decline in RSV cases in 2020, possibly due to reduced exposure to RSV as a result of the nationwide lockdown. (10).

The COVID-19 pandemic has presented a unique opportunity for the widespread implementation of public health interventions on a global scale for a limited period of time (1). However, there is a lack of evidence regarding the effectiveness of these public health measures in local and regional contexts, contributing to the decreased transmission of RSV during the pandemic. As a result, the widened epidemiology gap has intrigued researchers worldwide to comprehend the possible shift in RSV seasonality in assessing the timing and effectiveness of prophylactic and therapeutic interventions. To address this knowledge gap, it is crucial to establish large-scale surveillance that can improve our understanding of RSV epidemiology, particularly in the context of COVID-19. Therefore, our study represents a pioneering effort aimed at advancing the comprehension of disease epidemiology within the framework of the COVID-19 pandemic, particularly regarding RSV. We aim to potentially identify a larger scale or a shifted paradigm of RSV infections compared to previous years in Malaysia.

2 Materials and methods

2.1 Study design and sample size

This was a retrospective study conducted at Hospital Canselor Tuanku Muhriz (HCTM), a tertiary hospital located in the Klang Valley spanning three states namely Selangor, Kuala Lumpur and the Federal Territorial State of Kuala Lumpur (11). Based on the latest hospital audit report (2018–2021), we received an average of 518, 885.8 patients per year, with 14.9% (77, 300.8 patients) being pediatric patients aged 0 to 17 years old (12). For this study, we analyzed data collected over a six-year period, from 1st January 2017 to 31st December 2022. We included 4,096 hospitalized patients ranging from birth up to 12 years old who underwent nasopharyngeal analysis (NPA) due to acute respiratory tract infections (ARTIs). Additional demographic information, including date of birth, race, test date, and age at the time of the test, was extracted from patient databases. Children with mild respiratory symptoms and not requiring non-invasive ventilation (NIV) or without NPA analysis were excluded from this study. We further excluded 12 repeated RSV samples obtained from the same patient within a two-week timeframe, hypothesized to be from the same period of infection. Consequently, only the earlier RSV samples were included for analysis. Using the formula for population sampling by Krejcie and Morgan, a 95% confidence interval from a final sampling frame of 4,084 patients required at least 354 positive samples. This study received ethical approval and support from the Secretariat of Research and Innovation Universiti Kebangsaan Malaysia (UKM) (Project code: JEP-2021-780).

2.2 RSV infection detection

ARTI is defined as the presence of cough and cold with respiratory symptoms such as rapid breathing, tachypnoea above age limit, and/ or chest in-drawing, along with warning signs such as the inability to tolerate feeding, persistent vomiting, lethargy, or stridor (10). Symptomatic children with moderate to severe respiratory distress symptoms requiring NIV were admitted, and NPA was routinely obtained to detect various common respiratory viruses. We utilized the direct fluorescent antibody (DFA) method, specifically the D3 Ultra DFA Respiratory Virus Screening and Identification Kits (Diagnostic Hybrids, United States) to detect RSV, adenovirus, influenza A and B, and parainfluenza 1, 2, and 3 viruses (sensitivity: 95.5%, specificity: 98.3%) (13).

2.3 COVID-19 epidemiological data

The daily COVID-19 cases spanning from 1st March 2020 to 31st December 2022 were extracted from the Department of Statistics, Ministry of Health Malaysia (14). To depict the pinnacle of COVID-19 infections, our dataset exclusively encompassed the daily count of newly confirmed positive cases across all age groups, ranging from pediatric to adult populations. Moreover, our analysis refrained

from stratifying the data according to state-specific case aggregates, despite the study being conducted in Kuala Lumpur.

2.4 Public health measures in response to COVID-19 pandemic

On 4th February 2020, Malaysia recorded its first locally transmitted case of COVID-19, which subsequently increased sharply to a consistent daily count surpassing 100 in March 2020. As a result, the Malaysian government strictly implemented a nationwide movement control order (MCO) on 18th March 2020 to mitigate the spread of the virus. This measure included restrictions on mass movements and gatherings across all locations. Despite the initial measures, virus transmission persisted, leading to more stringent enforcement on 1st April 2020. As a result of improved compliance with the MCO, a notable decrease in daily new COVID-19 cases and an increase in recoveries were observed 14 days after its enforcement. To revive the national economy while continuing to manage the situation, more businesses were allowed to resume operations, leading to the revision as conditional MCO (CMCO) which was further relaxed to recovery MCO (RMCO) (15). Interestingly, COVID-19 detection rates increased exponentially from late 2021 to early 2022 due to an increased number of clusters, including prison inmates, foreigners, and mass gatherings related to elections. In response, the government proactively mapped and detect active cases, leading to several mass sampling areas nationwide. The Malaysian nationals also implemented the use of online contact tracing, "MySejahtera," to assist in COVID-19 outbreak (Figure 1) (16).

2.5 Statistical analysis

We performed the data analysis using the Statistical Package for the Social Sciences (SPSS) version 26 (IBM, Chicago, IL, United States). Descriptive analyses were utilized to present the demographic data, where categorical variables were expressed as frequencies and percentages, and continuous data as means. We stratified our RSV data into three cohorts: pre-COVID (2017–2019), during-COVID (2020 and 2021) and post-COVID (2022). To visualize the trends and relationships within each cohort, we plotted the number of RSV and COVID-19 cases on time-series graphs, using a weekly timeframe. Consequently, we calculated the positivity rate of RSV during two prominent seasons using the following equation:

$$(\text{Total positive cases} / \text{Total NPA samples collected}) \times 100\%$$

In addition, we performed a time series analysis to forecast the total counts of RSV cases for the years 2020, 2021, and 2022. This analysis was based on the weekly counts of RSV cases from 2017 to 2019, focusing on the two seasons of interest. To generate the predictions, we estimated the model parameters using the maximum likelihood method and 95% confidence intervals (CIs) to account for the uncertainty in the forecasted values. This was done by calculating the percentage difference between the two numbers, employing the following equation:

$$(\text{Observed frequency} / \text{Expected frequency}) \times 100\%$$

In order to establish the statistical association between COVID-19 and RSV cases, we conducted a Pearson correlation analysis. Subsequently, significant correlations were further examined using bivariate logistic regression analysis. To determine the significance of the correlations, a two-sided value of p of less than 0.05 was considered statistically significant.

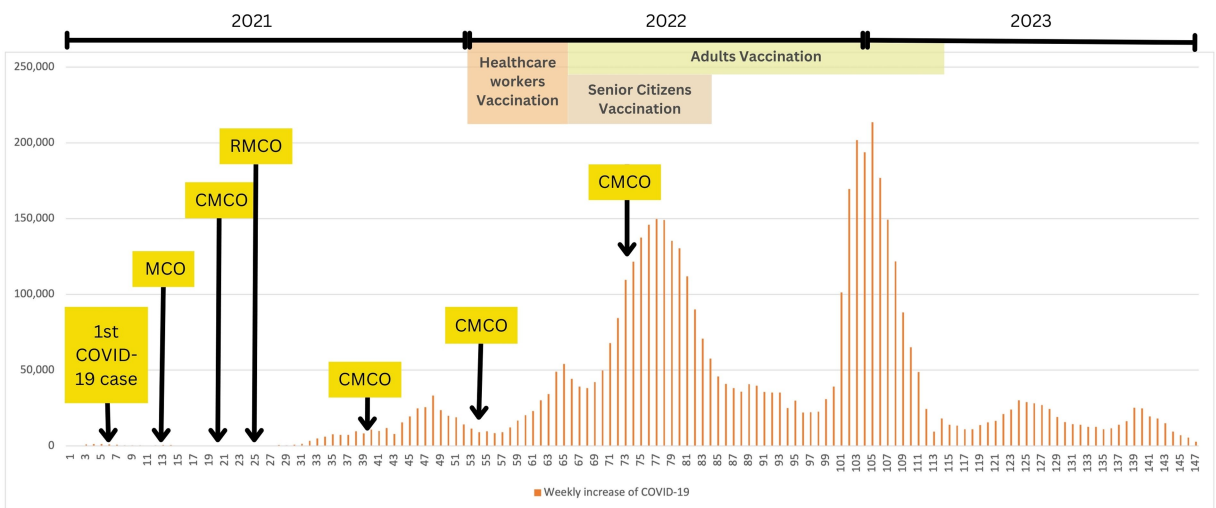

FIGURE 1
COVID-19 and public health measures timeline in Malaysia (1st March 2020 – 31st December 2022). This graph illustrates the weekly increase of COVID-19 cases within the stipulated timeframe, where number of weeks, $N = 147$. Data was obtained from the Department of Statistics, Ministry of Health Malaysia. MCO, movement control order; CMCO, controlled movement control order; RMCO, recovery movement control order.

3 Results

3.1 Demographic data of severe respiratory syncytial virus infections for the past 6-year period

Among these 4, 084 samples, 697 (17.1%) children tested positive for RSV. Demographically, the median age of positive cases was 1 year, with the majority of cases being under 2 years old. The highest RSV detection rate was observed among children under 6 months (23.9%). Analysis of the data showed a positive trend of increasing RSV cases from 2017 to 2022, except for a slight dip in 2020 (15.0%). This decline may be attributed to underreporting or possibly the impact of COVID-19 public health measures. Overall, Malay children exhibited a higher rate of RSV infection (17.7%). A summary of the demographic data for RSV-positive cases can be found in Table 1.

3.2 Prominent seasonality of respiratory syncytial virus infections before COVID-19

Visually, Figure 2 shows that RSV infections occurred consistently throughout the year, exhibiting two distinct periods of pronounced seasonality. The first period of seasonality spanned from week 26 to week 31, occurring in the middle of the year. The second period of seasonality was observed from week 45 to week 52, toward the year-end. Using this pattern as a baseline for comparison, the data from the years during-COVID (2020, 2021) and post-COVID (2022) were analyzed to illustrate the trend of the infectivity rate during these two seasons.

TABLE 1 Demographic information of children with severe RSV infections.

Indicator (N = 4,084)	RSV positive = 697, n (%)
Age	
<6 months	172 (23.9)
6 months to 2 years	397 (20.1)
2.1 years to 5 years	95 (8.9)
>6 years	33 (10.2)
Year	
2017	47 (8.9)
2018	56 (10.8)
2019	167 (17.7)
2020	80 (15.0)
2021	85 (20.2)
2022	262 (22.9)
Ethnicity	
Malay	653 (17.7)
Chinese	27 (13.9)
Indian	4 (8.2)
Other	13 (8.8)

Descriptive analysis was used to describe the demographic information of RSV-positive children based on (i) age groups (ii) year of analysis, and (iii) ethnicity. The results were expressed in n (%), where n represents the number of children.

3.3 The relationship between RSV and COVID-19 infections In subsequent 3 years (2020–2022)

Figure 3 illustrates an inverse relationship between COVID-19 and RSV infections. This relationship is particularly significant in Figure 3A (2020) and Figure 3B (2021), where RSV infections drastically declined as COVID-19 infections increased ($p < 0.001$). In 2020, the average number of RSV infections dropped significantly to approximately 1 case throughout the year. Overall, the highest RSV infection among our patients was observed at the beginning of the COVID-19 outbreak, coinciding with the implementation of lockdown measures. As strict lockdowns were enforced during the 2nd MCO, the number of COVID-19 cases gradually decreased and plateaued to less than 100 new cases daily from week 24 to 36. This led to a gradual easing of restrictions, transitioning to the CMCO and subsequently to the RMCO. Around 4 weeks after the RMCO, RSV infections resurfaced after remaining dormant since March 2020. However, the overall number of recorded RSV cases during this period was relatively lower than pre-COVID. Statistically, logistic regression supported this trend and predicted that with every increase of COVID-19 cases in 2020, we noted a significant drop in the number of RSV cases, approximately by 8-fold ($p < 0.014$).

Large-scale immunizations against COVID-19 were enforced nationwide in the first quarter of 2021 to all adults aged 18 and above, leading to herd immunity against COVID-19. Consequently, the infection rate of COVID-19 significantly reduced to approximately 10, 000 cases per week. However, this decrease in COVID-19 cases indirectly contributed to a surge in RSV infections among our patients. However, this phenomenon was only observed until the second half of 2021 when a sudden spike in COVID-19 cases necessitated the reimplementation of MCO. Interestingly, RSV rebounded to 8 cases at week 46, marking the highest count since the COVID-19 pandemic started. However, it declined sharply in the following week, potentially attributed to the detection of the Omicron variant of the virus in Malaysia. The presence of the Omicron variant may have played a role in the subsequent decrease in RSV cases until early 2022.

As the vaccination coverage among the adult population increased and the number of COVID-19 cases decreased, the MCO was fully lifted in the second half of 2022. As anticipated, there was a sharp rebound in the total number of RSV cases, with more than 10 cases per week reported in week 23. This high number of RSV cases persisted consistently for approximately 2 months, as depicted in Figure 3C.

3.4 The impact of COVID-19 on trend of RSV infections in two prominent seasonalities

To assess the impact of COVID-19 on RSV infections, we stratified the overall cases based on the two seasons of RSV epidemics. In pre-COVID era (2017–2019), RSV exhibited its highest positivity rate during the first season, with a rate of 20.6%. However, with the emergence of COVID-19, we observed a significant decline in the percentage positivity of RSV during the same season, dropping to 8.3% (Table 2). This decline is further supported by our time series

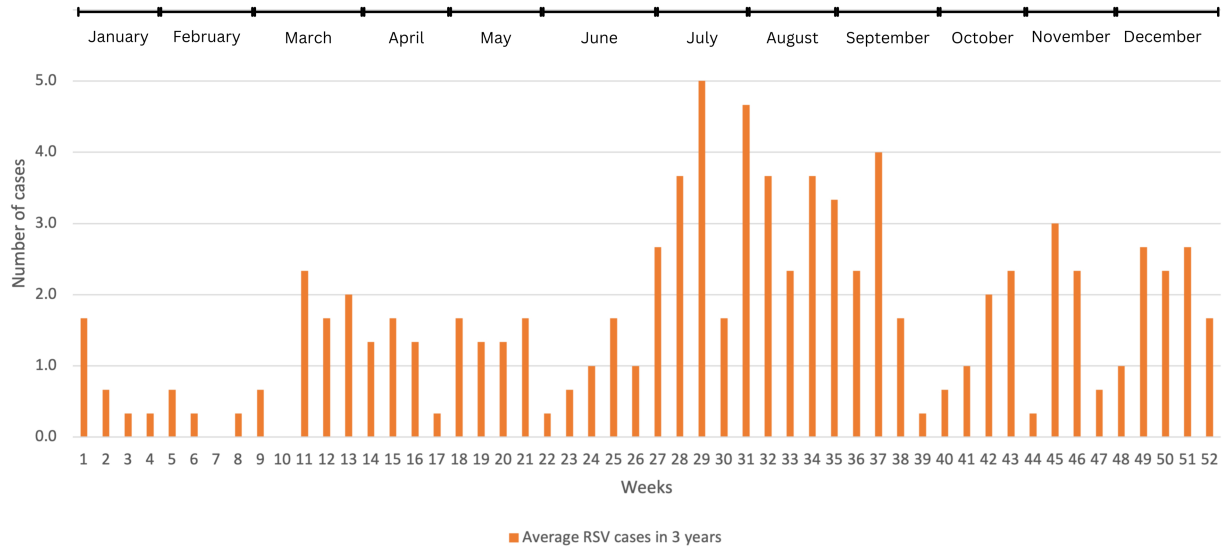

FIGURE 2
The average number of RSV cases before COVID-19 in weekly basis (2017–2019). We used descriptive analysis to compare the average number of RSV-positive cases within 52 weeks in these 3 years. The results were expressed as (*n*), where *n* represents the average number of cases.

model, indicating that the number of RSV cases in 2020 was 98.3% lower than the predicted value. A similar pattern was observed in the second season of 2020, resulting in 95.7% fewer cases than predicted.

As the restriction orders were gradually lifted in 2021, we observed a gradual increase in the trend of RSV infections. Towards the end of the year, total RSV infections peaked and surpassed the pre-COVID era, reaching a positivity rate of 22.2%. This realignment with the postulated trend resulted in the actual cases differed by 61.9% of the expected value, as shown in Table 2.

In 2022, when no movement restrictions were implemented, we observed the re-emergence of RSV with the recurrence of seasonality. Notably, the overall positivity rate of RSV during the first season of 2022 was significantly higher than the pre-COVID era, reaching a rate of 36.3%.

4 Discussion

Severe RSV infections have indirectly posed a substantial economic burden on healthcare systems, governments. and society (17). Many studies have shown that the disease burden extends beyond affected children, impacting caregivers, leading to a loss of work productivity and increased hospitalization costs (17). Butel et al. estimated an average total cost per patient of around EUR 2000 (equivalent to USD 2163) for the first episode of acute bronchiolitis, mainly attributed to hospitalization costs (18). Prior to COVID-19, RSV accounted for 5.4% of all detected positive respiratory pathogens in the United States between December 2019 and March 2020. However, during the implementation of public health measures to combat COVID-19, the RSV positivity rate dropped dramatically to 0.03% from December 2020 to March 2021 (19). Besides, studies in Spain and Germany reported that preventive measures implemented against COVID-19 resulted in fewer hospitalizations for RSV bronchiolitis during the autumn-winter season of 2020 to 2021 (20, 21). In Asia, Japanese investigators reported a significant reduction in RSV infections among children aged between 0 to 11 months, which had highest prevalence before the pandemic. South Korean researchers reported an 81 and 91% reduction in RSV-positive cases during 2020 (22, 23). Therefore, these findings align with a growing body of evidence suggesting that stringent public health measures can effectively reduce the spread of epidemic respiratory viruses (24).

In our latest epidemiological study, we identified two peaks of RSV seasonality occurring in distinct monsoon periods, specifically during July to August and October to December, coinciding with previous local and regional studies. (10, 25–27). Surprisingly, in this study, we observed an unusual increase of RSV cases throughout the first half of 2021. This is deemed as a global phenomenon as many countries reported a change in the seasonal variation of RSV during the COVID-19 pandemic (28). Typically, RSV infections peak during colder temperatures and reduced humidity, conditions favorable for the stability and transmission of the virus (10). However, in Shanghai, China, Ran Jia et al. documented an unusual increase in the RSV detection rate during the summer of 2021 (28). Interestingly, in Japan, there was a shift in RSV cases occurring in the spring of 2021, with a higher magnitude compared to the pre-COVID-19 period (29). Similarly, in Taiwan, Lee et al. observed a delay in the RSV season, with cases occurring during the winter of 2020–2021 instead of the usual peaks in spring and fall (30).

Several factors have been identified as responsible for the seasonality change in RSV and its unusual resurgence. Firstly, the relaxation of public health measures has revealed a strong association with increased RSV activity (31). The return of children to schools and the lifting of social gathering restrictions indirectly contribute to the transmission of RSV among children. Moreover, many studies have emphasized the role of adults as reservoirs for RSV, which was previously underestimated (31). During the COVID-19 period, public health measures were strictly implemented on older children and adults for better compliance than younger children (32). Consequently, while adults benefited from the easing of restrictions earlier in 2021, RSV cases increased significantly among younger children who remained

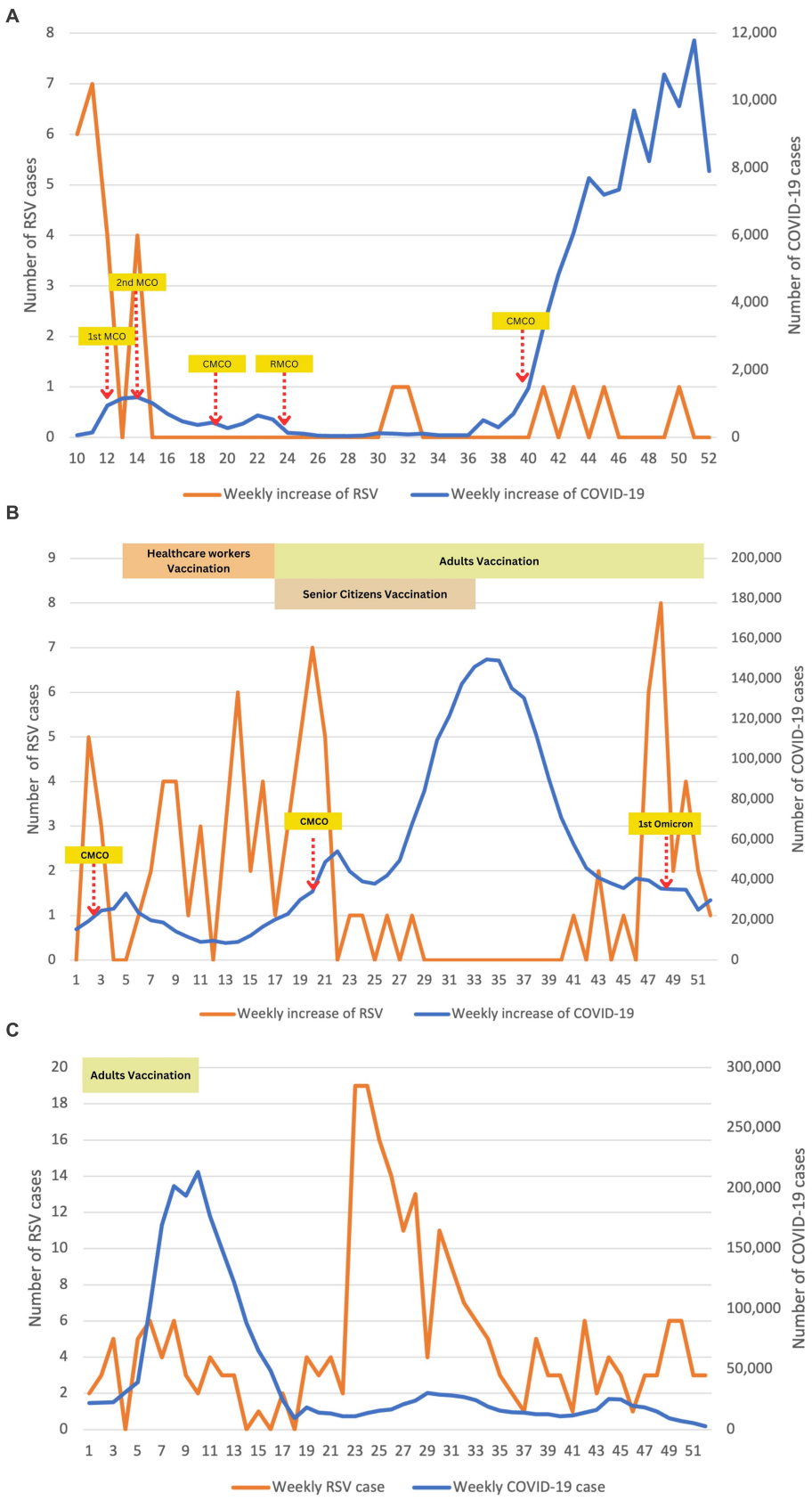

FIGURE 3
Epidemiological time-series plot of COVID-19 and RSV infections during COVID-19 (2020–2022). **(A)** Epidemiological data of COVID-19 and RSV in 2020. **(B)** Epidemiological data of COVID-19 and RSV in 2021. **(C)** Epidemiological data of COVID-19 and RSV in 2022. We performed descriptive analysis to demonstrate the weekly positive cases of RSV and COVID-19 during study periods from 2020 to 2022. Data were plotted on time-series

(Continued)

FIGURE 3 (Continued)
graphs to depict the relationship between the two viruses. Months (weeks) description: January (1–5); February (6–9); March (10–14); April (15–18); May (19–22); June (23–27); July (28–31); August (32–35); September (36–40); October (41–44); November (45–48); December (49–52). MCO, movement control order; CMCO, controlled movement control order; RMCO, recovery movement control order.

TABLE 2 Comparison of percentage positivity and time-series analysis for prediction of RSV cases in 3 years (2020–2022).

Year	Positivity rate[a] (%)	Actual number of cases	Predicted number of cases	Percentage difference[b] (%)	Upper limit (95% CI)	Lower limit (95% CI)	R^2
Week 26–31							
2017–2019	20.6						
2020	8.3	1	58	98.3	270	−153	0.715
2021	18.2	2	78	97.4	306	−149	
2022	36.3	62	98	63.3	341	−144	
Week 45–52							
2017–2019	14.8						
2020	5.9	2	47	95.7	165	−70	0.839
2021	22.2	24	63	61.9	189	−64	
2022	14.4	28	78	64.1	213	−57	

[a]Percentage positivity was calculated based on the total number of positive RSV cases and the total number of NPA samples collected. The rate was expressed in percentage (%). [b]Percentage difference was derived from analysis of two time series models between actual and predicted number of RSV cases in two seasons. The percentage difference was expressed in (%).

restricted due to closed childcare facilities. This raises speculation that adults play a major role in household chains of RSV transmission.

Secondly, the substantial decrease in protective immunity, termed as immunity debt, resulted from extended periods of low exposure to pathogens (33). The children's immune systems have now weakened due to reduced exposure to pathogens, a consequence of the public health response to the pandemic (34, 35). This immunity debt poses a particular concern for RSV in younger children, especially with the waning of maternal antibodies and a lack of seasonal exposure, rendering them susceptible to future and potentially more severe infections (35). In addition to public health measures, the phenomenon of viral interference may also help to explain the sudden disappearance of RSV in the context of COVID-19 (36). Briefly, it has long been hypothesized that respiratory viral infections can prevent superinfection of other respiratory pathogens through to the activation of innate immunity, mainly via interferon response. This is widely evidenced by the delay of Influenza Virus (H1N1) in 2009 during the first pandemic by Rhinovirus in September–October 2009 period (37). Accordingly, we agree with the hypothesis proposed by Raffaella et al. that the sharp decline in RSV circulation may have been partly contributed by the ongoing spread of the highly contagious and abundant COVID-19 Omicron variant surge in late 2021, particularly affecting unvaccinated children (36).

Although the implementation of public health measures has disrupted the transmission of RSV and COVID-19, it is unlikely that these measures can entirely eliminate the infections (31). One classic example to emulate is the Ebola Virus disease (EVD), where the African health authorities, despite being highly prepared to manage the endemic after several regional outbreaks over the last decade, still maintain extreme vigilance to avoid cross-border exportation of EVD and further international lockdowns triggered by COVID-19 (38). Moreover, it is crucial to highlight the importance of protecting immunocompromised children who are at a higher risk of RSV and other infections, while also being cautious of the ongoing prevalence and high contagiousness of COVID-19 within our communities (34, 39–42). To date, Palivizumab, a recombinant humanized monoclonal antibody targeting RSV F-protein, is the only passive immunization that could reduce the rate and severity of RSV infections when administered intramuscularly to children as pre-exposure prophylaxis (31). Over the years, Palivizumab has been clinically recommended for high-risk children under 2 years of age, including preterm infants and infants with congenital heart disease and chronic lung disease. Considering the shift in RSV seasonality following the emergence of COVID-19, the American Academy of Pediatrics (AAP) recommended more than five consecutive doses of Palivizumab for the best efficacy (43). Hence, it is imperative for clinicians to determine the optimal timing of RSV immunization, in light of the changed seasonality, as a promising pharmaceutical strategy for preventing RSV infections.

We have identified several limitations in our study. Firstly, due to the retrospective nature of the study spanning a period of 6 years, we were unable to ensure consistent nasal swab testing for all children with symptoms of ARTI. This may have resulted in underestimating the true burden of RSV infection, particularly during the COVID-19 pandemic period. Secondly, our reported data on COVID-19 cases were based on daily national statistics instead of state-focused data, which may limit the accuracy of assessing the relationship between COVID-19 and RSV cases in the exact locality. Nevertheless, it is worth noting that our study area, which covered Kuala Lumpur and Selangor, remained one of the highest contributors to daily COVID-19 cases throughout the three-year study period (44). Regretfully, we did not exclusively demonstrate pediatric COVID-19 infection as Malaysia was lagging in COVID-19 detection rate compared to other developed countries, thus the main catchment area was to aim at adults, rather than pediatric population. In addition, our study

focused solely on RSV infection, and we were unable to comprehensively assess the epidemiological characteristics of other common respiratory viruses before and during the pandemic. This includes the detection of any co-infections by our DFA kit, which could have influenced the clinical outcomes of the patients. Although our diagnostic RSV test used has commendable sensitivity (95.5%) and specificity (98.3%), false negatives and false positives of the results may affect the actual prevalence from what we reported. During the pandemic, COVID-19 was detected using real-time polymerase chain reaction (PCR), which have higher sensitivities than DFA detection of RSV, thus explained the large ratio between COVID-19 and RSV positive cases. The impact of these inaccuracies can vary depending on the context in which the test is used, the prevalence of RSV in the population, and the potential consequences of misdiagnosis.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

This study received ethical approval and support from the Secretariat of Research and Innovation Universiti Kebangsaan Malaysia (UKM) (Project code: JEP-2021-780).

Written informed consent for participation was not required for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AA: Conceptualization, Funding acquisition, Project administration, Supervision and Writing- review & editing; CM: Data curation, Formal analysis, Investigation, Methodology, Writing-original draft; AW: Conceptualization, Supervision, Writing- review & editing. All authors contributed to the manuscript revision, read, and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Garg I, Shekhar R, Sheikh AB, Pal S. Impact of COVID-19 on the changing patterns of respiratory syncytial virus infections. *Infect Dis Rep*. (2022) 14:558–68. doi: 10.3390/idr14040059

2. Pruccoli G, Castagno E, Raffaldi I, Denina M, Barisone E, Baroero L, et al. The importance of RSV epidemiological surveillance: a multicenter observational study of RSV infection during the COVID-19 pandemic. *Viruses*. (2023) 15:280. doi: 10.3390/v15020280

3. Pneumonia Etiology Research for Child Health (PERCH) Study Group. Causes of severe pneumonia requiring hospital admission in children without HIV infection from Africa and Asia: the PERCH multi-country case-control study. *Lancet*. (2019) 394:757–79. doi: 10.1016/S0140-6736(19)30721-4

4. Shi T, McAllister DA, O'Brien KL, Simoes EAF, Madhi SA, Gessner BD, et al. Global, regional, and national disease burden estimates of acute lower respiratory infections due to respiratory syncytial virus in young children in 2015: a systematic review and modelling study. *Lancet*. (2017) 390:946–58. doi: 10.1016/S0140-6736(17)30938-8

5. Wong JJM, Gan CS, Kaushal SH, Chuah SL, Sultana R, Tan NWH, et al. Pediatric COVID-19 risk factors in Southeast Asia-Singapore and Malaysia: a test-negative case-control study. *Am J Trop Med Hyg*. (2022) 106:1113–20. doi: 10.4269/ajtmh.21-1000

6. COVID-19 Excess Mortality Collaborators. Estimating excess mortality due to the COVID-19 pandemic: a systematic analysis of COVID-19-related mortality, 2020-21. 2022 16;399(10334):1468. *Lancet*. (2022) 399:1513–36. doi: 10.1016/S0140-6736(21)02796-3

7. Olsen SJ, Winn AK, Budd AP, Prill MM, Steel J, Midgley CM, et al. Changes in influenza and other respiratory virus activity during the COVID-19 pandemic - United States, 2020-2021. *MMWR Morb Mortal Wkly Rep*. (2021) 70:1013–9. doi: 10.15585/mmwr.mm7029a1

8. Saravanos GL, Hu N, Homaira N, Muscatello DJ, Jaffe A, Bartlett AW, et al. RSV epidemiology in Australia before and during COVID-19. *Pediatrics*. (2022) 149:e2021053537. doi: 10.1542/peds.2021-053537

9. Zhang M, Gao J, Guo Q, Zhang X, Zhang W. Changes of respiratory syncytial virus infection in children before and after the COVID-19 pandemic in Henan. *China J Infect*. (2023) 86:154–225. doi: 10.1016/j.jinf.2022.12.011

10. Chan CM, Wahab AA, Ali A. Determining the relationship of meteorological factors and severe pediatric respiratory syncytial virus (RSV) infection in central peninsular Malaysia. *Int J Environ Res Public Health*. (2023) 20:1848. doi: 10.3390/ijerph20031848

11. Saif MAM, Hussin N, Husin MM, Alwadain A, Chakraborty A. Determinants of the intention to adopt digital-only banks in Malaysia: the extension of environmental concern. *Sustain For*. (2022) 14:11043. doi: 10.3390/su141711043

12. Annual Report Hospital Canselor Tuanku Muhriz (HCTM). (2021). Available at: https://hctm.ukm.my/en/laporan-tahunan/ (Accessed May 25, 2023).

13. D3 Ultra DFA. Respiratory Virus Screening and Identification Kit. Package Insert. Quidel. (2010). Available at: https://www.quidel.com/sites/default/files/product/documents/PI1670001EN00_%2805_19%29_D3_Ultra_DFA_Respiratory_Virus_Screen_%26_ID_Kit.pdf (Accessed November 29, 2022)

14. Ministry of Health Malaysia (2023). Number of Daily Confirmed Cases of the Novel Coronavirus Infection (Covid-19) in Malaysia. Statista Research Department. Available at: https://www.statista.com/statistics/1110785/malaysia-covid-19-daily-cases/ (Accessed May 25, 2023).

15. Jayaraj VJ, Rampal S, Ng CW, Chong DWQ. The epidemiology of COVID-19 in Malaysia. *Lancet Reg Health West Pac*. (2021) 17:100295. doi: 10.1016/j.lanwpc.2021.100295

16. Hashim JH, Adman MA, Hashim Z, Mohd Radi MF, Kwan SC. COVID-19 epidemic in Malaysia: epidemic progression, challenges, and response. *Front Public Health*. (2021) 9:560592. Published 2021 May 7. doi: 10.3389/fpubh.2021.560592

17. Gastaldi A, Donà D, Barbieri E, Giaquinto C, Bont LJ, Baraldi E. COVID-19 lesson for respiratory syncytial virus (RSV): hygiene works. *Children (Basel)*. (2021) 8:1144. doi: 10.3390/children8121144

18. Butel T, Angoulvant F, Filipovic-Pierucci A, Milcent K, Teglas JP, Bellêttre X, et al. Clinical course and cost assessment of infants with a first episode of acute bronchiolitis presenting to the emergency department: data from the GUERANDE clinical trial. *Pediatr Pulmonol*. (2021) 56:3802–12. doi: 10.1002/ppul.25621

19. Uhteg K, Amadi A, Forman M, Mostafa HH. Circulation of non-SARS-CoV-2 respiratory pathogens and coinfection with SARS-CoV-2 amid the COVID-19 pandemic. *Open Forum Infect Dis*. (2021) 9:ofab618. doi: 10.1093/ofid/ofab618

20. Bermúdez Barrezueta L, Matías Del Pozo V, López-Casillas P, Brezmes Raposo M, Gutiérrez Zamorano M, Pino Vázquez MA. Variation in the seasonality of the respiratory syncytial virus during the COVID-19 pandemic. *Infection*. (2022) 50:1001–5. doi: 10.1007/s15010-022-01794-y

21. Stamm P, Sagoschen I, Weise K, Plachter B, Münzel T, Gori T, et al. Influenza and RSV incidence during COVID-19 pandemic-an observational study from in-hospital point-of-care testing. *Med Microbiol Immunol*. (2021) 210:277–82. doi: 10.1007/s00430-021-00720-7

22. Wagatsuma K, Koolhof IS, Shobugawa Y, Saito R. Decreased human respiratory syncytial virus activity during the COVID-19 pandemic in Japan: an ecological time-series analysis. *BMC Infect Dis*. (2021) 21:734. doi: 10.1186/s12879-021-06461-5

23. Kim JH, Roh YH, Ahn JG, Kim MY, Huh K, Jung J, et al. Respiratory syncytial virus and influenza epidemics disappearance in Korea during the 2020-2021 season of COVID-19. *Int J Infect Dis*. (2021) 110:29–35. doi: 10.1016/j.ijid.2021.07.005

24. Britton PN, Hu N, Saravanos G, Shrapnel J, Davis J, Snelling T, et al. COVID-19 public health measures and respiratory syncytial virus. *Lancet Child Adolesc Health*. (2020) 4:e42–3. doi: 10.1016/S2352-4642(20)30307-2

25. Teck KS, Mac Guad R, Van Rostenberghe AH, Hua GS. Prevalence, risk factors and clinical characteristics of res-piratory syncytial virus-associated lower respiratory tract infections in Kelantan. *Malays J Med Virol*. (2019) 91:1608–15. doi: 10.1002/jmv.25500

26. Khor CS, Sam IC, Hooi PS, Quek KF, Chan YF. Epidemiology and seasonality of respiratory viral infections in hospitalized children in Kuala Lumpur, Malaysia: a retrospective study of 27 years. *BMC Pediatr*. (2012) 12:32. doi: 10.1186/1471-2431-12-32

27. Thongpan I, Vongpunsawad S, Poovorawan Y. Respiratory syncytial virus infection trend is associated with meteoro-logical factors. *Sci Rep*. (2022) 10:10931. doi: 10.1038/s41598-020-67969-5

28. Jia R, Lu L, Su L, Lin Z, Gao D, Lv H, et al. Resurgence of respiratory syncytial virus infection during COVID-19 pandemic among children in Shanghai. *China Front Microbiol*. (2022) 13:938372. doi: 10.3389/fmicb.2022.938372

29. Ujiie M, Tsuzuki S, Nakamoto T, Iwamoto N. Resurgence of respiratory syncytial virus infections during COVID-19 pandemic, Tokyo. *Japan Emerg Infect Dis*. (2021) 27:2969–70. doi: 10.3201/eid2711.211565

30. Lee CY, Wu TH, Fang YP, Chang JC, Wang HC, Lin SJ, et al. Delayed respiratory syncytial virus outbreak in 2020 in Taiwan was correlated with two novel RSV-A genotype ON1 variants. *Influenza Other Respir Viruses*. (2022) 16:511–20. doi: 10.1111/irv.12951

31. Chuang YC, Lin KP, Wang LA, Yeh TK, Liu PY. The impact of the COVID-19 pandemic on respiratory syncytial virus infection: a narrative review. *Infect Drug Resist*. (2023) 16:661–75. doi: 10.2147/IDR.S396434

32. Fourgeaud J, Toubiana J, Chappuy H, Delacourt C, Moulin F, Parize P, et al. Impact of public health measures on the post-COVID-19 respiratory syncytial virus epidemics in France. *Eur J Clin Microbiol Infect Dis*. (2021) 40:2389–95. doi: 10.1007/s10096-021-04323-1

33. Cohen R, Pettoello-Mantovani M, Somekh E, Levy C. European pediatric societies call for an implementation of regular vaccination programs to contrast the immunity debt associated to coronavirus Disease-2019 pandemic in children. *J Pediatr*. (2022) 242:260–261.e3. doi: 10.1016/j.jpeds.2021.11.061

34. Needle RF, Russell RS. Immunity debt, a gap in learning, or immune dysfunction? *Viral Immunol*. (2023) 36:1–2. doi: 10.1089/vim.2022.0204

35. Hatter L, Eathorne A, Hills T, Bruce P, Beasley R. Respiratory syncytial virus: paying the immunity debt with interest. *Lancet Child Adolesc Health*. (2021) 5:e44–5. doi: 10.1016/S2352-4642(21)00333-3

36. Nenna R, Matera L, Licari A, Manti S, Di Bella G, Pierangeli A, et al. An Italian multicenter study on the epidemiology of respiratory syncytial virus during SARS-CoV-2 pandemic in hospitalized children. *Front Pediatr*. (2022) 10:930281. doi: 10.3389/fped.2022.930281

37. Wu A, Mihaylova VT, Landry ML, Foxman EF. Interference between rhinovirus and influenza a virus: a clinical data analysis and experimental infection study. *Lancet Microbe*. (2020) 1:e254–62. doi: 10.1016/s2666-5247(20)30114-2

38. Al-Tammemi AB, Sallam M, Rebhi A, Soliman L, Sarayrih LA, Tarhini Z, et al. The outbreak of Ebola virus disease in 2022: a spotlight on a re-emerging global health menace. *Narra J*. (2022) 2:e97. doi: 10.52225/narra.v2i3.97

39. Solís L, Nordin J, Prevot J, Mahlaoui N, Sánchez-Ramón S, Ali A, et al. The PID life index: an interactive tool to measure the status of the PID healthcare environment in any given country. *Orphanet J Rare Dis*. (2022) 17:11. doi: 10.1186/s13023-021-02161-0

40. Chan CM, Mahlaoui N, San'chez-Ramo'n S, Pergent M, Solis L, Prevot J, et al. Primary immunodeficiencies (PID) life index in Southeast Asia: a comparative analysis of PID principles of care (PoC). *Front Immunol*. (2023) 14:1151335. doi: 10.3389/fimmu.2023.1151335

41. Nordin J, Solís L, Prévot J, Mahlaoui N, Chapel H, Sánchez-Ramón S, et al. The PID principles of care: where are we now? A global status report based on the PID life index. *Front Immunol*. (2021) 12:780140. doi: 10.3389/fimmu.2021.780140

42. Adli A, Wahab AA, Abdul Latiff AH, Ismail IH, Zaki FM, Borhanuddin BK. Clinical and laboratory observation on immunoglobulin replacement therapy switching from an intravenous to a subcutaneous route in a Malaysian X-linked agammaglobulinemia patient. *Med J Malaysia*. (2022) 77:95–7.

43. American Academy of Pediatrics. (2023). Updated Guidance: Use of Palivizumab Prophylaxis to Prevent Hospitalization from Severe Respiratory Syncytial Virus Infection during the 2022–2023 RSV Season. Available from: https://www.aap.org/en/pages/2019-novel-coronavirus-covid-19-infections/clinical-guidance/interim-guidance-for-use-of-palivizumab-prophylaxis-to-prevent-hospitalization/. (Accessed January 18, 2023)

44. Tang KHD. Movement control as an effective measure against Covid-19 spread in Malaysia: an overview. *Z Gesundh Wiss*. (2022) 30:583–6. doi: 10.1007/s10389-020-01316-w

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The potential risks of equine serum therapy in transmitting new infectious diseases: lessons from a post-pandemic era

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KEYWORDS

infectious diseases, viral transmission, pandemic risks, infection control measures, health crisis, heterologous serum therapy

In the world of medical treatments, certain interventions carry hidden risks that are not always readily apparent. The use of equine serum in human therapies has raised substantial concerns, often overlooking, or minimizing the potential risks associated with these interventions. One prime example is the use of equine-derived antivenoms, crucial in treating venomous animal envenomations, such as those caused by snakes, scorpions, and spiders. A primary concern centers around the immunogenicity of equine serum components upon introduction into the human body. This can provoke immune responses ranging from mild allergic reactions to serum sickness and severe anaphylaxis, necessitating immediate medical intervention (1).

Likewise, equine serum-derived treatments may carry the risk of transmitting infections or diseases from the horse to the human recipient. The purification process involves pasteurizing horse IgGs. Typically, pasteurization occurs in the presence of stabilizers such as amino acids, sugars, or citrate to preserve protein functionality, preventing molecular changes and protein aggregation. These stabilizers also contribute to fortifying against viruses, underscoring the need to validate treatment conditions. Pasteurization can effectively deactivate a variety of viruses, both enveloped and non-enveloped (e.g., HIV, HBV, HCV, and HAV). However, there is limited data on the inactivation of resistant non-enveloped viruses like porcine parvovirus, SV 40, or reovirus type 3 in plasma products (2, 3). Thus, pasteurization has proven itself the only effective step toward assuring the virus safety of final product (4), although it is a process that do not have deliberately introduced viral inactivation, that could result in the parenteral transmission of zoonotic diseases. Caprylic acid treatment, formulation at acidic pH, and ion-exchange chromatography represent additional purification steps employed in the process. While these methods can effectively remove viruses from the serum, it's worth noting that they may not be as robust or comprehensive in their virus-removal capabilities (3, 5, 6).

Recent occurrences of zoonotic diseases serve as indicators of the interplay between humans and the reservoirs of biological agents harbored by animals (7). Moreover, these events underscore the inherent perils associated with the emergence of novel diseases such as the human immunodeficiency virus (HIV), Hantaan, Lassa, Ebola, Nipah, and a variety of paramyxoviruses. Furthermore, the list expands to encompass the equine morbilli virus, the West Nile virus, and notably, the strong likelihood of the inclusion of the severe acute respiratory syndrome coronavirus, including SARS-CoV-2 (8, 9). It is also important to highlight the actual endemic status of hepatitis E (HEV), which represents

a significant public health concern. The transmission dynamics of this disease have been found to extend beyond conventional routes, potentially including blood transfusions as an important mode of spread (10).

Burnouf et al. (3) have put forward a list of 19 viruses, with the capacity to cause diseases in horses, and notably, 11 of these can also induce diseases in humans. The shared characteristics, encompassing attributes such as being enveloped or non-enveloped, DNA or RNA-based, etc., are depicted in Figure 1. Notably, Bornouf has proposed to include screening for at least these specific pathogens for sera derived from horses; however, these recommendations have not been implemented to date (3).

In fact, diseases like Eastern equine encephalitis (EEE), Western equine encephalitis (WEE), and Venezuelan-equine encephalitis (VEE) are highly infectious, spreading through aerosols. Venezuela and Colombia have seen continuous, fatal cases of VEE-induced encephalitis in horses and humans (11). On the other hand, Vesicular Stomatitis (VS), common among North American horses, poses zoonotic risks by causing encephalitis in children (12); while Hendra Virus (HeV) leads to respiratory and neurological diseases, fatal for humans and horses (13). West Nile fever has recently spread across new territories globally (14), being considered a epizootic emergence (15). Although rare in horses, rabies remains a grave public health concern (16, 17), in contrast with Equine Influenza, which apparently do not affect humans (18). Regarding SARS-CoV-2, although there is little evidence for horse natural infection (19), it was evidenced that a COVID-19 patient infected a horse, demonstrating in the horse the seroconversion following dayle contact during the development of clinical disease (20).

However, the diseases mentioned earlier are only the ones we're aware of; there could be numerous others being transmitted. In fact, it is estimated that 60% of emerging infectious diseases that are reported globally are zoonoses and over 30 new human pathogens have been detected in the last three decades, 75% of which have originated in animals (21).

This situation brings up a puzzling question: If we're not sure about the different diseases that could be in equine-derived serum, how can we know what illnesses might spread from it? Screening equine-derived serum for potential diseases with zoonotic implications may requires a multifaceted approach encompassing various techniques, including virus-specific PCR assays, serological tests, metagenomic sequencing, mass spectrometry, viral culture, next-generation sequencing (NGS), microarray analysis, immunofluorescence assays (IFA), proteomic analysis, nucleic acid amplification techniques (NAAT), cell culture-based assays, bioinformatics and computational analysis, lateral flow assays, digital PCR, and/or biosensors. As they are not applied so far, using serum from horses might not just bring new diseases to human but also create big outbreaks like the recent pandemic of COVID-19. This careless use of animal-based serums, such horses, is like "playing a risky game with public health". Not being careful with these treatments ignores lessons from history and science.

We must not overlook the absence of transparency concerning these risks for patients and their families. Patients have the right to informed decision-making about their treatment choices, empowered by a thorough grasp of potential advantages and risks. And in this context, no information beyond the most common

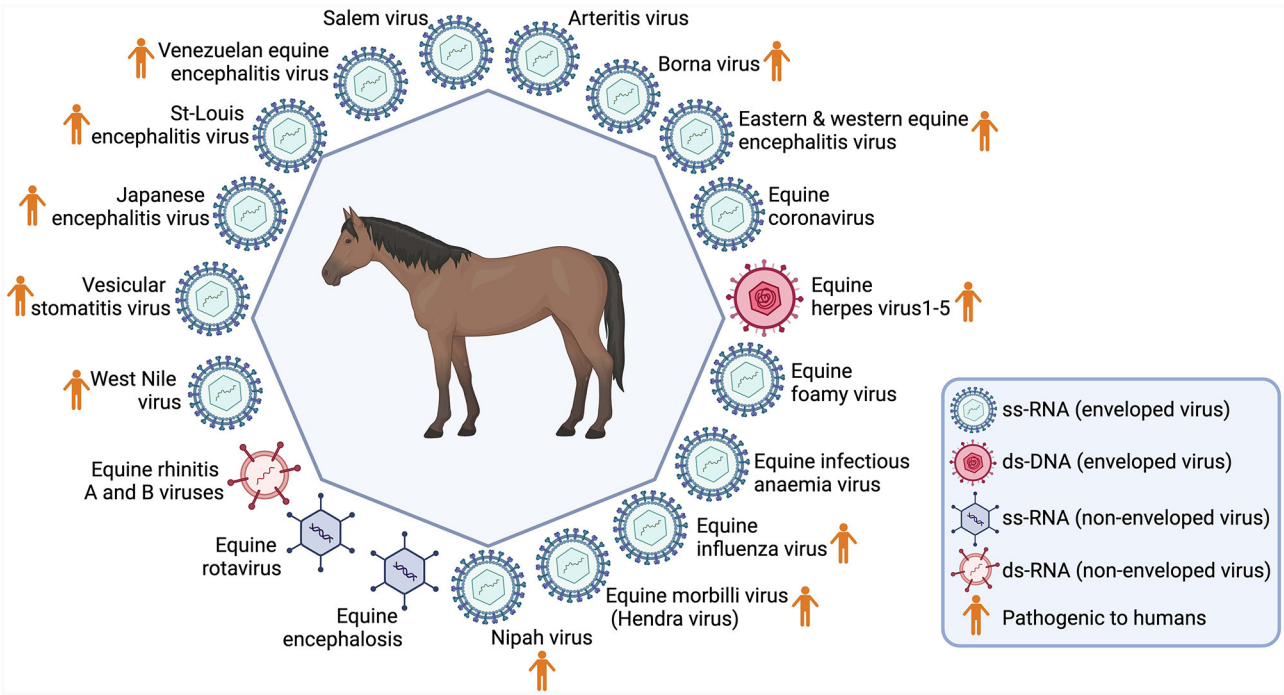

FIGURE 1
Main viruses identified in horses. Created with Biorender.com.

and rare side effects (i.e.,; allergies, serum sickness, fever, and anaphylaxis), that include the risks of contracting diseases, is described in the antivenom's label (22).

In the midst of the 21st century, with unprecedented achievements in science and pharmaceuticals, and with the glaring lessons imparted by the COVID-19 pandemic, one might wonder how we continue to endorse the archaic practice of using equine serum as a therapeutic option.

In the pursuit of alternatives to serum derived from horses, notable progress has been achieved, with a particularly promising pathway being the utilization of human monoclonal antibodies generated through phage display technology (23). The production of human monoclonal antibodies via phage display offers distinct advantages, including diminished immunogenicity and the capacity to customize antibodies for therapeutic applications (1). This methodology not only reduces dependence on equine serum but also provides a more individualized and human-centric solution, underscoring the transformative potential of cutting-edge technologies in advancing biotechnological alternatives (24). Currently, numerous researchers are actively exploring monoclonal antibodies and other alternatives to replace serum derived from horses (25–32).

While medical breakthroughs surge ahead, it is disconcerting that we overlook the pressing need to rigorously scrutinize the safety of treatments, particularly those produced by animal sources. Ignoring this matter not only dismisses the significant advancements in medical understanding but also puts the fundamental aspects of human health and wellbeing in danger.

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References

1. Laustsen AH, María Gutiérrez J, Knudsen C, Johansen KH, Bermúdez-Méndez E, Cerni FA, et al. Pros and cons of different therapeutic antibody formats for recombinant antivenom development. *Toxicon.* (2018) 146:151–75. doi: 10.1016/j.toxicon.2018.03.004

2. Burnouf-Radosevich M, Burnouf T, Huart JJ. A pasteurized therapeutic plasma. *Infusionsther Transfusionsmed.* (1992) 19:91–4. doi: 10.1159/000222590

3. Burnouf T, Griffiths E, Padilla A, Seddik S, Stephano MA, Gutiérrez J-M. Assessment of the viral safety of antivenoms fractionated from equine plasma. *Biologicals.* (2004) 32:115–28. doi: 10.1016/j.biologicals.2004.07.001

4. Gröner A, Broumis C, Fang R, Nowak T, Popp B, Schäfer W, et al. Effective inactivation of a wide range of viruses by pasteurization. *Transfusion.* (2018) 58:41–51. doi: 10.1111/trf.14390

5. Horowitz B, Piët MP, Prince AM, Edwards CA, Lippin A, Walakovits LA. Inactivation of lipid-enveloped viruses in labile blood derivatives by unsaturated fatty acids. *Vox Sang.* (1988) 54:14–20. doi: 10.1159/000461754

6. Burnouf T, Radosevich M. Affinity chromatography in the industrial purification of plasma proteins for therapeutic use. *J Biochem Biophys Methods.* (2001) 49:575–86. doi: 10.1016/S0165-022X(01)00221-4

7. Ivan A, Indrei LL. Emergence of transmissible disorders, a continuous process-a new type of viral meningoencephalitis. *Rev Med Chir Soc Med Nat Iasi.* (2000) 104:51–5.

8. Jo WK, de Oliveira-Filho EF, Rasche A, Greenwood AD, Osterrieder K, Drexler JF. Potential zoonotic sources of SARS-CoV-2 infections. *Transbound Emerg Dis.* (2021) 68:1824–34. doi: 10.1111/tbed.13872

9. Kaaden OR, Eichhorn W, Essbauer S. Recent developments in the epidemiology of virus diseases. *J Vet Med B Infect Dis Vet Public Health.* (2002) 49:3–6. doi: 10.1046/j.1439-0450.2002.00530.x

10. Arankalle VA, Chobe LP. Retrospective analysis of blood transfusion recipients: evidence for post-transfusion hepatitis E. *Vox Sang.* (2000) 79:72–4. doi: 10.1046/j.1423-0410.2000.7920072.x

11. Roy CJ, Reed DS, Wilhelmsen CL, Hartings J, Norris S, Steele KE. Pathogenesis of aerosolized Eastern Equine Encephalitis virus infection in guinea pigs. *Virol J.* (2009) 6:170. doi: 10.1186/1743-422X-6-170

12. Monath TP, Emerson JK, Kemp GE, Poland JD, Cholas G, Webb PA, et al. Epizootic vesicular stomatitis in Colorado, 1982: infection in occupational risk groups. *Am J Trop Med Hyg.* (1987) 36:177–82. doi: 10.4269/ajtmh.1987.36.177

13. Epstein JH, Baker ML, Zambrana-Torrelio C, Middleton D, Barr JA, DuBovi E, et al. Duration of maternal antibodies against canine distemper virus and hendra virus in pteropid bats. *PLoS ONE.* (2013) 8:e67584. doi: 10.1371/journal.pone.0067584

14. Chancey C, Grinev A, Volkova E, Rios M. The global ecology and epidemiology of West Nile Virus. *Biomed Res Int.* (2015) 2015:1–20. doi: 10.1155/2015/376230

15. Ziegler U, Santos PD, Groschup MH, Hattendorf C, Eiden M, Höper D, et al. West Nile virus epidemic in Germany triggered by epizootic emergence, 2019. *Viruses.* (2020) 12:448. doi: 10.3390/v12040448

16. Araujo FAA. *Raiva Humana no Brasil, 1992 - 2001*. (2002). Available online at: https://repositorio.ufmg.br/handle/1843/BUOS-8BWGGJ (accessed December 22, 2023).

17. Green SL, Smith LL, Vernau W, Beacock SM. Rabies in horses: 21 cases (1970-1990). *J Am Vet Med Assoc.* (1992) 200:1133–7. doi: 10.2460/javma.1992.200.08.1133

18. Kumar B, Manuja A, Gulati B, Virmani N, Tripathi BN. Zoonotic viral diseases of equines and their impact on human and animal health. *Open Virol J.* (2018) 12:80–98. doi: 10.2174/1874357901812010080

19. Legere RM, Allegro AR, Affram Y, da Silveira BP, Fridley JL, Wells KM, et al. Equine bronchial epithelial cells are susceptible to cell entry with a SARS-CoV-2 pseudovirus but reveal low replication efficiency. *Am J Vet Res.* (2023) 84:ajvr.23.06.0132. doi: 10.2460/ajvr.23.06.0132

20. Pusterla N, Chaillon A, Ignacio C, Smith DM, Barnum S, Lawton KOY, et al. SARS-CoV-2 seroconversion in an adult horse with direct contact to a COVID-19 individual. *Viruses.* (2022) 14:1047. doi: 10.3390/v14051047

21. Jones KE, Patel NG, Levy MA, Storeygard A, Balk D, Gittleman JL, et al. Global trends in emerging infectious diseases. *Nature.* (2008) 451:990–3. doi: 10.1038/nature06536

22. Butantan. *Portal do Butantan. Soros e Bulas*. (2023). Available online at: https://butantan.gov.br/soros-e-vacinas/soros (accessed August 31, 2023).

23. McCafferty J, Griffiths AD, Winter G, Chiswell DJ. Phage antibodies: filamentous phage displaying antibody variable domains. *Nature.* (1990) 348:552–4. doi: 10.1038/348552a0

24. Camphora A, Pucca M. Horses used for large-scale production of immunoglobulins: an inter-species approach. *Int Anim Health J.* (2022) 8:12–6. Available online at: https://international-animalhealth.com/horses-used-for-large-scale-production-of-immunoglobulins-an-inter-species-approach/

25. Pucca MB, Cerni FA, Peigneur S, Arantes EC, Tytgat J, Barbosa JE. Serrumab: a novel human single chain-fragment antibody with multiple scorpion toxin-neutralizing capacities. *J Immunotoxicol.* (2014) 11:133–40. doi: 10.3109/1547691X.2013.809175

26. Ahmadi S, Pucca MB, Jürgensen JA, Janke R, Ledsgaard L, Schoof EM, et al. An *in vitro* methodology for discovering broadly-neutralizing monoclonal antibodies. *Sci Rep.* (2020) 10:10765. doi: 10.1038/s41598-020-67654-7

27. Knudsen C, Ledsgaard L, Dehli RI, Ahmadi S, Sørensen CV, Laustsen AH. Engineering and design considerations for next-generation snakebite antivenoms. *Toxicon.* (2019) 167:67–75. doi: 10.1016/j.toxicon.2019.06.005

28. Sørensen CV, Fernández J, Adams AC, Wildenauer HHK, Schoffelen S, Ledsgaard L, et al. Antibody-dependent enhancement of toxicity of myotoxin II from Bothrops asper. *Nat Commun.* (2024) 15:173. doi: 10.1038/s41467-023-42624-5

29. Føns S, Ledsgaard L, Nikolaev MV, Vassilevski AA, Sørensen CV, Chevalier MK, et al. Discovery of a recombinant human monoclonal immunoglobulin G antibody against α-latrotoxin from the mediterranean Black Widow Spider (*Latrodectus tredecimguttatus*). *Front Immunol.* (2020) 11:587825. doi: 10.3389/fimmu.2020.587825

30. Ledsgaard L, Wade J, Jenkins TP, Boddum K, Oganesyan I, Harrison JA, et al. Discovery and optimization of a broadly-neutralizing human monoclonal antibody against long-chain α-neurotoxins from snakes. *Nat Commun.* (2023) 14:682. doi: 10.1038/s41467-023-36393-4

31. Campos LB, Pucca MB, Silva LC, Pessenda G, Filardi BA, Cerni FA, et al. Identification of cross-reactive human single-chain variable fragments against phospholipases A2 from Lachesis muta and Bothrops spp venoms. *Toxicon.* (2020) 184:116–21. doi: 10.1016/j.toxicon.2020.05.027

32. Albulescu L-O, Xie C, Ainsworth S, Alsolaiss J, Crittenden E, Dawson CA, et al. A therapeutic combination of two small molecule toxin inhibitors provides broad preclinical efficacy against viper snakebite. *Nat Commun.* (2020) 11:6094. doi: 10.1038/s41467-020-19981-6

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Impact of normalized COVID-19 prevention and control measures on lower respiratory tract infection pathogenesis in hospitalized children

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Objective: This study aimed to investigate the epidemiological characteristics of common pathogens contributing to childhood lower respiratory tract infections (LRTIs) in Xiangtan City, Hunan Province before and during the coronavirus disease 2019 (COVID-19) pandemic.

Methods: A total of 11,891 enrolled patients, aged 1 month to 14 years, diagnosed with LRTIs and admitted to Xiangtan Central Hospital from January 2018 to December 2021 were retrospectively reviewed in this study. Specifically, the epidemiological characteristics of these pathogens before and during the COVID-19 pandemic were analyzed.

Results: There was a significant decrease in the number of children hospitalized with LRTIs during the COVID-19 pandemic (2020–2021) compared to data from 2018 to 2019 (before the COVID-19 pandemic). Of these cases, 60.01% (7,136/11,891) were male and 39.99% (4,755/11,891) were female. 78.9% (9,381/11,891) cases occurred in children under 4years of age. The average pathogen detection rate among 11,891 hospitalized LRTIs children was 62.19% (7,395/11,891), with the average pathogen detection rate of 60.33% (4,635/7,682) and 65.57% (2,670/4,209) before and during COVID-19 pandemic, respectively. The detection rates of adenovirus (ADV), *bordetella pertussis* (BP) and *moraxella catarrhalis* (*M. catarrhalis*) decreased dramatically, while the detection rates of influenza viruses (IFV), parainfluenza viruses (PIV), respiratory syncytial virus (RSV), *haemophilus influenzae* (*H. influenzae*), *streptococcus pneumoniae* (*S. pneumoniae*), and *staphylococcus aureus* (*S. aureus*) increased significantly during the COVID-19 pandemic. Overall, RSV, *mycoplasma pneumoniae* (MP), *H. influenzae*, and IFV were the major pathogens causing LRTIs in hospitalized children before and during the COVID-19 pandemic.

Conclusion: Public health interventions for COVID-19 prevention are beneficial to reduce the incidence of LRTIs in children by limiting the prevalence of ADV, MP, BP, and *M. catarrhalis*, but which have limited restrictive effects on other common LRTIs-associated pathogens. Collectively, the data in this study comprehensively investigated the effects of COVID-19 pandemic on the epidemiological characteristics of respiratory pathogens, which will be beneficial for improving early preventive measures.

KEYWORDS

COVID-19 pandemic, respiratory pathogens, epidemiological characteristics, children, lower respiratory tract infection

1 Introduction

Lower respiratory tract infections (LRTIs) are responsible for a high level of morbidity and mortality in humans, which have been considered one of the most significant factors threatening public health worldwide (1). The rapid spread of LRTIs-related pathogens within a short time are frequently observed in some specific occasions (e.g., kindergarten and playground), owing to their high contagion (2). The outbreak or occurrence of LRTIs can be caused by a variety of pathogens, including viruses, bacteria, and *mycoplasma pneumoniae* (MP), while the epidemiological characteristics of these infectious agents vary from regions, seasons, and other factors (3). Thus, updating the information on the epidemiological features of LRTIs-related pathogens is essential for the early diagnosis and treatment of this disease.

Since the end of December 2019, a novel human coronavirus, called the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), emerged in Wuhan city, China (4). Subsequently, the disease (coronavirus disease 2019, COVID-19) caused by SARS-CoV-2 has been documented in all provinces or regions in China, the threats of which to public health received widespread concern (5). In view of these, a variety of strict non-pharmaceutical interventions (NPIs) measures were performed to limit the spread of SARS-CoV-2 nationwide, and which effectively restricted the prevalence of this infectious virus and saved the lives of thousands of people in China (6).

During the COVID-19 pandemic in Shanghai city of China in 2020, the decreased detection rates of Human rhinovirus (HRV), Human parainfluenza virus (HPIV), *Haemophilus influenzae* (*H. influenzae*), and MP, and the increased detection rates of Influenza B virus (FluB) and most of tested bacteria [including *Staphylococcus aureus* (*S. aureus*), *Escherichia coli* (*E. coli*), and *Klebsilla pneumoniae* (*K. pneumoniae*)] were observed compared with these in 2019 (7). In western China (Gansu, Qinghai, Xinjiang, and Inner Mongolia), the positive rates of influenza virus (IFV), and *Streptococcus pneumoniae* (*S. pneumoniae*) among patients with acute respiratory infections decreased, but other types of viruses and bacteria showed higher prevalence tendency during the COVID-19 pandemic (8). In Henan province of China, the positive detection rates of IFV, and human metapneumovirus (HMPV), and human respiratory syncytial virus (HRSV) decreased sharply, and the detection rates of HRV and human bocavirus significantly increased (9). These data suggested that NPIs have broad effects on the transmission of these respiratory pathogens, while the changes of different respiratory pathogens before and during COVID-19 pandemic varied in different regions (10, 11).

This study conducted a retrospective study from 2018 to 2021 to analyze the epidemiological characteristics of pathogen-associated LRTIs among inpatients (aged 1 month to 14 years) in Xiangtan Central Hospital, Hunan Province, China. In particular, the impact of the COVID-19 pandemic on the number of LRTIs-positive patients, the type of pathogens and their epidemiological characteristics were studied. The results will provide references for clinical diagnosis and treatment, and to help formulate strategies for the prevention and control of respiratory infections in children in the public health sector.

2 Methods

2.1 Study design

From January 2018 to December 2021, all hospitalized children from Xiangtan Central Hospital diagnosed with LRTIs were included in this retrospective study. This was a retrospective study that followed ethical standards, obtained informed consent from the children's families, and was approved by the Ethics Committee of Xiangtan Central Hospital (NO. 2023-KC-58-09-019).

Inclusion criteria: (1) Meet the diagnostic criteria for LRTIs according to the 8th edition of Zhufutang Practical Paediatrics (12); (2) Age from 1 month to 14 years; (3) The clinical samples from inpatients were collected for pathogen detection.

Exclusion criteria: (1) Presence of congenital airway malformations such as esophageal tracheal atresia and congenital tracheal chondrodysplasia; (2) Presence of intracranial infections, congenital immunodeficiencies, leukemias, and other systemic disorders as primary diagnosis; (3) Acute exacerbation of bronchial asthma with normal chest imaging.

2.2 Specimen collection

Nasal or throat swabs from enrolled patients were collected individually at the collection site within 24 h and then sent to the clinical laboratory center for antigenic or nucleic acid testing for common pathogens, including influenza A and B viruses (FluA and FluB), adenovirus (ADV), HPIV 1–3, HRSV, *Bordetella pertussis* (BP), and MP. Sputum samples were also collected for bacterial cloning and molecular characterization.

2.3 Pathogens detection

A multiplex direct immunofluorescence assay kit (Diagnostic Hybrids, Inc., United States and Bierce Spain Ltd.) was used to simultaneously detect the fluorescent antigens of RSV, ADV, PIV-1, PIV-2, PIV-3, Flu A, and Flu B. Using the commercially available reagent kits to individually extract total nucleic acid from collected samples and perform individual pathogen nucleic acid testing (Shenzhen Yicubic Biotechnology Co., Guangzhou Da'an Gene Co., Shengxiang Biotechnology Co., China).

The sputum samples were mixed with sterilized normal saline. The supernatants were then collected and plated on Columbia agar plates containing 5% defibrinated sheep blood. After 24 h of incubation at 37°C and 5% CO2, individual colonies on the plate were selected and purified. Finally, the 16S rRNA gene of the purified isolates was amplified by PCR using primers 27F 5′-AGAGTTT GATCCTGGCTCAG-3′ and 1492R 5′-TACCTTGTTACGACTT-3′ according to a recently published research (13).

2.4 Clinical data capture and management

Clinical data of children hospitalized with LRTIs from January 2018 to December 2021 were retrospectively analyzed. Data were retrieved from our clinical case database, which collected basic

information about children, including sex, age, admission time, discharge diagnosis (including bronchiolitis and community-acquired pneumonia), respiratory pathogen test results, etc. Two physicians independently validated the data and resolved any discrepancies through re-export and subsequent review, following a meticulous process to ensure data accuracy. A total of 11,891 cases were finally included in this analysis.

2.5 Statistical analysis

The data obtained were analyzed using SPSS 26.0 software, and count data were expressed as percentages (%), and comparisons between groups were made using the chi-squared test, *Post-hoc* pairwise comparisons were performed with partitions of the χ^2 method, with $p < 0.05$ was considered to be statistically different and the adjusted test level was $\alpha' = 0.0083$.

3 Results

3.1 Study population

A total of 11,891 hospitalized children diagnosed as LRTIs during 2018–2021 were included in this research, among which 7,136 (60.01%) cases were male and 4,755 (39.99%) cases were female. Considering the initial report of COVID-19 disease in the end of 2019 in China, we divided the collected samples into two groups based on the sampling time: 7,682 (64.60%) cases from 2018 to 2019 and 4,209 (35.40%) cases from 2020 to 2021. According to the patients' age, the children were divided into four groups, as follows: 0 ~ 1 year (3,882, 32.65%), 1 years (2,300, 19.34%), 2 years (1,327, 11.16%), 3 years (1,872, 15.74%), 4 years (1,033, 8.69%), 5 years (534, 4.49%), 6 years (360, 3.03%) and 6 ~ 14 years (583, 4.90%). In addition, the cases in spring, summer, autumn, and winter during 2018–2021 were 2,844 (23.92%), 2,182 (18.35%), 3,068 (25.80%), and 3,797 (31.93%), respectively. In contrast, the rate of pathogen-positive tests for children hospitalized in 2018–2019 was lower than in 2020–2021, with significant differences by age 3 years and in winter and spring at two different times ($p < 0.05$) (Table 1). However, the number of LRTIs cases by age, gender, and season was significantly higher in 2018–2019 than in 2020–2021, but there was no significant difference in the proportion of child gender, summer, and multiple infections between the two periods ($p > 0.05$) (Table 2; Figure 1).

3.2 Overall detection of LRTIs-associated pathogens

Of 11,891 tested samples/patients, 62.19% (7,395/11,891) of LRTIs-patients were diagnosed with at least one respiratory pathogen. Among 7,395 cases, 4,425 (59.84%) cases had single pathogen infection, 2,970 cases (40.16%) had two or more than two pathogen infection. According to pathogen characteristics, bacterial infection (30.73%, 3,654/11,891) was the most common factors resulting in LRTIs, followed by viral infection (26.22%, 3,118/11,891), and MP infection (13.05%, 1,552/11,891). However, the number of cases documented in 2018–2019 ($n = 7{,}682$) was much higher that these during CDVID-19 pandemic ($n = 4{,}209$), the detection rates of these pathogens greatly changed during these two periods. By comparing infections with LRTIs-associated pathogens before and during the COVID-19 pandemic, we found that the case number of FluA, ADV,

TABLE 1 Demographic characteristics and pathogen detection profile of LRTI in hospitalized children before and during the COVID-19 pandemic (2018–2021).

Characteristics	Total (*n*, %)	2018–2019 (*n*, %)	2020–2021 (*n*, %)	χ^2	*p*-value
	11,891 (7,395, 62.19%)	7,682 (4,635, 60.33%)	4,209 (2,760, 65.57%)	31.73	<0.001
Gender				1.95	0.163
Male	7,136 (4,474, 62.70%)	4,644 (2,832, 60.98%)	2,492 (1,642, 65.89%)	16.71	<0.001
Female	4,755 (2,921, 61.43%)	3,038 (1,803, 59.35%)	1,717 (1,118, 65.11%)	15.39	<0.001
Age				57.11	<0.001
0–1 years	3,882 (2,386, 61.46%)	2,703 (1,608, 59.49%)	1,179 (778, 65.99%)	14.64	<0.001
1 years	2,300 (1,317, 57.26%)	1,397 (731, 52.33%)	903 (586, 64.89%)	35.40	<0.001
2 years	1,327 (805, 60.66%)	753 (424, 56.31%)	574 (381, 66.38%)	13.84	<0.001
3 years	1,872 (1,199, 64.05%)	1,147 (720, 62.77%)	725 (479, 66.07%)	2.10	0.148
4 years	1,033 (689, 66.70%)	656 (442, 67.38%)	377 (247, 65.52%)	0.37	0.541
5 years	534 (353, 66.10%)	372 (250, 67.20%)	162 (103, 63.58%)	0.66	0.416
6 years	360 (247, 68.61%)	251 (175, 69.72%)	109 (72, 66.10%)	0.47	0.491
>6 years	583 (399, 68.44%)	403 (285, 70.72%)	180 (114, 63.33%)	3.14	0.076
Season				28.26	<0.001
Spring (Mar-May)	2,844 (1,784, 62.73%)	2,037 (1,236, 60.67%)	807 (548, 67.91%)	12.92	<0.001
Summer (Jun-Aug)	2,182 (1,350, 61.87%)	1,421 (852, 59.58%)	761 (498, 65.44%)	6.31	0.012
Autumn (Sep-Nov)	3,068 (1,799, 58.64%)	1,860 (1,083, 58.23%)	1,208 (716, 59.27%)	0.33	0.566
Winter (Jan-Feb, Dec)	3,797 (2,462, 64.84%)	2,364 (1,464, 61.93%)	1,433 (998, 69.64%)	23.29	<0.001

TABLE 2 Detection rates of various pathogens in hospitalized children with LRTI before and during the COVID-19 pandemic (2018–2021).

Characteristics	Total	2018–2019	2020–2021	χ^2	*p*-value
	n = 11,891	*n* = 7,682	*n* = 4,209		
Diagnosis					
Single infection	4,425 (37.21%)	2,728 (35.51%)	1,697 (43.41%)	21.78	<0.001
Multiple infections	2,970 (24.98%)	1,907 (24.82%)	1,063 (27.19%)	0.27	0.604
Pathogenic uncertainty	4,496 (37.81%)	3,047 (39.67%)	1,449 (37.07%)	31.73	<0.001
Pathogen					
Viral	3,118 (26.22%)	1,631 (21.23%)	1,487 (35.33%)	279.34	<0.001
FluA	435 (3.65%)	290 (3.78%)	145 (3.44%)	0.84	0.359
FluB	464 (3.90%)	177 (2.30%)	287 (6.82%)	147.80	<0.001
PIV1	82 (0.69%)	33 (0.43%)	49 (1.16%)	21.43	<0.001
PIV2	40 (0.34%)	8 (0.10%)	32 (0.76%)	34.92	<0.001
PIV3	237 (1.99%)	63 (0.82%)	174 (4.13%)	152.87	<0.001
ADV	658 (5.53%)	536 (6.98%)	122 (2.90%)	86.54	<0.001
RSV	1,202 (10.11%)	524 (6.82%)	678 (16.11%)	272.66	<0.001
Mycoplasma pneumoniae	1,552 (13.05%)	1,034 (13.46%)	518 (12.31%)	3.19	0.074
Bacterial	3,654 (30.73%)	2,256 (29.38%)	1,398 (33.21%)	18.91	<0.001
H. influenzae	1,153 (9.70%)	637 (8.29%)	516 (12.26%)	48.88	<0.001
S. aureus	398 (3.35%)	237 (3.09%)	161 (3.83%)	4.60	0.032
S. pneumoniae	536 (4.51%)	304 (3.96%)	232 (5.51%)	15.27	<0.01
K. pneumoniae	308 (2.59%)	193 (2.51%)	115 (2.73%)	0.52	0.470
M. catarrhalis	404 (3.40%)	325 (4.23%)	79 (1.88%)	45.90	<0.001
P. aeruginosa	222 (1.87%)	117 (1.52%)	105 (2.49%)	14.70	<0.001
E. coli	458 (3.85%)	285 (3.71%)	173 (4.11%)	1.18	0.278
B. pertussis	175 (1.47%)	158 (2.06%)	17 (0.40%)	51.23	<0.001

MP, and bacterial infection significantly decreased during COVID-19 pandemic. Analysis of positive LRTIs pathogen test results showed only the prevalence of ADV, BP, and *M. catarrhalis* remarkably decreased during COVID-19 pandemic, while the detection rates of FluB, PIV, RSV, *H. influenzae*, *S. pneumoniae*, and *P. aeruginosa* significantly increased (Table 2).

3.3 Influence of age on LRTIs incidence

To further characterize the relationship between age and pathogen infection, data were stratified by age. There was a more statistically significant difference between infants and toddlers (0–2 years), who had a higher relative rate of pathogen-positive detection during the COVID-19 pandemic. In contrast, there was no statistically significant difference between the two groups in pathogen-positive detection rates among children aged 3 years and older (Table 1). Among these, RSV and bacterial infections were the most common causes of LRTIs in infants under 4 years of age, and RSV was also the predominant epidemiological pathogen in infants and children after normative control of COVID-19 pandemic, whereas the prevalence of MP and IFV remained relatively stable in all age groups, and LRTIs in school-aged children was predominantly characterized by MP, IFV, and ADV (Table 2; Figure 2). Thus, during the COVID-19 pandemic, the pathogens of respiratory infections in children shifted to a viral spectrum, with the age of infection progressively favoring younger children, especially those under 2 years of age.

3.4 Influence of seasonal changes on the prevalence of respiratory pathogens

The overall prevalence of LRTIs in hospitalized children in the four seasons was lowest in summer, highest in winter, and intermediate in spring and autumn, while the rates of positive tests were spring (1,784/2,844, 62.73%), summer (1,350/2,182, 61.87%), autumn (1,799/3,068, 58.64%), and winter (2,462/3,797, 64.84%), respectively. The data results showed a decrease in the prevalence of LRTIs in children in the spring before and during the COVID-19 pandemic, no significant difference in prevalence between the summer months, and a relative increase in prevalence in the autumn and winter months (Figure 1), but the rate of pathogen-positive tests increased except in the autumn, and the difference was statistically significant (Table 1). We again analyzed the seasonal epidemiological characteristics of the different pathogens and found that RSV and MP had higher detection rates in the perennial year compared with other pathogens, while ADV had an outbreak in the summer of 2019 (Figure 3A), and there was a general increase in the rate of positive tests for IFV in the winter,

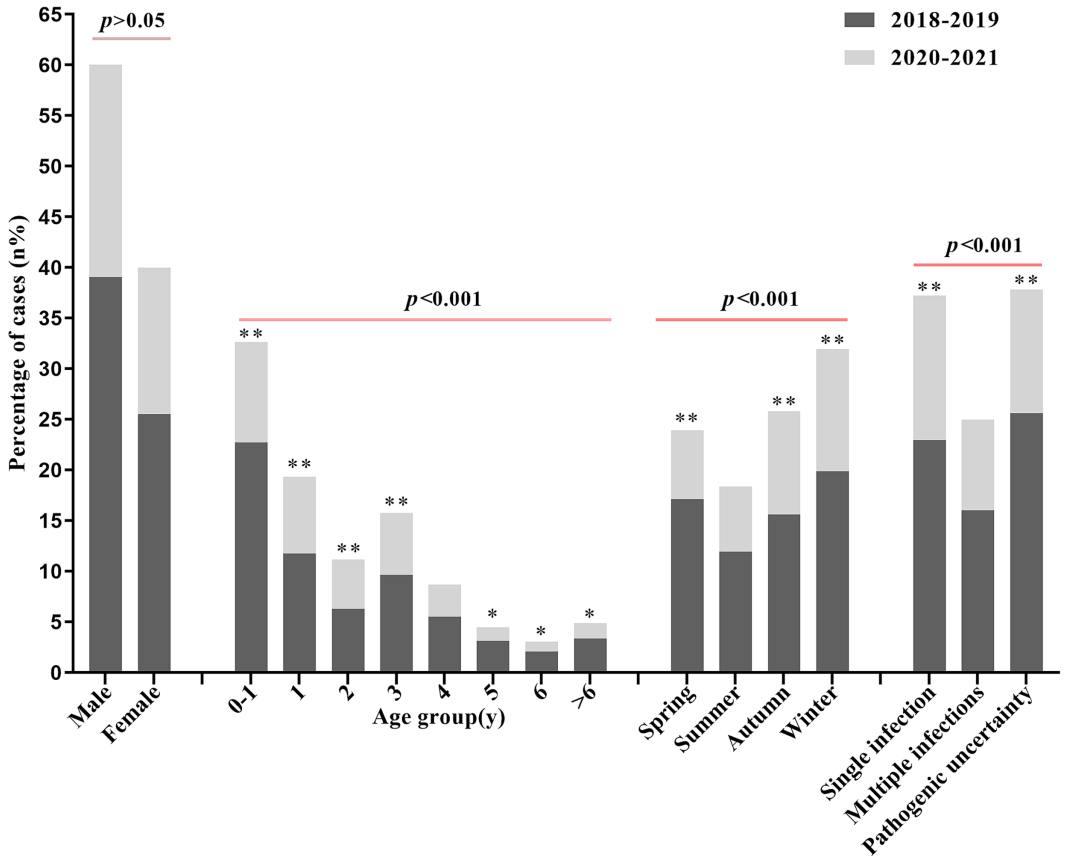

FIGURE 1
Percentage comparison of the number of LRTIs in hospitalized children by gender, age, season, and etiologic diagnosis in two different time periods. * indicates $p < 0.05$ and ** indicates $p < 0.01$ for comparison between the two groups (2018–2019, 2020–2021).

whereas the positive rates of FluB and PIV increased significantly in 2020–2021 (Table 2; Figure 3B).

4 Discussion

COVID-19 pandemic has inflicted significant global human and economic losses (5). The main modes of respiratory pathogen transmission are droplet and contact, since the initial outbreak of COVID-19 in late 2019 (14), China has implemented a series of pandemic prevention and control measures (15). Strict NPIs, including social distancing and wearing masks, were beneficial in minimizing the spread of respiratory pathogens in both adult and pediatric populations. These measures have not only effectively contained the large-scale spread of COVID-19, but have also contributed to a reduction in infections and hospitalizations related to common respiratory pathogens in children in China. As a result, the epidemiological characteristics of childhood LRTIs have been affected and the spectrum of common pathogens has changed (16–19). Notably, the incidence of LRTIs was significantly reduced after the COVID-19 outbreak, suggesting the effectiveness of COVID-19 prevention and control measures in reducing childhood respiratory infections (16, 19). Detection rates for different pathogens show significantly variation with age, with the highest rates, particularly for viral infections (17–21), occurring before the age of 3 years, this is attributed to lower immunity and increased environmental contact in young children, coupled with challenges in adhering to preventive measures liking handwashing and mask-wearing (18, 19). While the incidence rate of LRTI usually decreases with age, the incidence rate of LRTI increases significantly at the age of 3 years, which may be due to the fact that children begin to learn in early childhood settings at this age (22). The closure of schools during the COVID-19 pandemic, a crucial measure to reduce transmission, particularly in schools, significantly lowered cross-infections (15).

Firstly, our study indicated that the infection rate of LRTIs was observed to be higher in boys than in girls (Table 1; Figure 1), a trend possibly linked to the demographic composition of children in Xiangtan. This finding aligns with domestic investigations (23, 24). Compared to 2018–2019, the incidence of LRTIs decreased significantly in 2020–2021, but pathogen positivity and single pathogen positivity increased over the same period, this observation may be attributed to the preventive measures implemented for COVID-19 pandemic (Table 2). Before and during the COVID-19 pandemic, there was no statistically significant difference in the incidence of LRTIs among children in summer (Figure 1), but there was no statistically significant difference in the rate of pathogen positivity compared with that in autumn (Table 1), and the difference in seasonal incidence was attributed to the change in temperature in autumn and winter, which is favorable for pathogen multiplication, and the peak of pathogen infection started in autumn (25). The

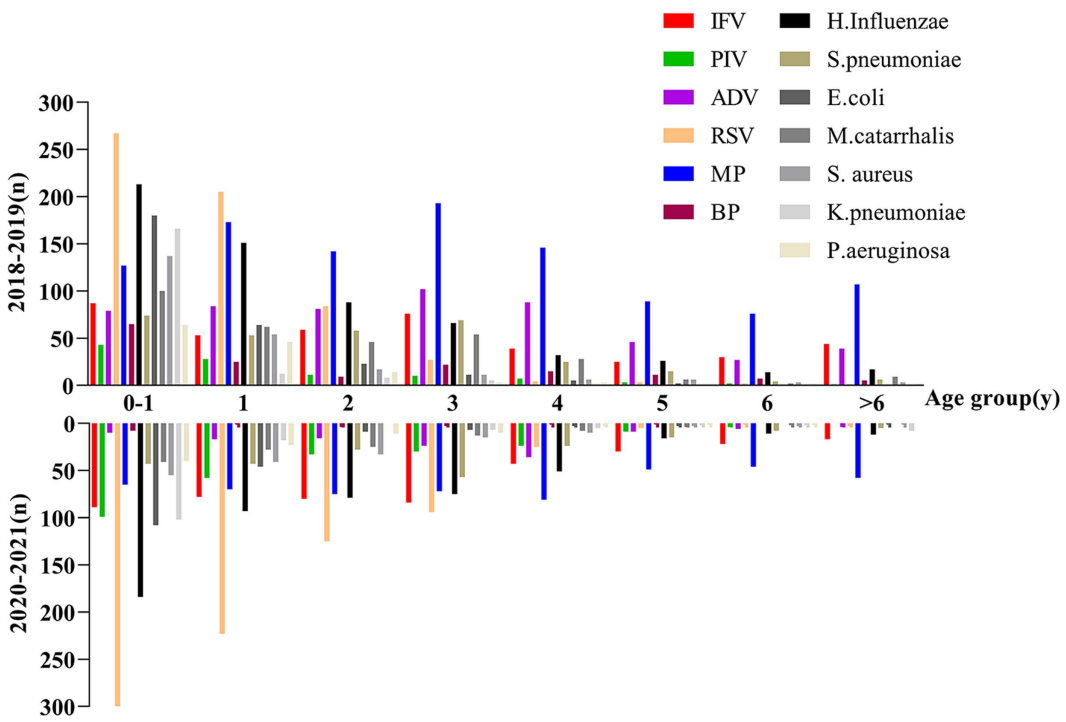

FIGURE 2
Comparison of detection of different pathogens in hospitalized children with LRTIs in different age groups before and during the COVID-19 pandemic.

increase in the pathogen-positive detection rate may be due to the systematic training of all sampling personnel in health care facilities on respiratory specimen collection during the COVID-19 pandemic, and the prevention and control measures that allowed children to attend the hospital relatively late (17).

Secondly, our study identified the three most common pathogens for LRTIs in children as RSV, MP, and *H. influenzae* (Table 2; Figure 2). Overall detection rates for common viral infections were significantly different before and during the COVID-19 pandemic, and the outbreak of ADV infections in the summer of 2019 was contained during the COVID-19 pandemic, but the rate of positive tests for IFV, PIV, and RSV was elevated due to the fact that ADV predominantly infects children over 3 years of age and correlates with average temperatures and humidity during the ADV season (25, 26). Although the overall LRTIs positive rate decreased during routine COVID-19 prevention and control activities. However, viral prevalence actually increased in relative terms. The RSV pandemic remained prevalent, while IFV and PIV showed some outbreaks in autumn or winter (Figure 3A), with variations in different subtypes (Figure 3B), these results are inconsistent other studies (16–19). Post-COVID-19 prevention and control, the overall incidence of LRTIs decreased, but the prevalence of MP and IFV remained relatively stable across all age groups (Figure 2).

Finally, we found that there were outbreaks of ADV, IFV, RSV, and MP infections in children with LRTIs in 2019, the prevention and control of COVID-19 have also controlled the outbreak and infection of various pathogens (Figure 3). However, during the COVID-19 pandemic, MP and RSV were common pathogens causing LRTIs in hospitalized children, while IFV and PIV continued to outbreak during the autumn and winter seasons, thus requiring continued attention. While bacterial infections in LRTIs in infants and hospitalized children are often secondary to viral infections, some pathogens (e.g., *H. influenzae* and *S. pneumoniae*) also show a relatively high rate of positive detection, making viral and bacterial infections the main causes of LRTIs in children under 3 years of age (27). Therefore, we must continue to emphasize the importance of prevention and control of viral and bacterial respiratory infections in infants and young children, and pay more attention to MP, IFV, and ADV infections in school-aged children. It emphasizes the need for focused preventive measures in early childhood centers or schools where younger children engage in group learning, potentially increasing the risk of cross-infections. Measures such as environmental disinfection and indoor air circulation should be reinforced in these settings.

Overall, pathogenetic studies conducted before and after COVID-19 not only contribute to a more accurate medical basis for clinical diagnosis and treatment, and evidence-based medicine, but also enhance healthcare professionals' comprehension of regional respiratory infection characteristics caused by common pathogens. The study acknowledges limitations such as not testing for other common or rare respiratory pathogens in children such as HRV, Chlamydia and HMPV, and not testing for different subtypes of common viruses, thus not reflecting the epidemiological status of these pathogens (20–26). To enhance the surveillance of respiratory pathogens in children, more comprehensive multiplex assays are needed to monitor LRTIs for common respiratory pathogens as well as infections with different viral subtypes. Consequently, vigilance against potential pandemics of rare pathogens such as HMPV is crucial in addition to preventing and controlling outbreaks of

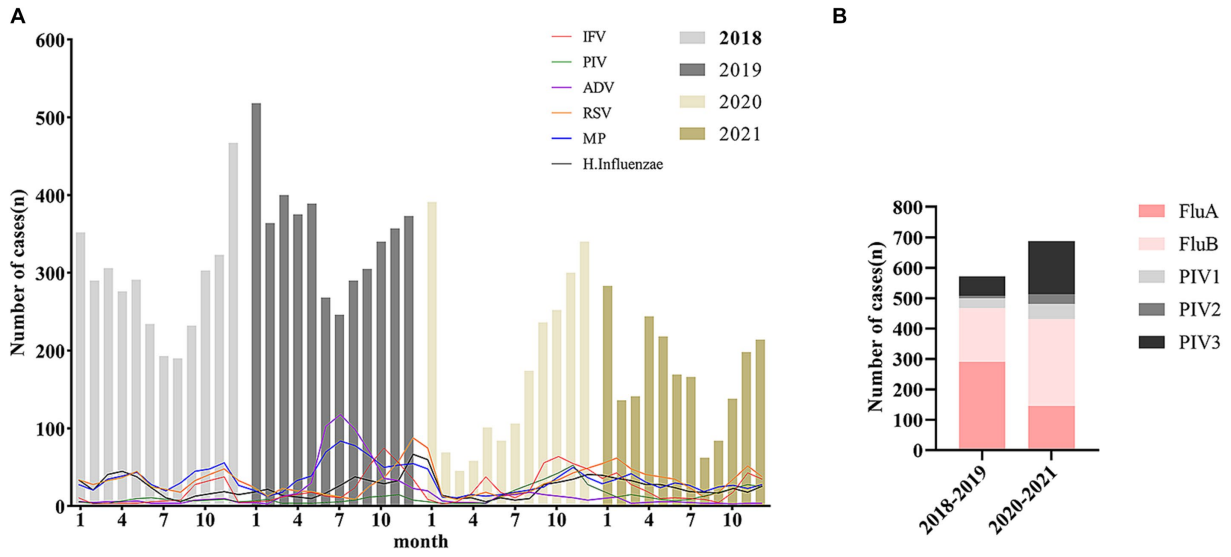

FIGURE 3
Seasonal epidemiological trends of common viruses and *Haemophilus influenzae* in pediatric LRTIs **(A)**. Detection of influenza and parainfluenza virus subtypes in children with LRTIs before and during the COVID-19 pandemic **(B)**.

common childhood respiratory infections and MP. Targeted measures to prevent and control pandemic outbreaks of common respiratory pathogens in children are essential, with particular emphasis on the persistent challenge of preventing and controlling RSV. This remains a major concern and an urgent priority in the current landscape.

5 Conclusion

Our results suggest that standardized prevention and control measures implemented during the COVID-19 pandemic reduced the incidence of LRTIs in children, while also influencing the pathogen profile of LRTIs in hospitalized children. Overall, this impact has resulted in a reduction in outbreaks of common pathogens and more infectious pathogens while tilting toward viral infections and affecting younger age groups. Therefore, attention needs to be paid to potential outbreaks and pandemics of common pathogens, especially viral respiratory infections in infants and young children. In addition, after the relaxation of pandemic-related measures, continuous surveillance and targeted prevention and control efforts are essential to manage and mitigate the evolving situation of respiratory infections in children.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by Ethics Committee of Xiangtan Central Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation in this study was provided by the participants' legal guardians/next of kin.

Author contributions

YF: Funding acquisition, Investigation, Software, Writing – original draft, Writing – review & editing. HZ: Data curation, Writing – original draft. BZ: Data curation, Writing – original draft. YZ: Data curation, Funding acquisition, Validation, Writing – review & editing. HY: Supervision, Visualization, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Wan L, Li L, Zhang H, Liu C, Li R, Wu X, et al. The changing pattern of common respiratory viruses among children from 2018 to 2021 in Wuhan, China. *Arch Virol*. (2023) 168:291. doi: 10.1007/s00705-023-05891-7

2. Hong S, Li D, Wei Y, Zheng Y, Cai J, Zheng H, et al. Epidemiology of respiratory pathogens in patients with acute respiratory tract infection in Xiamen, China: A retrospective survey from 2020 to 2022. *Heliyon*. (2023) 9:e22302. doi: 10.1016/j.heliyon.2023.e22302

3. Lauinger IL, Bible JM, Halligan EP, Bangalore H, Tosas O, Aarons EJ, et al. Patient characteristics and severity of human rhinovirus infections in children. *J Clin Virol*. (2013) 58:216–20. doi: 10.1016/j.jcv.2013.06.042

4. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5

5. Chen K, Zhang L, Fang Z, Li J, Li C, Song W, et al. Analysis of the protective efficacy of approved COVID-19 vaccines against Omicron variants and the prospects for universal vaccines. *Front Immunol*. (2023) 14:1294288. doi: 10.3389/fimmu.2023.1294288

6. Chow EJ, Uyeki TM, Chu HY. The effects of the COVID-19 pandemic on community respiratory virus activity. *Nat Rev Microbiol*. (2023) 21:195–210. doi: 10.1038/s41579-022-00807-9

7. Tang M, Dong W, Yuan S, Chen J, Lin J, Wu J, et al. Comparison of respiratory pathogens in children with community-acquired pneumonia before and during the COVID-19 pandemic. *BMC Pediatr*. (2023) 23:535. doi: 10.1186/s12887-023-04246-0

8. Shi T, Zhao X, Zhang X, Meng L, Li D, Liu X, et al. Immediate and long-term changes in the epidemiology, infection spectrum, and clinical characteristics of viral and bacterial respiratory infections in Western China after the COVID-19 outbreak: a modeling study. *Arch Virol*. (2023) 168:120. doi: 10.1007/s00705-023-05752-3

9. Yan H, Zhai B, Yang F, Wang P, Zhou Y. The impact of non-pharmacological interventions measures against COVID-19 on respiratory virus in preschool children in Henan. *China J Epidemiol Glob Health*. (2023). doi: 10.1007/s44197-023-00168-3 [Online ahead of print].

10. Li ZJ, Yu LJ, Zhang HY, Shan CX, Lu QB, Zhang XA, et al. Chinese Centers for Disease Control and Prevention (CDC) etiology surveillance study team of acute respiratory infections. Broad Impacts of Coronavirus Disease 2019 (COVID-19) pandemic on acute respiratory infections in china: an observational study. *Clin Infect Dis*. (2022) 75:e1054–62. doi: 10.1093/cid/ciab942

11. Yeoh DK, Foley DA, Minney-Smith CA, Martin AC, Mace AO, Sikazwe CT, et al. Impact of coronavirus disease 2019 public health measures on detections of influenza and respiratory syncytial virus in children during the 2020 Australian winter. *Clin Infect Dis*. (2021) 72:2199–202. doi: 10.1093/cid/ciaa1475

12. Jiang ZF, Shen KL, Shen Y. *Zhu Futang Practice of Pediatrics*. Beijing: People's Medical Publishing House; (2015):1251–1253.

13. Khasapane NG, Koos M, Nkhebenyane SJ, Khumalo ZTH, Ramatla T, Thekisoe O. Detection of staphylococcus isolates and their antimicrobial resistance profiles and virulence genes from subclinical mastitis cattle milk using MALDI-TOF MS, PCR and sequencing in free state province, South Africa. *Animals (Basel)*. (2024) 14:154. doi: 10.3390/ani14010154IF: 3.0 Q1

14. Zhu N, Zhang D, Wang W, Li X, Yang B, Song J, et al. China novel coronavirus investigating and research team. A novel coronavirus from patients with pneumonia in China, 2019. *N Engl J Med*. (2020) 382:727–33. doi: 10.1056/NEJMoa2001017

15. Li J, Yong Y. *Guidance for Corona Virus Disease 2019: Prevention, Control, Diagnosis and Managements*. Beijing, China: People's Medical Publishing House (2020) Available from CIP-Database China, Available at: http://www.pmph.com/.

16. Su C, Zhang Z, Zhao X, Peng H, Hong Y, Huang L, et al. Changes in prevalence of nosocomial infection pre- and post-COVID-19 pandemic from a tertiary Hospital in China. *BMC Infect Dis*. (2021) 21:693. doi: 10.1186/s12879-021-06396-x

17. Serigstad S, Markussen DL, Ritz C, Ebbesen MH, Knoop ST, Kommedal Ø, et al. CAPNOR study group. The changing spectrum of microbial aetiology of respiratory tract infections in hospitalized patients before and during the COVID-19 pandemic. *BMC Infect Dis*. (2022) 22:763. doi: 10.1186/s12879-022-07732-5

18. Zhang J, Yang T, Zou M, Wang L, Sai L. The epidemiological features of respiratory tract infection using the multiplex panels detection during COVID-19 pandemic in Shandong province, China. *Sci Rep*. (2023) 13:6319. doi: 10.1038/s41598-023-33627-9

19. Ren L, Lin L, Zhang H, Wang Q, Cheng Y, Liu Q, et al. Epidemiological and clinical characteristics of respiratory syncytial virus and influenza infections in hospitalized children before and during the COVID-19 pandemic in Central China. *Influenza Other Respir Viruses*. (2023) 17:e13103. doi: 10.1111/irv.13103

20. Perez A, Lively JY, Curns A, Weinberg GA, Halasa NB, Staat MA, et al. New vaccine surveillance network collaborators. respiratory virus surveillance among children with acute respiratory illnesses - new vaccine surveillance network, United States, 2016-2021. *MMWR Morb Mortal Wkly Rep*. (2022) 71:1253–9. doi: 10.15585/mmwr.mm7140a1

21. Dallmeyer LK, Schüz ML, Fragkou PC, Omony J, Krumbein H, Dimopoulou D, et al. Epidemiology of respiratory viruses among children during the SARS-CoV-2 pandemic: A systematic review and meta-analysis. *Int J Infect Dis*. (2023) 138:10–8. doi: 10.1016/j.ijid.2023.10.023

22. Perin J, Mulick A, Yeung D, Villavicencio F, Lopez G, Strong KL, et al. Global, regional, and national causes of under-5 mortality in 2000-19: an updated systematic analysis with implications for the Sustainable Development Goals. *Lancet Child Adolesc Health*. (2022) 6:106–15. doi: 10.1016/S2352-4642(21)00311-4

23. Liu YN, Zhang YF, Xu Q, Qiu Y, Lu QB, Wang T, et al. Chinese Center for Disease Control and Prevention Etiology Surveillance Study Team of Acute Respiratory Infections. Infection and co-infection patterns of community-acquired pneumonia in patients of different ages in China from 2009 to 2020: a national surveillance study. *Lancet Microbe*. (2023) 4:e330–9. doi: 10.1016/S2666-5247(23)00031-9

24. Zhu L, Luo T, Yuan Y, Yang S, Niu C, Gong T, et al. Epidemiological characteristics of respiratory viruses in hospitalized children during the COVID-19 pandemic in southwestern China. *Front Cell Infect Microbiol*. (2023) 13:1142199. doi: 10.3389/fcimb.2023.1142199

25. Zhao H, Feng Q, Feng Z, Zhu Y, Ai J, Xu B, et al. Clinical characteristics and molecular epidemiology of human metapneumovirus in children with acute lower respiratory tract infections in China, 2017 to 2019: A multicentre prospective observational study. *Virol Sin*. (2022) 37:874–82. doi: 10.1016/j.virs.2022.08.007

26. Zhu G, Xu D, Zhang Y, Wang T, Zhang L, Gu W, et al. Epidemiological characteristics of four common respiratory viral infections in children. *Virol J*. (2021) 18:10. doi: 10.1186/s12985-020-01475-y

27. Yan Y, Sun J, Ji K, Guo J, Han L, Li F, et al. High incidence of the virus among respiratory pathogens in children with lower respiratory tract infection in northwestern China. *J Med Virol*. (2023) 95:e28367. doi: 10.1002/jmv.28367

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"Disease X" and prevention policies

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KEYWORDS

Disease X, pandemic, prevention, government bodies, WHO

Background

"Disease X" refers to an unexpected and unknown outbreak of a contagious or infectious disease. It is a concept that a serious global epidemic could possibly be caused by a "pathogen X," which is presently unidentified and capable of infecting humans. The pathogen X, which is most likely a zoonotic agent, is supposed to be the etiological agent of Disease X with epidemic or pandemic potential (1, 2). According to the World Health Organization (WHO) diseases directory, Disease X is considered among highly contagious diseases such as Ebola, Zika, and COVID-19 (2). As an elusive pathogen, we are unable to prevent the occurrence of Disease X. However, by implementing preventative measures, we may be able to impede or minimize its transmission and possible health risks. In order to achieve this, a universal scientific protocol for managing Disease X would be required.

The goal of the study is to draw attention to the essential protocol elements that can assist the scientific community in creating an all-encompassing protocol to combat Disease X.

Opinion

Recently, we witnessed the world rocked by an X disease "severe acute respiratory coronavirus virus 2 (SARS-CoV-2)," and yet more to come. Pandemics in the past are a grave reminder that future pandemics may present even greater challenges, which would swing medical confidence. As an old saying goes, "Prevention is better than cure." It is imperative that we proactively equip ourselves for the emergence of Disease X, far in advance before its potential global impact. History is the spectator that we never properly prepare ourselves for Disease X, and the absence of appropriate etiquette consistently coincided with the outbreak of Disease X worldwide. Even commendable organizations such as WHO and others also fall short when it comes to taking prompt, timely, decisive, and tough action to minimize the spread of contagious diseases (3). As an illustration, consider the 2014 Ebola outbreak, which began in Guinea, West Africa, and quickly spread to Sierra Leone, Liberia, Italy, Mali, Nigeria, Senegal, Spain, the United Kingdom, and the United States of America (USA). It took 2 years for the outbreak to end, resulting in 28,600 cases overall and 11,325 (40%) fatal cases (4, 5).

In 2016, Adam Kamradt-Scott conducted research to determine where the responsibility lies for the delayed control of the 2014 Ebola outbreak. The study concluded that the delayed responses from the World Health Organization (WHO) and the lack of comprehension and collaboration among various nations, together with delayed financing, exacerbated the situation (6). Failing to heed the counsels of history, global organizations and countries worldwide, including those with robust economies, failed again to react to

the most recent outbreak of Disease X (COVID-19) on time. For instance, within just 10 months (March to November 2020), the USA confirmed over 262,000 deaths and approximately 13 million cases of COVID-19 (7). Furthermore, the WHO reports that between 3 January 2020 and 30 November 2023, there were 103,436,829 confirmed cases of COVID-19 in the USA, along with 1,144,877 confirmed deaths (8). After the COVID-19 pandemic, engaging in procedural discussions with partner nations, the WHO commenced a new program, "Preparedness and Resilience for Emerging Threats (PRET)," to improve pandemic awareness (9). PRET introduced the initial Preparedness and Resilience Plan, called "Module 1: Planning for Respiratory Pathogen Pandemics Version 1.0," which focuses only on respiratory infections. This plan was developed using previous knowledge and guidance from past pandemic experiences (10). While this is a commendable initiative from the WHO, much more has to be done.

Proposed intervention strategies

The suggested intervention strategies are aimed at preventing the onset and reducing the snag of a manifested disease X through endorsing the liabilities of stakeholders and authoritative bodies to remain vigilant and respond quickly to pathogen X.

Government bodies liabilities

National strategies

Individual government bodies must (a) strengthen health policies for Disease X and allocate enough funding for epidemic preparation in the annual budgets; (b) timely effectual measures must be taken by governments by providing the funds without delaying epidemic preparation; and (c) there must be a section for Disease X in the national healthcare systems and management that is purely responsible for the prevention and control of Disease X.

International strategies

(a) Advice, recommendations, and suggestions from global academics and scientists must be sought sensibly without any political conflicts among nations; (b)The measures shall be taken to prevent the cross-border transmission of Disease X in the form of pre or on-the-spot airport screening of passengers in any suspicious Disease X situations; (c) In case of pathogen X confirmation, instant and suitable travel restrictions must be implemented to prevent the cross-border spread of pathogen X.

World health organization liabilities

Being the premier health organization, WHO needs to (a) establish a medical/clinical laboratories surveillance unit to inspect the worldwide pathogenic laboratories and pharmaceutical companies on a weekly basis to prevent the accidental spread of natural or engineered pathogen/s X. (b) WHO must provide an easily accessible collaborative platform for the world's scientists, clinicians, and infectious disease experts where they can freely and efficiently exhibit their views, expertise, and suggestions for a timely control and elimination of pathogen X. (c) Its WHO's responsibility to provide funds for epidemic preparation to economically poor countries for prevention and establish a minimum fighting health system before Disease X pandemic. (d) Being the executive united organization, it should be WHO's responsibility to provide enough resources for diagnostics, vaccines, clinical trials, etc., in case of a pandemic.

Conclusion

The Ebola, COVID-19, and any previous pandemics were not the last to cause havoc in the world, and there will likely be many more in the future that pose serious threats to worldwide health. Thus, we need to get ready together for the upcoming outbreak as soon as possible, deploying timely measures to save lives.

Limitations

The study lacks the approach of medical countermeasures, vaccine production, drug development, and prompt supply chain of medical equipment for managing subsequent Disease X.

Author contributions

MZ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing. NA: Methodology, Validation, Visualization, Software, Writing—review & editing. MI: Data curation, Resources, Writing—review & editing, Software, Validation.

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References

1. Simpson S, Kaufmann MC, Glozman V, Chakrabarti A. Disease X: accelerating the development of medical countermeasures for the next pandemic. *Lancet Infect Dis.* (2020) 20:e108–e15. doi: 10.1016/S1473-3099(20)30123-7

2. Tahir MJ, Sawal I, Essar MY, Jabbar A, Ullah I, Ahmed A. Disease X: a hidden but inevitable creeping danger. *Infect Control Hospital Epidemiol.* (2022) 43:1758–9. doi: 10.1017/ice.2021.342

3. Iserson KV. The next pandemic: prepare for "Disease X". *West J Emerg Med.* (2020) 21:756–8. doi: 10.5811/westjem.2020.5.48215

4. Kaner J, Schaack S. Understanding Ebola: the 2014 epidemic. *Global Health.* (2016) 12:53. doi: 10.1186/s12992-016-0194-4

5. WHO. *Ebola outbreak 2014-2016 - West Africa.* Available online at: https://www.who.int/emergencies/situations/ebola-outbreak-2014-2016-West-Africa (accessed November 30, 2023).

6. Kamradt-Scott A. WHO's to blame? The World Health Organization and the 2014 Ebola outbreak in West Africa. *Third World Q.* (2016) 37:401–18. doi: 10.1080/01436597.2015.1112232

7. Alexander M, Unruh L, Koval A, Belanger W. United States response to the COVID-19 pandemic, January-November 2020. *Health Econ Policy Law.* (2022) 17:62–75. doi: 10.1017/S1744133121000116

8. WHO. *Health Emergency Dashboard, COVID-19.* Available online at: https://covid19.who.int/region/amro/country/us (accessed November 30, 2023).

9. WHO. *WHO launches new initiative to improve pandemic preparedness.* Available online at: https://www.who.int/news/item/26-04-2023-who-launches-new-initiative-to-improve-pandemic-preparedness (accessed February 8, 2024).

10. WHO. *Preparedness and Resilience for Emerging Threats Module 1: Planning for respiratory pathogen pandemics Version 1.0 (draft).* Available online at: https://www.who.int/publications/m/item/preparedness-and-resilience-for-emerging-threats-module-1-planning-for-respiratory-pathogen-pandemics-version-1 (accessed February 8, 2024).

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Case report: Neglected subacute thyroiditis: a case following COVID-19 vaccination

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We report a case of overlooked Subacute Thyroiditis (SAT) potentially induced by the administration of a COVID-19 vaccine. This case prompted a thorough review of the existing literature to elucidate possible mechanisms by which immune responses to the COVID-19 vaccine might precipitate thyroid damage. The primary objective is to enhance the clinical understanding and awareness of SAT among healthcare professionals. Subacute thyroiditis is a prevalent form of self-limiting thyroid disorder characterized by fever, neck pain or tenderness, and palpitations subsequent to viral infection. The development of numerous SARS-CoV-2 vaccines during the COVID-19 pandemic was intended to mitigate the spread of the virus. Nevertheless, there have been documented instances of adverse reactions arising from SARS-CoV-2 vaccines, such as the infrequent occurrence of subacute thyroiditis. While the majority of medical practitioners can discern classic subacute thyroiditis, not all cases exhibit typical characteristics, and not all systematic treatments yield positive responses. In this study, we present a rare case of subacute thyroiditis linked to the administration of the SARS-CoV-2 vaccine. A previously healthy middle-aged female developed fever and sore throat 72 h post-inoculation with the inactivated SARS-CoV-2 vaccine. Initially attributing these symptoms to a common cold, she self-administered ibuprofen, which normalized her body temperature but failed to alleviate persistent sore throat. Suspecting a laryngopharyngeal disorder, she sought treatment from an otolaryngologist. However, the pain persisted, accompanied by intermittent fever over several days. After an endocrinology consultation, despite the absence of typical neck pain, her examination revealed abnormal thyroid function, normal thyroid antibodies, heterogeneous echogenicity on thyroid ultrasonography, and elevated levels of Erythrocyte Sedimentation Rate (ESR) and C-Reactive Protein (CRP). These findings led to a consideration of the diagnosis of SAT. Initially, she was treated with non-steroidal anti-inflammatory drugs (NSAIDs) for her fever, which proved effective, but her neck pain remained uncontrolled. This suggested a poor response to NSAIDs. Consequently, steroid therapy was initiated, after which her symptoms of fever and neck pain rapidly resolved.

KEYWORDS

COVID-19, subacute thyroiditis, SARS-CoV-2 vaccine, autoimmunity, fever

Introduction

Subacute thyroiditis, also known as granulomatous thyroiditis or De Quervain's thyroiditis, represents a relatively rare yet significant thyroid disorder characterized by its self-limiting nature (1). It leads to thyroid destruction, resulting in thyrotoxicosis, which can exacerbate comorbid conditions such as respiratory distress and diabetes, and in severe cases, lead to multi-organ failure and potentially evolve into permanent hypothyroidism (2). Patients typically present with classic symptoms of upper respiratory tract infection, fever, anterior neck pain, and thyroid dysfunction. Despite decades of research, the pathogenesis and critical factors influencing the clinical course of this disease remain incompletely understood. Reports of SAT following COVID-19 vaccination are scarce; however, there have been instances of SAT following other vaccinations, including influenza and hepatitis B vaccines (3, 4).

Since the onset of the COVID-19 pandemic in 2020, an unparalleled global public health emergency has been instigated. In light of this crisis, scientists, medical professionals, and researchers worldwide have been diligently engaged in the pursuit of an efficacious vaccine against this rampant infectious ailment. Over time, numerous COVID-19 vaccines have been triumphantly formulated, prompting governments across the globe to actively initiate large-scale vaccination campaigns, thereby fostering renewed optimism in curtailing the epidemic. During the process of vaccination, adverse reactions induced by vaccines are a matter of significant apprehension, necessitating continuous surveillance and comprehensive investigation (5). Among these reactions, the occurrence of subacute thyroiditis subsequent to vaccination has sparked extensive deliberation and thorough exploration into the possible correlation between vaccines and thyroid disease.

Case presentation

The patient is a middle-aged woman who has not received any other vaccines within the past six months and has no history of COVID-19 infection. There was no history of thyroid disease diagnosis in the patient's immediate family, encompassing both parents and offspring. After receiving the first dose of the SARS inactivated vaccine (BBIBP-CorV), she developed a sore throat and fever 72 h later. Her body temperature reached up to 38.6°C and she did not experience coughing, sputum production, palpitations, or dyspnea. She self-administered cephalosporin antibiotics and ibuprofen, but her pain symptoms did not significantly improve. An examination at the otolaryngology department confirmed that her tonsils were enlarged at I°, the posterior pharyngeal and lateral pharyngeal walls were congested and swollen, and the thyroid gland was mildly tender but not significantly enlarged. Other tests, including COVID-19 nucleic acid, antibodies, influenza A and B viruses, and respiratory pathogens, were negative. Nevertheless, it was observed that the WBC and neutrophil ratios exhibited a slight increase, whereas the levels of CRP and ESR were significantly elevated and displayed a strong correlation with SAT. Her liver and kidney function were normal, and there were no obvious abnormalities found in abdominal color ultrasound, chest CT, and electrocardiogram. She received intravenous infusion of cefazolin sodium, vitamin C, and nebulization of the throat as treatment. During the treatment, she experienced recurrent fever and repeated COVID-19 nucleic acid tests were negative. After consultation with the endocrinology department, it was discovered that her thyroid-stimulating hormone (TSH) was decreased, while free thyroxine (FT4), free triiodothyronine (FT3), and thyroglobulin (Tg) were increased. Thyroglobulin antibody (TgAb) and anti-thyroid peroxidase antibody (TPOAb) were not found to be special (Table 1). Thyroid ultrasound revealed hypoechoic areas with blurred edges, irregular shapes, and decreased blood vessels, indicating thyroiditis and cysts in the right lobe of the thyroid gland (TI--RADS class 2) (Figure 1). The patient independently administered ibuprofen prior to hospitalization. Although her body temperature briefly improved, her symptoms of a sore throat persisted, and she encountered recurrent fevers and a sore throat throughout her hospitalization. After excluding other infections and potential sources of fever and sore throat, subacute thyroiditis was contemplated based on the patient's medical history and examination findings. Once the diagnosis was verified, it was established that the ibuprofen consumed prior to admission did not alleviate the symptoms of a sore throat. The therapeutic efficacy of non-steroidal drugs was deemed inadequate for the patient, thus prompting the administration of prednisone 10 mg qd. Following the administration of prednisone, the patient experienced alleviation of neck pain symptoms, normalization of body temperature, and absence of subsequent recurrence during the subsequent outpatient follow-up.

Discussion

Subacute thyroiditis is a thyroid disorder that typically manifests 2–8 weeks following viral infection and exhibits a higher prevalence among women, particularly within the 40–50 age bracket (6). The classic initial clinical manifestations of SAT include neck pain or tenderness, fever, palpitations, accompanied by a range of thyroid dysfunction indicators, with early-stage thyrotoxicosis as the primary clinical feature (7). These symptoms result from a regressive process affecting the thyroid follicular epithelium, leading to the release of thyroglobulin, thyroxine, and an assortment of other iodine-rich molecular fragments into the systemic circulation, thus indicating a potential trend towards hypothyroidism in this patient subgroup (1, 7, 8). The confirmation of SAT diagnosis depends on a synergistic assessment encompassing clinical symptomatology, an array of laboratory diagnostic techniques, and radiological imaging findings. Typical laboratory markers comprise thyroid functional abnormalities, identifiable thyroid lesions via ultrasound, and increased titers of serum thyroid antibodies, with the majority of patients testing positive for thyroid peroxidase (TPO) antibodies and a minority for thyroglobulin antibodies. The diagnostic process for SAT invariably involves systematically ruling out other thyroid pathologies, including hyperthyroidism and subclinical hyperthyroid variants. The etiology of SAT is inherently multifaceted, encompassing factors such as viral invasion of the thyroid, unique immune response mechanisms, and genetic predispositions (8–10).

The primary approach to managing subacute thyroiditis involves alleviating clinical symptoms and addressing potential complications. Patients frequently necessitate sufficient rest, analgesics, and nonsteroidal anti-inflammatory drugs to mitigate pain and elevated body temperature. In severe cases, oral corticosteroids may be administered to alleviate symptoms of thyroiditis, if deemed

TABLE 1 Laboratory investigations.

Variable	Reference range adult	Initial visit	Follow-up		
			2 weeks	4 weeks	2 months
TSH	0.28–4.12 nmol/L	0.02	1.19	3.23	3.38
TT3	1.20–3.15 nmol/L	3.06			
TT4	61.13–164.74 nmol/L	174.06			
FT3	3.05–6.85 pmol/L	11.61	6.72	5.53	5.24
FT4	12.0–21.50 pmol/L	37.43	20.46	17.32	17.79
Anti-TPO	<9 IU/mL	9.0			7.4
Anti-TG	<4 IU/mL	0.1			0.1
Tg	1.15–130.77 ng/mL	266.96			103.29
ESR	<20 mm/h	108	13		3
CRP	<5.0 mg/L	62.43	5.13		1.42
WBC	$4.0–10.0 \times 10^9$/mL	11.63	7.48		3.25

WBC, white bood cell; CRP, c-reactive protein; ESR, erythrocyte sedimentation rate; TSH, thyroid stimulating hormone; TT3, total Triiodothyronin; TT4, total thyroxine; FT3, tree triiodothyronine; FT4, tree thyroxine; Anti-TPO, anti-thyroid teroxidase ntibody; Anti-TG, anti-thyroglobulin; Tg, thyroglobulin.

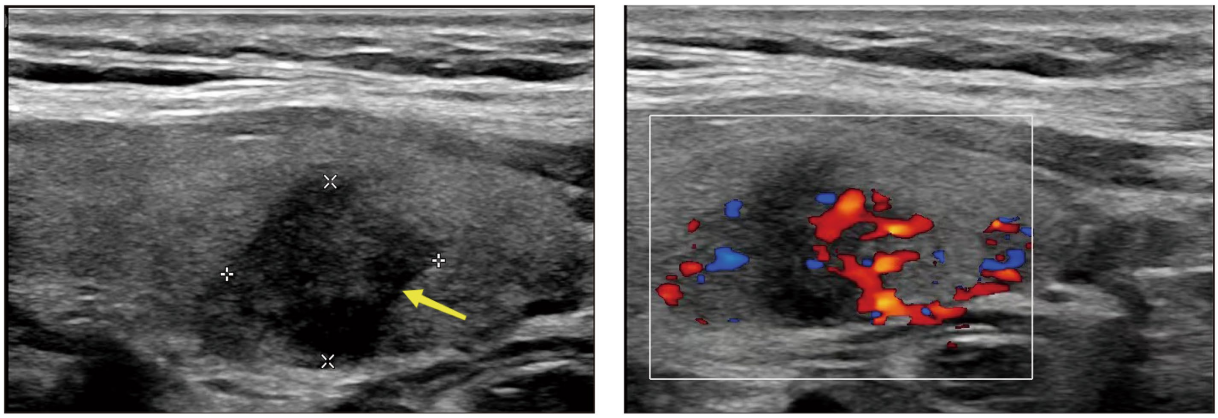

FIGURE 1
The echo of thyroid is decreased, and the density is uneven.

necessary (11, 12). The majority of patients are anticipated to experience recovery within a span of weeks to months, although a subset of individuals may experience a delay in the onset of chronic thyroiditis (11).

In light of the emergence of the novel coronavirus, various SARS-CoV-2 vaccines have been formulated, encompassing mRNA vaccines (Pfizer/BioNTech, Moderna), viral vector-based vaccines (ChAdOx1 nCoV-19 vaccine, CanSino Biologics, Gamaleya Institute, Johnson & Johnson), inactivated vaccines (CoronaVac, Sinovac, Bharat Biotech BBV 152, Sinopharm BIBP), and protein subunit-based vaccines (Novax, Chinese Academy of Sciences) (13–17).

Globally, there have been reported incidents of adverse reactions from diverse vaccine types, but on the whole, instances of subacute thyroiditis ensuing from vaccination are uncommon (17–20). Such occurrences frequently manifest within a few days to weeks post-vaccination and exhibit characteristic symptoms of thyroiditis, encompassing severe neck pain, thyroid swelling, and thyroid function disruption (15). These reports meticulously detail patients presenting with thyroiditis symptoms post-vaccination, dismiss other influencing factors, and provide comprehensive information concerning their clinical manifestations, laboratory examination outcomes, and additional related details. The objective of these reports is to elucidate potential correlations between vaccination and thyroid disorders and alert potential risks. Postpondered from case reports (5, 17, 19), SARSCoV-2 vaccination can induce 70% of mRNA-inducing individuals vaccinated, with viral vector-based vaccines accounting for 18% of reported cases. Conversely, SARSCoV-2 inactivated vaccines infrequently evoke SAT. An analysis of relevant case data indicates that 55% of patients experienced SAT following their first dose of SARS-CoV-2 vaccine, while this proportion rises to 44% after the second dose (5, 6, 17). Concerning the duration between administration of the vaccine and symptom appearance, a median time of 10 days was identified, ranging from as short as 12 h to as long as 84 days, which varies from the typical presentation of SAT (15, 20).

The precise etiology of subacute thyroiditis following vaccination remains uncertain; however, several studies propose that vaccines may elicit immune system activation, particularly the autoimmune response, resulting in potential damage to thyroid tissue (10). This

observation implies that vaccine-related thyroid complications may pose a rare yet tangible hazard, particularly among individuals with pre-existing thyroid conditions or genetic susceptibility (21–25).

Autoimmunity has been explained by several hypotheses, including molecular mimicry (26, 27). Some scholars are positing that the immune response triggered by the SARS-CoV-2 spike protein and the SARS-CoV-2 nucleoprotein results in the generation of cross-reactive antibodies (23, 26, 28). These antibodies, in turn, engage with various tissue antigens, including thyroid tissue, thereby instigating the development of autoimmune diseases, such as SAT (29, 30). The mechanism is SARS-CoV-2 single-stranded RNA viruses with similar structures to the novel coronavirus, and different types of COVID19 vaccines also share a common feature, namely molecular mimicry between S protein, viral protein, and human tissue, and the immune response to SARSCoV2 spike protein and Nucleo protein leads to cross-reactivity to produce antibodies, and their interaction with different tissue antigens, including thyroid tissue, leads to autoimmune thyroid disease (25–32). Simultaneously, the S protein's interaction with the angiotensin-converting enzyme 2 (ACE2) receptor situated on the cellular membrane facilitates the virus's adherence to said receptor, subsequently initiating viral entry into the cell and consequent infection (28, 33, 34).

Furthermore, adjuvants have been found to augment the immunogenicity of vaccines, bolster innate and autoimmune responses, and potentially trigger the production of autoantibodies or local/systemic inflammation (35–40). Nevertheless, when adjuvants are present, viruses have the capability to elicit diverse inflammatory and autoimmune reactions in genetically vulnerable populations while interacting with host cells (37, 40–43). COVID-19 vaccines incorporate various excipients, including aluminum hydroxide or aluminum salts (found in the Coronavac vaccine), polysorbate 80 (used in the AstraZeneca vaccine), and polyethylene glycol (PEG) lipid conjugates, stable lipid nanoparticles, among others (29, 36). These excipients may serve as adjuvants in mRNA vaccines (such as Pfizer/BioNTech) and water–oil emulsion formulations, potentially leading to autoimmune or allergic responses following COVID-19 vaccination (27, 44, 45).

Moreover, research has demonstrated that metabolites derived from SARS-CoV-2 disrupt the configuration and operation of human leukocyte antigen (HLA), a phenomenon associated with the resemblance between human leukocyte antigen (HLA) genes and SARS-CoV-2 antigens (43, 46–49). This resemblance renders certain individuals more prone to thyroid disease (47, 50). Simultaneously, specific variants of HLA (such as HLAB35) have exhibited heightened susceptibility to this virus-susceptible antigen (43, 48). The activation of the HLAB35 antigen complex has the potential to initiate immune-mediated damage to thyroid follicular cells (5, 51). Additionally, there is a noteworthy concern regarding certain factors previously identified as risk factors (such as individual or familial autoimmune disease or pregnancy) or predictors (such as smoking, high-pressure environment, or drug intake), as they can influence the development of autoimmunity following COVID-19 vaccination (22, 49). In many instances, this can result in the manifestation of inflammatory thyroid disease.

According to the inflammatory factor storm theory, subacute thyroiditis caused by most SARS-CoV-2 vaccines typically presents with inflammatory symptoms such as neck pain, myalgia, and fever. Additionally, thyroid color ultrasound examination may reveal structural abnormalities and hypoechoic areas in the thyroid, and some patients may experience thyroid enlargement. Blood biochemical tests have shown that almost all patients exhibit thyrotoxicosis and elevated levels of serum inflammatory markers like CRP/ESR. Most patients respond well to treatment with NSAIDs or prednisolone (or other steroids), indicating an inflammatory response. The mechanism behind this inflammatory response may be associated with the release of cytokines, which can initiate a chain reaction resulting in increased levels of circulating interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1RA), chemokine 2 (CCL2), chemokine 8 (CCL8), as well as chemical antagonists of T cells or natural killer (NK) cells including chemoattractant 9 (CXCL9) and chemokine 16 (CXCL16) (24, 38, 39, 52).

However, due to the frequent overlap in clinical manifestations between SAT and other diseases, there is a potential for misdiagnosis or missed diagnosis. Consequently, clinicians must be highly vigilant when encountering patients who exhibit thyroid-related symptoms post-vaccination, particularly in cases following COVID-19 vaccination. If vaccine-induced SAT is suspected, the priority should be to conduct timely and comprehensive clinical assessments along with thyroid function tests, to lay a solid foundation for accurate diagnosis and the prompt implementation of an appropriate treatment plan. Additionally, healthcare professionals need to be aware of the potential link between vaccinations and thyroid issues in order to provide more precise medical guidance.

Conclusion

This article aims to explore the possible relationship between vaccines and subacute thyroiditis by examining a case of misdiagnosis due to overlooked SAT, as well as reviewing recent case reports from various countries. It suggests that vaccines may trigger an immune response leading to the development or exacerbation of SAT, especially in females and individuals with existing thyroid disorders or genetic predispositions. However, the incidence of SAT associated with COVID-19 vaccines appears to be relatively low, which may be due to challenges in accurate diagnosis as typical clinical symptoms are often overlooked or masked and mistaken for other conditions, and are mostly transient in nature. Conclusive assessment can be aided by certain biochemical tests, such as elevated serum inflammatory markers and thyrotoxicosis. Ultrasound examination may reveal thyroid enlargement, structural changes, and hypoechoic areas, while reduced blood flow can be observed through thyroid Doppler imaging. Treatment typically involves the use of non-steroidal anti-inflammatory drugs or corticosteroids. Notably, the likelihood of SAT induced by inactivated SARS-CoV-2 vaccines during the COVID-19 pandemic seems lower. Patients with fever and neck pain may incorrectly use non-steroidal anti-inflammatory drugs, potentially affecting the presentation of temperature and neck pain. Moreover, relying solely on symptomatic treatment with oral medications may mask the progression of the disease. Although most mild cases resolve spontaneously, some individuals may experience delayed progression, leading to hypothyroidism, thyroid storm, or other serious complications, potentially exacerbating the condition or posing a life-threatening risk. Therefore, medical experts must remain vigilant when assessing individuals with thyroid symptoms, particularly following COVID-19 vaccination, to ensure accurate diagnosis and timely treatment, as early clinical and thyroid function assessments are crucial for confirming diagnosis and expediting treatment. Vaccination is a critical public health intervention that significantly

combats the spread of novel coronavirus and mitigates the severity of the pandemic. Thus, vaccination programs should closely monitor and document any adverse reactions related to thyroid complications to fully understand their incidence and characteristics. Future research should further investigate the association between vaccination and thyroid disorders to strengthen the scientific basis for formulating immunization strategies that prioritize public health and welfare.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

SY: Writing – original draft. TG: Data curation, Writing – review & editing. HY: Formal analysis, Writing – review & editing. YH: Conceptualization, Supervision, Writing – review & editing. YZ: Conceptualization, Project administration, Supervision, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1349615/full#supplementary-material

References

1. Kojima M, Nakamura S, Oyama T, Sugihara S, Sakata N, Masawa N. Cellular composition of subacute thyroiditis. An immunohistochemical study of six cases. *Pathol Res Pract*. (2002) 198:833–7. doi: 10.1078/0344-0338-00344

2. Pearce EN, Farwell AP. Thyroiditis In: J Wass, W Arlt and R Semple, editors. *Oxford textbook of endocrinology and diabetes 3e. 3rd* ed. Oxford: Oxford University Press (2021). 443–53.

3. Toft J, Larsen S, Toft H. Subacute thyroiditis after hepatitis B vaccination. *Endocr J*. (1998) 45:135.

4. Altay FA, Güz G, Altay M. Subacute thyroiditis following seasonal influenza vaccination. *Hum Vaccin Immunother*. (2016) 12:1033–4. doi: 10.1080/21645515.2015.1117716

5. Oğuz SH, Şendur SN, İremli BG, Gürlek A, Erbas T, Ünlütürk U. SARS-CoV-2 vaccine–induced thyroiditis: safety of revaccinations and clinical follow-up. *J Clin Endocrinol Metabol*. (2022) 107:e1823–34. doi: 10.1210/clinem/dgac049

6. Hsiao J, Hsin S, Hsieh M, Hsia PJ, Shin SJ. Subacute thyroiditis following influenza vaccine (Vaxigrip®) in a young female. *Kaohsiung J Med Sci*. (2006) 22:297–300. doi: 10.1016/S1607-551X(09)70315-8

7. Alfadda AA, Sallam RM, Elawad GE, AlDhukair H, Alyahya MM. Subacute thyroiditis: clinical presentation and long term outcome. *Int J Endocrinol*. (2014) 2014:1–7. doi: 10.1155/2014/794943

8. Muller I, Cannavaro D, Dazzi D, Covelli D, Mantovani G, Muscatello A, et al. SARS-CoV-2-related atypical thyroiditis. *Lancet Diabetes Endocrinol*. (2020) 8:739–41. doi: 10.1016/S2213-8587(20)30266-7

9. Desailloud R, Hober D. Viruses and thyroiditis: an update. *Virol J*. (2009) 6:5. doi: 10.1186/1743-422X-6-5

10. Olivieri B, Betterle C, Zanoni G. Vaccinations and autoimmune diseases. *Vaccine*. (2021) 9:815. doi: 10.3390/vaccines9080815

11. Mundy-Baird G, Kyriacou A, Syed AA. De Quervain subacute thyroiditis. *Can Med Assoc J*. (2021) 193:E1007–7. doi: 10.1503/cmaj.202787

12. Lanzo N, Patera B, Fazzino G, Gallo D, Lai A, Piantanida E, et al. The old and the new in subacute thyroiditis: an integrative review. *Endocrine*. (2022) 3:391–410. doi: 10.3390/endocrines3030031

13. Bornemann C, Woyk K, Bouter C. Case report: two cases of subacute thyroiditis following SARS-CoV-2 vaccination. *Front Med*. (2021) 8:8.737142. doi: 10.3389/fmed.2021.737142

14. Franquemont S, Galvez J. Subacute thyroiditis after mRNA vaccine for Covid-19. *J Endocr Soc*. (2021) 5:A956–7. doi: 10.1210/jendso/bvab048.1954

15. Oyibo SO. Subacute thyroiditis after receiving the adenovirus-vectored vaccine for coronavirus disease (COVID-19). *Cureus*. (2021) 1:2–10. doi: 10.7759/cureus.16045

16. İremli BG, Şendur SN, Ünlütürk U. Three cases of subacute thyroiditis following SARS-CoV-2 vaccine: postvaccination ASIA syndrome. *J Clin Endocrinol Metabol*. (2021) 106:2600–5. doi: 10.1210/clinem/dgab373

17. Jafarzadeh A, Nemati M, Jafarzadeh S, Nozari P, Mortazavi SMJ. Thyroid dysfunction following vaccination with COVID-19 vaccines: a basic review of the preliminary evidence. *J Endocrinol Investig*. (2022) 45:1835–63. doi: 10.1007/s40618-022-01786-7

18. Jara LJ, Vera-Lastra O, Mahroum N, Pineda C, Shoenfeld Y. Autoimmune post-COVID vaccine syndromes: does the spectrum of autoimmune/inflammatory syndrome expand? *Clin Rheumatol*. (2022) 41:1603–9. doi: 10.1007/s10067-022-06149-4

19. Şendur SN, Oğuz SH, Ünlütürk U. COVID-19 vaccination and thyroiditis. *Best Pract Res Clin Endocrinol Metab*. (2023) 37:101759. doi: 10.1016/j.beem.2023.101759

20. Bahçecioğlu AB, Karahan ZC, Aydoğan Bİ, Kalkan İA, Azap A, Erdoğan MF. Subacute thyroiditis during the COVID-19 pandemic: a prospective study. *J Endocrinol Investig*. (2022) 45:865–74. doi: 10.1007/s40618-021-01718-x

21. Şendur SN, Özmen F, Oğuz SH, İremli BG, Malkan ÜY, Gürlek A, et al. Association of Human Leukocyte Antigen Genotypes with severe acute respiratory syndrome coronavirus 2 vaccine-induced subacute thyroiditis. *Thyroid*. (2022) 32:640–7. doi: 10.1089/thy.2022.0010

22. Rossetti CL, Cazarin J, Hecht F, Beltrão FEL, Ferreira ACF, Fortunato RS, et al. COVID-19 and thyroid function: what do we know so far? *Front Endocrinol*. (2022) 13:1041676. doi: 10.3389/fendo.2022.1041676

23. Vera-Lastra O, Ordinola Navarro A, Cruz Domiguez MP, Medina G, Sánchez Valadez TI, Jara LJ. Two cases of graves' disease following SARS-CoV-2 vaccination: an

autoimmune/inflammatory syndrome induced by adjuvants. *Thyroid*. (2021) 31:1436–9. doi: 10.1089/thy.2021.0142

24. Shah VK, Firmal P, Alam A, Ganguly D, Chattopadhyay S. Overview of immune response during SARS-CoV-2 infection: lessons from the past. *Front Immunol*. (2020) 11:1949. doi: 10.3389/fimmu.2020.01949

25. Vojdani A, Kharrazian D. Potential antigenic cross-reactivity between SARS-CoV-2 and human tissue with a possible link to an increase in autoimmune diseases. *Clin Immunol*. (2020) 217:108480. doi: 10.1016/j.clim.2020.108480

26. Rowley D, Jenkin CR. Antigenic cross-reaction between host and parasite as a possible cause of pathogenicity. *Nature*. (1962) 193:151–4. doi: 10.1038/193151a0

27. Vojdani A, Vojdani E, Kharrazian D. Reaction of human monoclonal antibodies to SARS-CoV-2 proteins with tissue antigens: implications for autoimmune diseases. *Front Immunol*. (2021) 11:617089. doi: 10.3389/fimmu.2020.617089

28. Rotondi M, Coperchini F, Ricci G, Denegri M, Croce L, Ngnitejeu ST, et al. Detection of SARS-COV-2 receptor ACE-2 mRNA in thyroid cells: a clue for COVID-19-related subacute thyroiditis. *J Endocrinol Investig*. (2021) 44:1085–90. doi: 10.1007/s40618-020-01436-w

29. Zhang Z, Shen Q, Chang H. Vaccines for COVID-19: a systematic review of immunogenicity, current development, and future prospects. *Front Immunol*. (2022) 13:843928. doi: 10.3389/fimmu.2022.843928

30. Liang Z, Zhu H, Wang X, Jing B, Li Z, Xia X, et al. Adjuvants for coronavirus vaccines. *Front Immunol*. (2020) 11:589833. doi: 10.3389/fimmu.2020.589833

31. Finkel Y, Mizrahi O, Nachshon A, Weingarten-Gabbay S, Morgenstern D, Yahalom-Ronen Y, et al. The coding capacity of SARS-CoV-2. *Nature*. (2020) 589:125–30. doi: 10.1038/s41586-020-2739-1

32. Kanduc D. Peptide cross-reactivity the original sin of vaccines. *Front Biosci-Schol*. (2012) S4:1393–401. doi: 10.2741/s341

33. Hoffmann M, Kleine-Weber H, Schroeder S, Krüger N, Herrler T, Erichsen S, et al. SARS-CoV-2 cell entry depends on ACE2 and TMPRSS2 and is blocked by a clinically proven protease inhibitor. *Cell*. (2020) 181:271–280.e8. doi: 10.1016/j.cell.2020.02.052

34. Marazuela M, Giustina A, Puig-Domingo M. Endocrine and metabolic aspects of the COVID-19 pandemic. *Rev Endocr Metab Disord*. (2020) 21:495–507. doi: 10.1007/s11154-020-09569-2

35. Exley C, Siesjö P, Eriksson H. The immunobiology of aluminium adjuvants: how do they really work? *Trends Immunol*. (2010) 31:103–9. doi: 10.1016/j.it.2009.12.009

36. Li Q, Li Z, Deng N, Ding F, Li Y, Cai H. Built-in adjuvants for use in vaccines. *Eur J Med Chem*. (2022) 227:113917. doi: 10.1016/j.ejmech.2021.113917

37. Watad A, Bragazzi NL, McGonagle D, Adawi M, Bridgewood C, Damiani G, et al. Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) demonstrates distinct autoimmune and autoinflammatory disease associations according to the adjuvant subtype: insights from an analysis of 500 cases. *Clin Immunol*. (2019) 203:1–8. doi: 10.1016/j.clim.2019.03.007

38. Cohen Tervaert JW, Martinez-Lavin M, Jara LJ, Halpert G, Watad A, Amital H, et al. Autoimmune/inflammatory syndrome induced by adjuvants (ASIA) in 2023. *Autoimmun Rev*. (2023) 22:103287. doi: 10.1016/j.autrev.2023.103287

39. Perricone C, Alessandri C, Valesini G. 'ASIA' – autoimmune/inflammatory syndrome induced by adjuvants: even and odd. *Reumatismo*. (2011) 63:63–6. doi: 10.4081/reumatismo.2011.63

40. Krammer F. SARS-CoV-2 vaccines in development. *Nature*. (2020) 586:516–27. doi: 10.1038/s41586-020-2798-3

41. Zhang N, Li K, Liu Z, Nandakumar KS, Jiang S. A perspective on the roles of adjuvants in developing highly potent COVID-19 vaccines. *Viruses*. (2022) 14:387. doi: 10.3390/v14020387

42. Kupferschmidt K, Cohen J. Race to find COVID-19 treatments accelerates. *Science*. (2020) 367:1412–3. doi: 10.1126/science.367.6485.1412

43. Soriano A, Nesher G, Shoenfeld Y. Predicting post-vaccination autoimmunity: who might be at risk? *Pharmacol Res*. (2015) 92:18–22. doi: 10.1016/j.phrs.2014.08.002

44. Holm MR, Poland GA. Critical aspects of packaging, storage, preparation, and administration of mRNA and adenovirus-vectored COVID-19 vaccines for optimal efficacy. *Vaccine*. (2021) 39:457–9. doi: 10.1016/j.vaccine.2020.12.017

45. Ippolito S, Gallo D, Rossini A, Patera B, Lanzo N, Fazzino GFM, et al. SARS-CoV-2 vaccine-associated subacute thyroiditis: insights from a systematic review. *J Endocrinol Investig*. (2022) 45:1189–200. doi: 10.1007/s40618-022-01747-0

46. Stasiak M, Tymoniuk B, Adamczewski Z, Stasiak B, Lewiński A. Sonographic pattern of subacute thyroiditis is HLA-dependent. *Front Endocrinol*. (2019) 10:3. doi: 10.3389/fendo.2019.00003

47. Stasiak M, Lewiński A. New aspects in the pathogenesis and management of subacute thyroiditis. *Rev Endocr Metab Disord*. (2021) 22:1027–39. doi: 10.1007/s11154-021-09648-y

48. Sahin Tekin M, Yorulmaz G, Yantir E, Gunduz E, Colak E. A novel finding of an HLA Allele's and a Haplotype's relationship with SARS-CoV-2 vaccine-associated subacute thyroiditis. *Vaccine*. (2022) 10:1986. doi: 10.3390/vaccines10121986

49. Tutal E, Ozaras R, Leblebicioglu H. Systematic review of COVID-19 and autoimmune thyroiditis. *Travel Med Infect Dis*. (2022) 47:102314. doi: 10.1016/j.tmaid.2022.102314

50. Stasiak M, Zawadzka-Starczewska K, Lewiński A. Clinical manifestation of subacute thyroiditis triggered by SARS-CoV-2 infection can be HLA-dependent. *Viruses*. (2021) 13:2447. doi: 10.3390/v13122447

51. Stasiak M, Tymoniuk B, Michalak R, Stasiak B, Kowalski M, Lewiński A. Subacute thyroiditis is associated with HLA-B*18:01, -DRB1*01 and -C*04:01—the significance of the new molecular background. *J Clin Med*. (2020) 9:534. doi: 10.3390/jcm9020534

52. Mehta P, McAuley DF, Brown M, Sanchez E, Tattersall RS, Manson JJ. COVID-19: consider cytokine storm syndromes and immunosuppression. *Lancet*. (2020) 395:1033–4. doi: 10.1016/S0140-6736(20)30628-0

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Current knowledge of human Mpox viral infection among healthcare workers in Cameroon calls for capacity-strengthening for pandemic preparedness

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Introduction: An increased incidence of human Monkeypox (Mpox) cases was recently observed worldwide, including in Cameroon. To ensure efficient preparedness and interventions in the health system, we sought to assess the knowledge of Mpox's transmission, prevention, and response among healthcare workers (HCWs) in Cameroon.

Methods: A cross-sectional online survey was conducted among HCWs in Cameroon using 21-item questions adapted from the United States Centers for Disease Control and Prevention (US-CDC) standard questionnaire on Mpox. The

overall knowledge of Mpox was assessed by cumulative score and categorized as excellent (≥80%, 17/21) or good (≥70%, ≥15/21) knowledge. The regression analysis was used to identify the predictors of Mpox knowledge.

Results: The survey enrolled 377 participants, but only responses from 342 participants were analyzed. Overall, 50.6% were female participants, and 59.6% aged 30 years or younger. The majority of the participants were medical doctors (50.3%); most worked in central-level hospitals (25.1%) and had 1–5 years of experience (70.7%). A total of up to 92.7% were aware of Mpox, with social media (58.7%) and radio/television (49.2%) as the main sources. The mean knowledge score was 14.0 ± 3.0 (4 to 20), with only 12.9% having excellent knowledge (≥80%) and 42.1% having good knowledge of Mpox. Younger age (26–30 years old) was associated with good knowledge, while workplace type was associated with excellent knowledge of Mpox (aOR [95% CI]: 4.01 [1.43–11.24]). Knowledge of treatment/management of Mpox was generally poor across the different professional categories.

Conclusion: Knowledge of Mpox among HCWs is substandard across different professionals. Thus, for optimal preparedness and immediate interventions for Mpox and similar emerging pathogens, capacity-strengthening programs should be organized for HCWs while encouraging scientific literature and organizational social media websites.

KEYWORDS

monkeypox (Mpox), knowledge, healthcare workers, emerging pathogens, Cameroon

1 Introduction

Human monkeypox (Mpox) is a viral zoonosis caused by the monkeypox (Mpox) virus belonging to the orthopoxvirus genus of the Poxviridae family (the same family as the virus that caused smallpox, which has now been eradicated). The virus is endemic in West and Central Africa, where it is thought to exist primarily in different types of rodents. There are two groups or "clades" of Mpox, one found in the Congo Basin of Central Africa with a case fatality of up to 10% and the other in West Africa with a case fatality rate of <3% (1, 2).

Mpox can be transmitted via direct contact with infected body fluids, sexual contacts, lesion material from humans or animals, or indirect contact with contaminated material (3, 4). Human-to-human transmission occurs primarily through large respiratory droplets (5). The symptoms include fever, headache, malaise, muscle aches, swollen lymph nodes, and proctitis (6), followed by a rash a few days later that begins on the face and spreads to other parts of the body. The complications of monkeypox infections include secondary infections, bronchopneumonia, sepsis, encephalitis, and infection of the cornea with ensuing loss of vision. The illness can last up to 4 weeks but starts to fade when the skin lesions begin to subside (7). The virus is known to evade detection by the inhibition of the host antiviral immune response (antiviral chemokines, cytokines, and antigen presentation) and the suppression of the activation of T-cells (8).

Mpox was first identified in 1958 during an outbreak of Mpox in the Asian monkey *Macaca fascicularis,* which was used for polio vaccine research at an animal facility in Copenhagen, Denmark (9). The first Mpox case in humans was reported in the Democratic Republic of Congo (DRC, previously known as Zaire) in 1970, and the disease has remained endemic in the country and other African countries (2).

Since 2016, cases have appeared in the Central African Republic, Liberia, Nigeria, and Sierra Leone(1). In 2017, the largest outbreak of Mpox was reported in Nigeria, with 197 suspected cases and 68 confirmed cases, and by the end of 2018, the number of confirmed cases increased to 89, with a case fatality rate of 6.7% (1, 10). Human Mpox cases have also been previously reported in the United States in June 2003 (11, 12), in the UK (13) in September 2018, and in Israel (14) on 4 October 2018. In the case of the United States, Mpox was transmitted from infected native prairie dogs that were housed with infected exotic pets imported from Africa (11, 12), while in the UK (13) and Israel (14), patients were travelers who had returned from Nigeria.

As an epicenter or endemic country for Mpox, the Democratic Republic of the Congo conducts routine Mpox surveillance and clinical trials on potential Mpx vaccines among HCWs (15, 16). One of the important aspects of the surveillance system is to enhance the capacity of healthcare workers (HCWs) to identify and report cases and improve patient management (16). For an optimal response strategy, HCWs, particularly medical doctors and nurses, should have knowledge about the transmission patterns and clinical symptoms of Mpox to be able to quickly identify, report, and manage new cases to prevent further community-related or nosocomial transmission.

The Africa CDC outbreak brief on the MPox pandemic in January 2023 indicated that between January 2022 and January 2023, 1,296 cases and 228 deaths (CFR: 17.6%) in 13 African Union (AU) member states were reported. These countries

include Cameroon (18 confirmed cases; 3 confirmed deaths), Benin (3 confirmed cases;0 confirmed deaths), Central African Republic (CAR) (13 confirmed cases;3 confirmed deaths), Congo (5 confirmed cases;3 confirmed deaths), the Democratic Republic of Congo (DRC) (319 confirmed cases;204 confirmed deaths), Ghana (116 confirmed cases;4 confirmed deaths), Liberia (6 confirmed cases;0 confirmed deaths), Nigeria (756 confirmed cases;7 confirmed deaths), Egypt (4 confirmed cases;0 confirmed deaths), Morocco (3 confirmed cases;0 confirmed deaths), Mozambique (1 confirmed cases;1 confirmed deaths), South Africa (5 confirmed cases;0 confirmed deaths), and Sudan (18 confirmed cases;1 confirmed deaths) (17).

In Cameroon, between 30 April and 30 May 2018, a total of 16 suspected cases (1 confirmed and 15 suspected cases) were reported to the Department of Disease, Epidemic and Pandemic Control of the Ministry of Public Health (18). These cases were identified in five health districts (HD) within five regions of Cameroon, namely, Njikwa HD ($n = 6$ suspected, $n = 1$ confirmed), Akwaya HD ($n = 6$ suspected), Biyem-Assi HD ($n = 1$ suspected), Bertoua HD ($n = 1$ suspected), and Fotokol HD ($n = 1$ suspected), with newer hot spots identified in other geographical locations, particularly, in the South West region (18). To mitigate this emerging global threat at the country level, the government of Cameroon developed and implemented a public health response strategy, which included the training of HCWs on infection prevention and control (IPC), with emphasis on the use of personal protective equipment, hand hygiene, and physical distancing, where necessary. Information related to the isolation of cases, symptomatic case management, and hand-washing techniques has been shared widely by the IPC workforce within hot spots and high-risk settings (19). A recent outbreak was reported in Cameroon in September 2022, in the South West region (20), and as of 19 April 2023, Cameroon had recorded 106 suspected cases, 18 confirmed cases, and 3 deaths related to Mpox (21, 22). These confirmed cases were found in four out of the five regions (South, Centre, North West, and South West), which called for the strengthening of the response strategy to stop its spread (23).

The increased number of human Mpox cases demonstrates the need and the importance of IPC, early detection, quick response, and the management of disease from HCWs. A report by the WHO and Africa CDC showed that one of the challenges faced in preventing the re-emergence of Mpox is the lack of sufficient knowledge about Mpox among HCWs in several countries, including high- and low-income settings (2).

Shafaati et al. (8) emphasized the importance of awareness and training campaigns to address the risks of sexual transmission of Mpox and prevent stigmatization of certain groups. A recent cross-sectional study assessing Mpox knowledge and attitudes of HCWs in some hospitals in Southern Italy in 2022 reported an unsatisfactory knowledge assessment, with a reported mean score of only 3.4 (0–9) (24). Furthermore, in a systematic review conducted by Mohamed L. and Abanoub A. in 2022, the overall knowledge of Mpox was unsatisfactory among nine articles, especially when assessing the knowledge of Mpox in Europe, the Middle East, and Asia (25). More precisely, poor knowledge of Mpox can lead to a large circulation of undiagnosed infections and thus skew epidemiological trends in resource-limited settings (RLS). Hence, in order to support the national response against Mpox at the country level, we sought to assess the knowledge of Mpox's transmission and management among HCWs in Cameroon.

2 Methods

2.1 Study design and settings

Within the framework of the country's response to Mpox, a cross-sectional online survey was conducted from August to October 2022 to assess the knowledge of Mpox viral infection among HCWs who are on service within the health system in Cameroon. The design and setting of this study were based on previous studies (26–28).

To achieve our intended goal, we used a random sampling method (self-administered online survey). According to Cameroon's Ministry of Public Health, the country has 39,720 health workers (29). Considering a 5% margin of error and a 95% confidence interval, a minimum of 381 participants were needed for this study. To ensure diversity, target participants, mainly medical doctors, nurses, and other HCWs (pharmacists, dermatologists, laboratory scientists, and nursing assistants) working at various levels of the healthcare system (central-level hospitals, district hospital (primary healthcare facilities), medicalized health centers, private hospitals, and other types of health facilities) were selected. The recruitment strategy involved reaching out to healthcare workers through social media, emails, and professional networks. Efforts were made to ensure diversity and representation by direct phone calls for participation and targeting underrepresented groups where necessary. We acknowledge that online surveys in Knowmedge, attitude and practice (KAP) studies are susceptible to some inherent biases including self-selection, non-response, social desirability, recall, sampling, access, and misinterpretation biases. These biases might have led to an unrepresentative sample, inaccurate responses, and underrepresentation of certain groups. To mitigate these potential biases, we used standardized assessment tools and provided clear instructions to minimize subjective interpretation. The Cameroonian health system has a National Public Health Emergency Coordination Centre with strategic and operational plans in response to infectious diseases of epidemics and pandemic potentials, including COVID-19, Cholera, Mpox, and viral hemorrhagic fevers. Field activities were conducted with the interventions of several stakeholders with a multi-sectorial approach in every hot spot and high-risk geographical location.

2.2 Survey instrument

A pre-tested and standard questionnaire was developed before the commencement of the study. The questionnaire consisted of questions to assess knowledge of Mpox and to collect a range of potential explanatory variables, with a total of 21 item multiple choice questions which were adapted from the United States Centers for Disease Control and Prevention (CDC) questionnaire (30) (see Appendix). The questionnaire was developed in both English and French, which are the two official languages of

Cameroon. For maximal efficiency (validation), pre-testing (pilot) was performed among 20 independent HCWs who were not included in the study (10 medical doctors, 5 nurses, and 5 clinical laboratorians). The questionnaire was then finalized and validated using various feedback obtained from the pilot testing phase. After administering the survey with this pilot group of respondents and repeating the survey with the same group at a later point in time, there was a complete agreement (reliability) between the two time points (kappa = 1). The questionnaire content validity was approved by a majority of independent HCWs (90%, 18/20).

2.3 Data collection

Invitation to complete the anonymous online survey was sent using social media (mainly WhatsApp) or e-mails. Efforts were made to ensure the participation of HCWs from the rural areas, especially in the southern region where people were sensitized during meetings to take up the survey, and up to two reminders were sent after the initial message. The questionnaire entailed detailed features and contacts of the principal investigators for any further clarification, as well as the purpose of the study for informed consent prior to enrolment. The survey was estimated to take ∼7–10 min to complete and without using any documentation. As the selection criteria, this study was limited to only active Cameroonian HCWs practicing in Cameroon, and those who were willing to participate and completed the questionnaire in ≤ 10 min without using any documentation were retained for analysis. The participants who fell short of the aforementioned requirements, as well as those who submitted incomplete responses, who submitted duplicate answers, with inconsistencies in their answers, and whose variables for assessing their level of knowledge were not clearly defined, were excluded from the study.

To ensure confidentiality, the names of the participants were not collected, and only the principal investigator had access to the survey account. At the end of the survey period, the raw data were extracted and imported into statistical software for analysis. Data were protected using specific anonymous and unique identifiers with a password-protected computer. To control and avoid resubmission, duplication, or multiple participation, we used unique identifiers such as email addresses or participant IDs. The study fulfilled the CHERRIES criteria (31).

2.4 Study variables

The response variable in this study was the knowledge of Mpox viral infection among HCWs in Cameroon. The questionnaire included knowledge of Mpox transmission, clinical features, and treatment/management. The questionnaire consisted of a 21-item questionnaire in which a correct response was scored one (1) and an incorrect response was scored zero (0). The scores were summed to give a total score ranging from 0 to 21. Two different cut-off scores were defined: ≥80% (at least 17/21) and ≥70% (at least 15/21), representing excellent and good knowledge of Mpox, respectively. Although previous studies used Bloom's cut-off point of 80–100% as good scores, 60–79% as moderate scores, and <60% and below as poor scores (32), our team decided to create two subdivisions instead of three. Here, we chose to use two scenarios based on the 80% and 70% thresholds and considered scores <70% as indicative of poor knowledge of Mpox. This decision was made to better distribute the survey's scores into more distinct categories given the volume of questions.

To facilitate the analysis and interpretation of data, we operationalized variables into specific categories and ranges. Four main groups of explanatory variables that could affect knowledge were categorized and assessed: sociodemographic characteristics, workplace characteristics, the characteristics of the medical specialty, and exposure to and/or sources of Mpox-related information. According to the distribution of participants, age was categorized into four specific ranges (20–25, 26–30, 31–39, and ≥40 years). The medical profession, defined as the completed/graduate medical or paramedical training, was grouped into the following: medical doctors, nurses, and other HCWs, which represent the three main categories of health workers in Cameroon. Workplace characteristics included the types of health facilities: central-level hospitals, district hospital (primary healthcare facilities), medicalized health centers, private hospitals, and other health facilities which represent the Ministry of Public Health's classification of health facilities. To assess the characteristics of the medical professionals, information on HCWs' job locations (rural or urban), their professional experiences (1–5, 6–10, 11–15, and ≥16 years), and whether they had attended any continuous education or training (local, national, and international conferences in the last 5 months) were collected. To assess exposure to or sources of Mpox-related information, the respondents were asked whether they had ever received Mpox information during their professional training and whether they had heard about Mpox prior to the interview. This categorization allowed for the capture of meaningful differences within these characteristics.

2.5 Statistical analysis

Frequencies, proportions, and confidence intervals were computed, and data were summarized using tables and figures. The associations between the explanatory variables and the dependent variables were assessed using a two-step logistic regression model for both ≥70% and ≥80% cut-off scores, representing good and excellent Mpox knowledge, respectively. Initially, all explanatory variables were analyzed separately in a univariate model, and variables with a p-value of ≤ 0.25 were then included in the multivariable logistic regression analysis to assess the impact of multiple independent variables on the likelihood of good knowledge of Mpox. Good knowledge of Mpox was the baseline variable used for comparison (outcome), and specific variables were chosen for inclusion based on their theoretical relevance to the outcome and existing evidence of their association with good knowledge of Mpox. For comparison, females were used as the reference for the "gender" variable; young HCWs aged 20–25 for the "age" variable, medical doctors for the "medical profession" category, the central hospital (tertiary healthcare facilities) for the "level of health facility", and HCWs with 1–5 years of experience for "years of experience" category.

To ease result interpretations, the estimated crude odds ratio (OR) of unadjusted analyses and the adjusted OR (aOR) were interpreted in relation to a reference category. The significance

was assessed at $p = 0.05$, and analyses were conducted using Statistical Package of Social Sciences version 22.0 software (SPSS Inc., Chicago, IL, USA).

2.6 Ethical considerations

In accordance with the Declaration of Helsinki on good clinical practices and ethical considerations, the present study was approved within the frame of multisectoral surveillance and in response to public health emergencies of zoonotic origin (authorization Ref. N° E2–168/L/MINSANTE/SG/DLMEP/SDLEP from the Ministry of Public Health in Cameroon). Prior to enrollment, the study information sheet was provided to each potential participant, and informed consent was then obtained from each participant. Data confidentiality and privacy of participants were ensured by the use of anonymized unique identifiers, and the data were secured in an encrypted password-protected computer. Only authorized individuals, such as the principal and co-principal investigators, had access to the survey account. The generated data were used to strengthen the capacity of the target population on better outbreak preparedness and response through result dissemination and exploitation.

3 Results

3.1 Respondents' characteristics

During the survey, a total number of 377 responses were received from study respondents, but 35 were excluded due to incomplete information and longer or shorter time of completing the questionnaire (i.e., <5 min to mitigate the risk of bias or more than 15 min to limit events of answers following consultations of information from different sources before responding). Respondents were expected to complete the questionnaire between 7–10 min. The final analysis included 342 (90.7%) respondents, which represents ∼90% (342/381) of the participation rate for the minimum sample size, with a margin of error of 5.3%. The characteristics of the surveyed HCWs are presented in Table 1.

Of the participants enrolled, 8 of the 10 regions of Cameroon were represented. More specifically, 42.6% (146/342) were from Yaounde, 11.9% (41/342) were from Douala, 10.8% were (37/342) from Bafoussam, 9.4% (32/342) were from Ngaoundere, 8.7% (30/342) were from Buea, 7.8% (27/342) were from Bertoua, 6.4% (22/342) were from Ebolowa, and 2.0% (7/342) were from Garoua. More than half of the participants, i.e., 172 (50.3%), were medical doctors. Concerning the gender of the participants, 50.6% (173/342) were female participants; for age, 59.6% were 30 years old or younger. Approximately 25.1% (86/342) of the respondents worked in central-level hospitals, 23.7% (81/342) in medicalized health centers, 10.2% (35/342) in private hospitals, and 27.5% (94/342) in other health facilities (research centers and non-governmental organization). Most of the HCWs (70.7%, 242/342) had a professional experience between 1 and 5 years (Table 1).

3.2 Source of information

In this study, 92.7% (317/342) of the participants reported having heard about Mpox infection; of these, 58.7% (186/317) of them received their information from online media, and 49.2% (156/317) of them received their information from radio/television. Furthermore, 30% (95/317) of the participants gained their information during their medical training, 24% from colleagues, 13.2% from peer-review articles, 17.7% from newspapers or magazines, 18.6% from national or international conferences, and 12.3% from other sources (Figure 1).

3.3 Knowledge of mpox and associated determinants

The median score on Mpox knowledge was 14 (95% CI: 13–15), and the score ranged from 4 to 20. Using the 80% cut-off score, only 44 (12.8%) out of 342 respondents had an excellent knowledge of Mpox. When the cut-off was reduced to 70%, 42.1% (144 out of 342) of respondents had a good knowledge.

Across some domains, the majority of the respondents had accurate knowledge of Mpox. For example, most (91.8%; 314/342) respondents stated that Mpox is caused by a virus, and more than 80% of them stated that Mpox and smallpox have similar signs and symptoms. Approximately 36.1% (218/342) of the respondents stated that some human Mpox cases were detected in Cameroon. Assessing respondents' "knowledge on[sic] transmission," those in the "Others" category [68.3% (67/98)] had poor knowledge of human-to-human transmission (Figure 2). Concerning the zoonotic transmission of Mpox, the majority of participants had at least a good knowledge of ≥70% (Figure 2). Participant's knowledge of clinical features was generally good (≥70%) (Figure 3). However, no professional category had a good knowledge of the presence of vesicles and papules, which are key clinical features of Mpox (Figure 3). Knowledge of treatment/management was generally poor across the different professional categories (<70%) (Figure 4).

The association of Mpox knowledge and some explanatory variables was assessed using both cutoff scores (i.e., 70% and 80%). Using the 80% cutoff score, at the univariate level, the age group of 31–39 years (17.3%) and the "Others" type of workplace were associated with excellent knowledge (OR:4.82 [95% CI:1.0–4.6s], $p = 0.041$; and OR:3.05 [95% CI:1.21–7.63], $p = 0.017$, respectively) compared to those aged 20–25 years and those who worked in central-level hospitals, respectively (Table 2). However, the multivariable analysis showed that the "Others" professional category (OR: 0.32 [95% CI: 0.26–0.82], $p = 0.018$) and the "Others" type of workplace category (OR: 4.01 [95% CI: 1.43–11.24], $p = 0.008$) were independently associated with excellent knowledge of Mpox.

With the lower cut-off score (70%), the age groups 26–30 (46.6%) and 31–39 years (41.8%) were associated with good knowledge of Mpox (OR: 2.63 [95% CI: 1.20–5.40], $p = 0.009$; and OR: 2.1 [95% CI: 1.0–4.6], $p = 0.04$, respectively), when compared to those aged 20–25 years. However, in the multivariate analysis, only the age group 26–30 years was associated with a good

TABLE 1 Factors associated with an excellent knowledge (80% threshold) of human Mpox infection among HCWs.

	Good knowledge		Unadjusted		Adjusted	
Variables	Overall *N* (%)	*n* (%)	OR (95% CI)	*P*–value	aOR (95% CI)	P–value
Gender						
Woman (R.)	173 (50.6)	19 (11.0)	1			
Man	169 (49.4)	25 (14.8)	1.40 (0.74–2.66)	0.294		
Age group (year)						
20–25 (R.)	48 (14.0)	2 (4.2)	1		1	
26–30	156 (45.6)	20 (12.8)	3.38 (1.20–5.40)	0.109	3.03 (0.66–13.83)	0.152
31–39	98 (28.7)	17 (17.3)	4.82 (1.00–4.60)	**0.041**	3.82 (0.75–19.39)	0.105
≥40	40 (11.7)	5 (12.5)	3.28 (0.60–17.94)	0.170	1.63 (0.17–15.27)	0.669
Medical profession						
Medical doctors (R.)	172 (50.3)	23 (13.4)	1		1	
Nurses	72 (21.1)	13 (18.1)	1.42 (0.67–3.00)	0.348	1.65 (0.85–3.18)	0.790
Others	98 (28.6)	8 (8.2)	0.57 (0.24–1.34)	0.201	0.32 (0.26–0.82)	**0.018**
Level of health facility						
Central hospital (tertiary healthcare facilities) level (R.)	86 (25.1)	7 (8.1)	1		1	
District hospital (primary healthcare facilities)	46 (13.5)	4 (8.7)	1.07 (0.29–3.88)	0.912	1.03 (0.27–3.81)	0.968
Medicalized health center	81 (11.1)	9 (11.1)	1.41 (0.50–3.98)	0.516	1.51 (0.51–4.42)	0.448
Private hospital	35 (23.7)	4 (11.4)	1.45 (0.39–5.32)	0.570	1.37 (0.36–5.17)	0.637
Others	94 (27.5)	20 (21.3)	3.05 (1.21–7.63)	**0.017**	4.01 (1.43–11.24)	**0.008**
Years of experience						
1–5 (R)	242 (70.8)	27 (11.2)	1		1	
6–10	48 (14.0)	10 (20.8)	2.09 (0.93–4.67)	0.071	1.49 (0.55–3.97)	0.426
11–15	31 (9.1)	5 (16.1)	1.53 (0.54–4.32)	0.421	1.49 (0.31–7.01)	0.613
≥16	21 (6.1)	2 (9.5)	0.83 (0.18–3.79)	0.819	1.01 (0.12–8.53)	0.993

p-values in bold indicate those that are statistically significant. The multivariable model was adjusted for age group, the medical profession, level of health facility, and years of experience. OR, odds ratio; aOR, adjusted odds ratio; CI, confidence Interval; R., reference category; HCW, healthcare workers.

knowledge of Mpox (OR: 2.74; 95% [CI: 1.2–5.8], $p = 0.008$) when compared to the age group 20–25 years.

4 Discussion

Responding to outbreaks, such as Mpox, requires a strong collaboration between all stakeholders, including frontline healthcare workers. In Cameroon, both event-based and case-based surveillance are put in place, but the current surveillance system mainly relies on case-based surveillance. Therefore, it is paramount that HCWs (particularly medical doctors and nurses) get a good mastery of the knowledge and case definitions and the management of potential epidemic diseases. This is because they are responsible for the early detection and management of cases at health facility levels. For this reason, our study aimed to assess the knowledge of HCWs in Cameroon on the ongoing Mpox infection, considering the transmission, clinical features, and management/treatment of the infection.

Data generated from this study revealed that, in general, the knowledge of HCWs on Mpox in Cameroon was poor (42%). Less than 15% of the participants were able to answer correctly to 80% of the 21 questions. When looking at some of the factors associated with knowledge of Mpox at an 80% cut-off score, we found that HCWs other than medical doctors and nurses had especially poor knowledge of Mpox. It was worrisome to observe that <20% of medical doctors and nurses recorded an excellent understanding because they are directly involved with patient care.

It was interesting to note that those in the categories of other health facility levels, including research centers and non-governmental organizations (NGOs), showed a slightly higher knowledge than those in hospital settings, which might be partly justified by the fact that several respondents in this category are involved with the design or implementation of public health policies related to the Mpox response. It was, for example, reported that public health NGOs have specific missions, with most largely embodying epidemiological surveillance of infectious diseases,

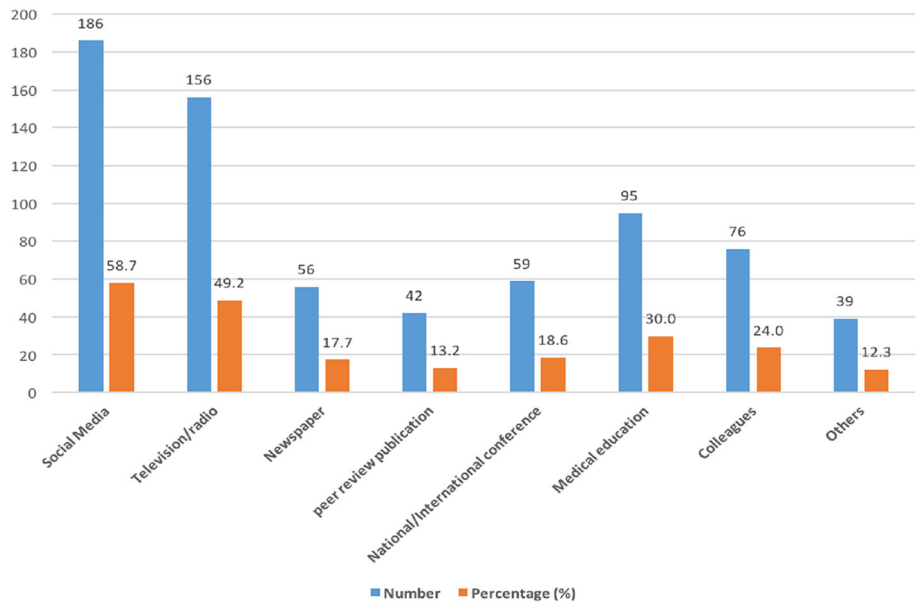

FIGURE 1
Sources of information on Mpox among HCWs. Some HCWs obtained Mpox information from multiple sources.

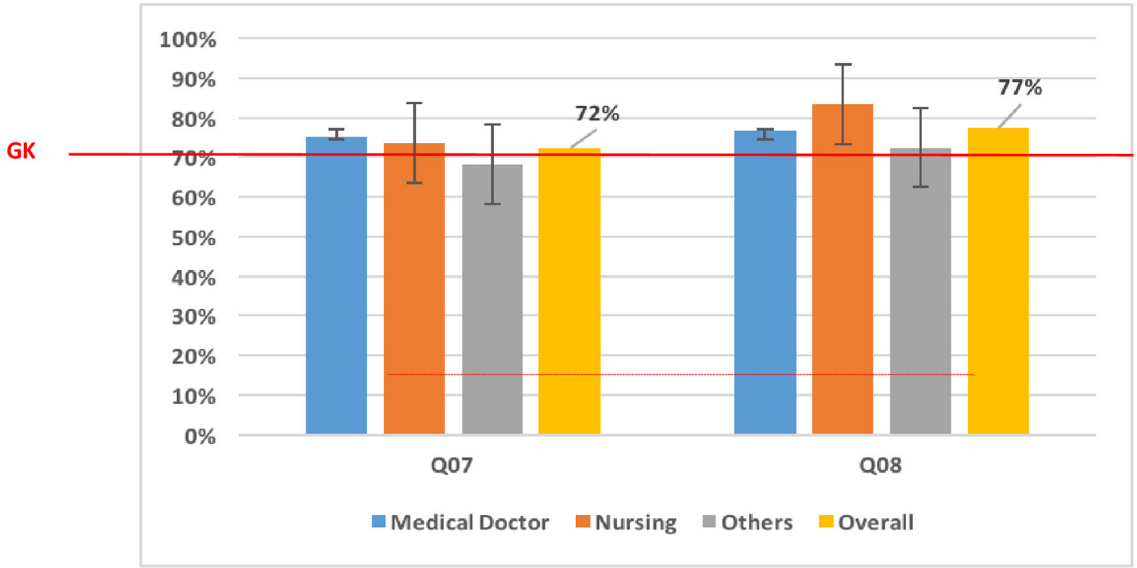

FIGURE 2
Knowledge of Mpox transmission means. "Others" (laboratory scientists, epidemiologists, pharmacy technicians, radiographers, physiotherapists, and dental technicians); "Overall" (mean of knowledge among medical doctors, nursing, and other categories); Q07 and Q08 represent questions 07 and 08 in the questionnaire used to assess the level of knowledge; Q07: Monkeypox is easily transmitted from human-to-human. Q08: Monkeypox could be transmitted through a bite of an infected monkey. GK, Good Knowledge (70% of good response).

which perhaps exposes them more to new emerging and re-emerging health conditions (33). The other variables including age, gender, and the number of years of work experience did not seem to show a significant difference in the Mpox knowledge. This finding indicates a uniformly low level of Mpox knowledge across these variables. This low knowledge of Mpox among HCWs is not only limited to Cameroon, as a previous study found a uniformly low knowledge among general practitioners in Indonesia (34). Moreover, a cross-sectional study conducted in 2022 to assess the knowledge and attitudes of HCWs in some hospitals in Southern Italy reported unsatisfactory knowledge (24). A systematic review by Mohamed L. and Abanoub A. showed that the overall understanding of Mpox was poor among nine articles, which exclusively assessed Mpox knowledge in Europe, the Middle East, and Asia (25). As Mpox was a rare disease, it received less attention. The recent pandemic of Mpox spread faster at a large scale and affected the most vulnerable populations, therefore indicating that more attention should be given to it.

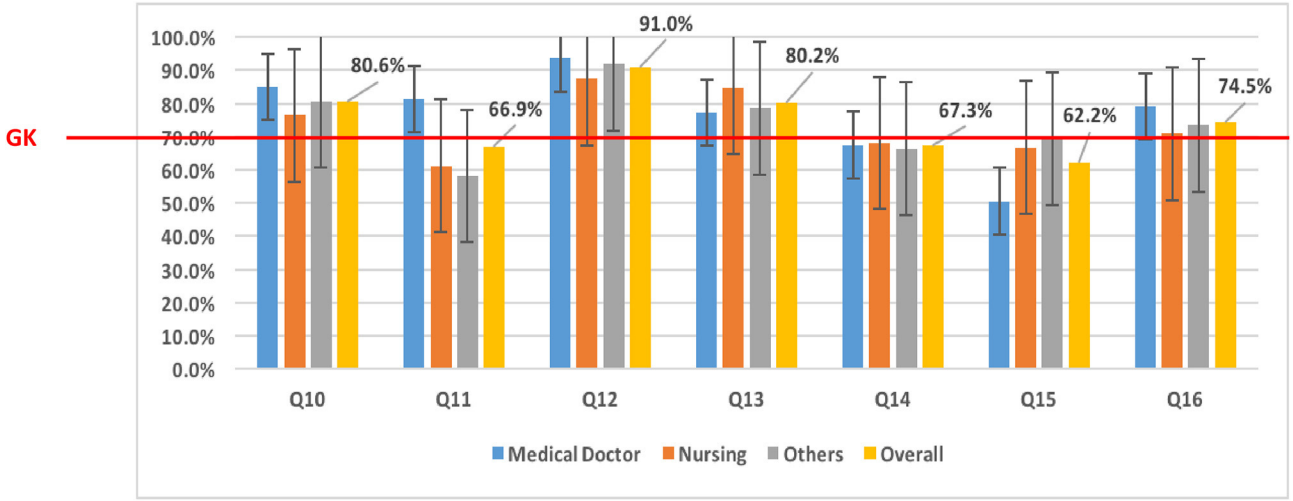

FIGURE 3
Knowledge of Mpox clinical diagnostics among HCWs. "Others" (laboratory scientists, epidemiologists, pharmacy technicians, radiographers, physiotherapists, and dental technicians); "Overall" (mean of knowledge among medical doctors, nurses, and Others categories); Q10, Q11, Q12, Q13, Q14, Q15, and Q16 represent questions 10 to 16 in the questionnaire used to assess the level of knowledge; Q10: Monkeypox and smallpox have similar signs and symptoms. Q11: Monkeypox and smallpox have the same signs and symptoms. Q12: Flu-like syndrome is one of the early signs or symptoms of human Monkeypox. Q13: Rashes on the skin are one of the signs or symptoms of human Monkeypox. Q14: Papules on the skin are one of the signs or symptoms of human Monkeypox. Q15: Vesicles on the skin are one of the signs or symptoms of human Monkeypox. Q16: Pustules on the skin are one of the signs or symptoms of human Monkeypox. GK, Good Knowledge (70% of good response).

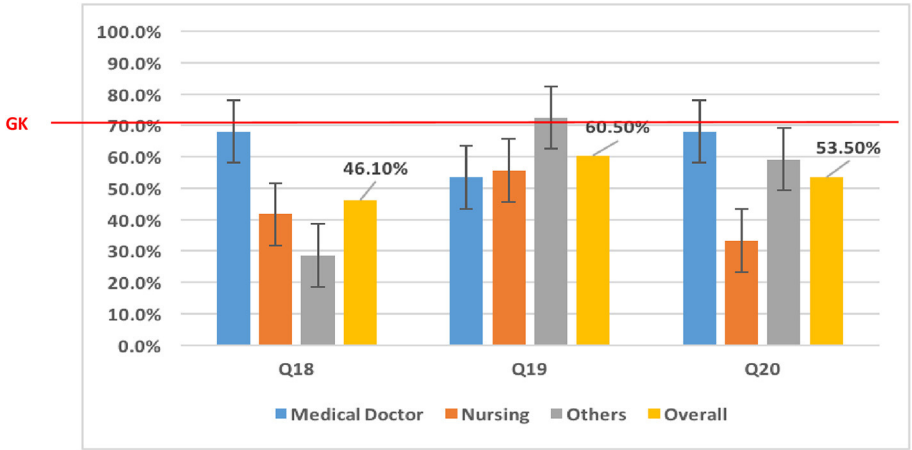

FIGURE 4
Knowledge on treatment/management of Mpox among HCWs. "Others" included laboratory scientists, epidemiologists, pharmacy technicians, radiographers, physiotherapists, and dental technicians. Q18, Q19, and Q20 represent questions 18 to 20 in the questionnaire used to assess the level of knowledge. Q18: One management option for patients with Monkeypox who are symptomatic is to use paracetamol. Q19: Antivirals are required in the management of human Monkeypox patients. Q20: Antibiotics are required in the management of human Monkeypox patients. GK, Good Knowledge (70% of good response).

In the present study, even at a threshold of 70% (here referred to as good knowledge), <50% of the participants had good knowledge. Most of the participants, including medical doctors, had poor knowledge (<70%) of the evolution and presentation of the classic clinical features of Mpox and case management. It should be noted that most of the HCWs who participated in this study were still in their early career, with only 1–5 years of working experience, which could have impacted their poor knowledge.

An exploratory analysis based on the cut-off score knowledge of 70% was equally carried out. A multivariable analysis indicated that those aged 26–30 years had a higher level of knowledge (47%) than those in other age groups. The age group of 26–30 years is part of the social media-friendly group; consequently, they might be more likely to get Mpox-related information. Of note, ~58% of the participants reported receiving information about Mpox via online media platforms (Facebook, WhatsApp, podcast, etc). It was reported elsewhere that young HCWs tend to prefer to consult social media networks for information because of their rapid accessibility (35). Despite some information lacking validity, social networks have the particularity of transmitting data in record time and with a larger coverage. In this digital era, social media can represent an effective communication channel that can provide

TABLE 2 Factors associated with good knowledge (70% threshold) of human Mpox infection among HCWs.

Variables	Overall *N* (%)	Good knowledge *n* (%)	Unadjusted		Adjusted	
			OR (95% CI)	*P*–value	aOR (95% CI)	*P*–value
Gender						
Woman (R.)	173 (50.6)	76 (43.9)	1			
Man	169 (49.4)	68 (40.2)	0.78 (0.45–1.35)	0.388		
Age group (year)						
20–25 (R.)	48 (14.0)	12 (25.0)	1		1	
26–30	156 (45.6)	73 (46.6)	2.63 (1.20–5.40)	**0.009**	2.74 (1.29–5.80)	**0.008**
31–39	98 (28.7)	41(41.8)	2.10 (1.00–4.60)	**0.049**	1.96 (0.84–4.54)	0.117
≥40	40 (11.7)	18 (45.0)	2.40 (0.90–6.10)	0.051	2.69 (0.68–10.58)	0.155
Medical profession						
Medical doctors (R.)	172 (50.3)	64 (37.2)	1		1	
Nurses	72 (21.1)	36 (50.0)	1.68 (0.96—2.94)	0.065	1.65 (0.85–3.18)	0.136
Others	98 (28.7)	44 (44.8)	1.37 (0.83–2.27)	0.216	1.42 (0.81–2.49)	0.218
Level of health facility						
Central hospital (tertiary healthcare facilities) (R.)	86 (25.1)	30 (34.9)	1			
District hospital (primary healthcare facilities)	46 (13.5)	18 (39.1)	1.09 (0.45–2.59)	0.844		
District medical centers (primary healthcare facilities)	35 (10.2)	31 (38.3)	2.89 (0.90–9.30)	0.751		
Private hospital	81 (23.7)	20 (57.1)	1.35 (0.65–2.83)	0.416		
Others	94 (27.5)	45 (47.9)	1.53 (0.70–3.34)	0.286		
Years of experience						
1–5 (R)	242 (70.8)	101 (41.7)	1			
6–10	48 (14.0)	22 (45.8)	1.18 (0.63–2.20)	0.600		
11–15	31 (9.1)	13 (41.9)	1.00 (0.47–2.15)	0.983		
≥16	21 (6.1)	8 (38.1)	0.85 (0.34–2.14)	0.745		

p-values in bold indicate those that are statistically significant. The multivariable model was adjusted for age group and medical profession. OR, odds ratio; aOR, adjusted odds ratio; CI, confidence Interval; R., reference category; HCW, healthcare workers.

continuous education to HCWs (36). There was uniformly low knowledge of Mpox, considering other variables such as gender, type of workplace, work experience, and medical training. This finding suggests that, in such a context, the infection can spread unnoticed in the community without being detected/reported timeously. Therefore, strategies for enhancing the knowledge of HCWs on the detection and management of zoonotic Mpox are needed, including sensitization of HCWs via online platforms to respond adequately to such outbreaks (37). These strategies are particularly important as they resonate with the One Health approach for sustainable infection prevention and control (38).

In the frame of pandemic preparedness and interventions, considering the reported pitfalls among HCWs would guide global health agencies (WHO, Africa CDC, etc) in tailoring capacity-building or strengthening programs for optimal efficiency in epidemic/pandemic preparedness and response at the continental and global levels.

This study has some limitations. This was an online survey that required an internet connection; as such, there was a potential selection bias in relation to the availability of internet access, especially in rural areas (39).

5 Implications and recommendations

The study's findings highlight the critical need for targeted training programs to enhance healthcare workers' (HCWs) understanding of epidemic diseases, such as Mpox, particularly among medical doctors and nurses. The uniformly low level of Mpox knowledge across various demographic and professional variables highlights the potential impact on outbreak response and the urgent need for comprehensive capacity-building efforts. To address these challenges, it is recommended to leverage coordinated social media and online platforms for

continuous education and sensitization of HCWs, considering their accessibility and potential to reach a wider audience. In addition, there is a need to conduct representative studies to ensure a comprehensive understanding of HCWs' knowledge levels nationwide (to overcome potential selection biases related to internet access, especially in rural areas), thereby guiding the development of capacity-building initiatives and pandemic preparedness strategies. These implications and recommendations are crucial for guiding the development of capacity-building initiatives and pandemic preparedness strategies at both national and global levels.

6 Conclusion

Knowledge of Mpox among HCWs within the health system of Cameroon is uniformly low across sociodemographic, workplace, and medical professional characteristics. Thus, for optimal preparedness and interventions on IPC, case management, and surveillance of Mpox and similar emerging pathogens, capacity-strengthening programs should be reinforced in the Cameroonian context and similar settings, with a particular focus on HCWs in clinical facilities and the older adults, while encouraging scientific literature and organizational social media web sites. Such evidence-based interventions could also support response in several African settings.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the participants was not required to participate in this study in accordance with the national legislation and the institutional requirements. In accordance with the Declaration of Helsinki on good clinical practices and ethical considerations, the present study was approved within the frame of multisectoral surveillance and in response to public health emergencies of zoonotic origin (authorization Ref. N° E268/L/MINSANTE/SG/DLMEP/SDLEP from the Ministry of Public Health in Cameroon). Prior to enrollment, the study information sheet was provided to each potential participant, and informed consent was then obtained from each participant. Data confidentiality and privacy of participants were ensured by the use of anonymized unique identifiers, and the data were secured in an encrypted passwordprotected computer. Only authorized individuals, such as the principal and co-principal investigators, had access to the survey account. The generated data were used to strengthen the capacity of the target population on better outbreak preparedness and response through result dissemination and exploitation.

Author contributions

ADN: Conceptualization, Data curation, Formal analysis, Writing—review & editing. YB: Data curation, Methodology, Writing—original draft, Writing—review & editing, Validation. JF: Conceptualization, Data curation, Formal analysis, Supervision, Validation, Visualization, Writing—original draft, Writing—review & editing. AK: Data curation, Investigation, Software, Writing—original draft. JG: Conceptualization, Formal analysis, Writing—review & editing. NM: Methodology, Project administration, Software, Supervision, Writing—original draft. DM: Formal analysis, Investigation, Methodology, Writing—review & editing. CA: Conceptualization, Formal analysis, Methodology, Visualization, Writing—review & editing. M-LM: Conceptualization, Data curation, Investigation, Visualization, Writing—review & editing. TD: Conceptualization, Data curation, Formal analysis, Writing—review & editing. BT: Data curation, Formal analysis, Project administration, Software, Writing—review & editing. DA: Data curation, Investigation, Resources, Writing—original draft. DN: Investigation, Writing—review & editing. SM: Investigation, Writing—review & editing. EN: Data curation, Formal analysis, Investigation, Writing—review & editing. AT: Investigation, Writing—review & editing. BF: Investigation, Writing—review & editing. IS: Investigation, Writing—review & editing. MT: Investigation, Writing—review & editing. DT: Investigation, Writing—review & editing. WP: Investigation, Writing—review & editing. SS: Funding acquisition, Investigation, Writing—review & editing. ET: Formal analysis, Writing—original draft, Writing—review & editing. LE: Investigation, Writing—review & editing. GE: Investigation, Writing—review & editing. A-CZ-K: Investigation, Writing—review & editing. H-EG: Investigation, Writing—review & editing. NN: Data curation, Methodology, Writing—original draft, Writing—review & editing. VC: Data curation, Writing—original draft, Writing—review & editing. C-FP: Conceptualization, Data curation, Writing—original draft, Writing—review & editing. AN: Writing—original draft, Writing—review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. *WHO Informal Consultation on Monkeypox 2017*. Geneva: WHO (2018).

2. Mpox (Monkeypox) Outbreak 2022 - Global. Available online at: https://www.who.int/emergencies/situations/monkeypox-oubreak-2022 (accessed February 16, 2023).

3. Sahay RR, Relhan V, Shete AM, Patil DY, Kumar LP, Kaur A, et al. Persistence of infectious mpox virus on the surfaces of isolation ward in a hospital setting, India. *J Hosp Inf.* (2023) 137:86–8. doi: 10.1016/j.jhin.2023.03.026

4. Low N, Bachmann LH, Ogoina D, McDonald R, Ipekci AM, Quilter LA, et al. Mpox virus and transmission through sexual contact: Defining the research agenda. *PLoS Med.* (2023) 20:e1004163. doi: 10.1371/journal.pmed.1004163

5. *How It Spreads, Mpox, Poxvirus, CDC*. (2023). Available online at: https://www.cdc.gov/poxvirus/mpox/if-sick/transmission.html (accessed August 20, 2023).

6. Alizadeh I, Shafaati M, Zandi M. Is it possible that arthropods could be an accidental reservoir for the outbreak of mpox virus in 2022? *New Microbes New Infect.* (2023) 53:1–14. doi: 10.1016/j.nmni.2023.101138

7. Brown K, Leggat PA. Tropical medicine and infectious disease human monkey pox: current state of knowledge and implications for the future. *Trop Med Infect Dis.* (2016) 1:8. doi: 10.3390/tropicalmed1010008

8. Shafaati M, Zandi M, Choudhary OP. Monkeypox virus crosstalk with HIV; where do we stand now? *Int J Surg.* (2022) 105:106897. doi: 10.1016/j.ijsu.2022.106897

9. *Rapports d'activités – CNLS _ NACC*. (2022). Available online at: https://cnls.cm/index.php/rapports-dactivites/ (accessed October 28, 2022).

10. Durski KN, McCollum AM, Nakazawa Y, Petersen BW, Reynolds MG, Briand S, et al. Emergence of Monkeypox — West and Central Africa, 1970–2017. *MMWR.* (2019) 67:306–10. doi: 10.15585/mmwr.mm6710a5

11. Sejvar JJ, Chowdary Y, Schomogyi M, Stevens J, Patel J, Karem K, et al. Human monkeypox infection: a family cluster in the midwestern United States. *The J Infect Dis.* (2004) 190:1833–40. doi: 10.1086/425039

12. *Multistate Outbreak of Monkeypox—Illinois, Indiana, and Wisconsin, 2003*. (2023). Available online at: https://www.cdc.gov/mmwr/preview/mmwrhtml/mm5223a1.htm (accessed February 16, 2023).

13. Vaughan A, Aarons E, Astbury J, Balasegaram S, Beadsworth M, Beck CR, et al. Two cases of monkeypox imported to the United Kingdom. *Eurosurveillance Eur Centre Dis Prev Control.* (2018) 23:38. doi: 10.2807/1560-7917.ES.2018.23.38.1800509

14. Erez N, Achdout H, Milrot E, Schwartz Y, Wiener-Well Y, Paran N, et al. Diagnosis of Imported Monkeypox, Israel, 2018. *Emerg Inf Dis Centers Dis Prev.* (2019) 25:980. doi: 10.3201/eid2505.190076

15. Osadebe L, Hughes CM, Shongo Lushima R, Kabamba J, Nguete B, Malekani J, et al. Enhancing case definitions for surveillance of human monkeypox in the Democratic Republic of Congo. *PLoS Negl Trop Dis.* (2017) 11:9. doi: 10.1371/journal.pntd.0005857

16. Bass J, Tack DM, McCollum AM, Kabamba J, Pakuta E, Malekani J, et al. Enhancing health care worker ability to detect and care for patients with monkeypox in the Democratic Republic of the Congo. *Int Health.* (2013) 5:237–43. doi: 10.1093/inthealth/iht029

17. *Outbreak Brief 26: Mpox in Africa Union Member States. Africa CDC*. (2023). Available online at: https://africacdc.org/disease-outbreak/outbreak-brief-26-mpox-in-africa-union-member-states/ (accessed August 20, 2023).

18. *Monkeypox – Cameroon*. (2023). Available online at: https://www.who.int/emergencies/disease-outbreak-news/item/05-june-2018-monkeypox-cameroon-en (acccessed December 29, 2023).

19. *Monkeypox – Cameroon*. (2023). Available online at: https://www.who.int/emergencies/disease-outbreak-news/item/05-june-2018-monkeypox-cameroon-en (accessed February 16, 2023).

20. *Rapport Situation MonkeyPox Cameroun N°4 - CCOUSP*. (2023). Available online at: https://www.ccousp.cm/download/rapport-situation-monkeypox-cameroun-n4/ (accessed March 2, 2023).

21. Mathieu E, Spooner F, Dattani S, Ritchie H, Roser M. *Mpox (monkeypox). Our World in Data*. (2022). Available online at: https://ourworldindata.org/monkeypox (accessed April 25, 2023).

22. *Mpox in the U.S. Centers for Disease Control and Prevention*. (2023). Available online at: https://www.cdc.gov/poxvirus/mpox/response/2022/world-map.html (accessed April 25, 2023).

23. *Rapport Situation MonkeyPox Cameroun N°5 - CCOUSP*. (2023). Available online at: https://www.ccousp.cm/download/rapport-situation-monkeypox-cameroun-n5/ (accessed March 2, 2023).

24. Miraglia del Giudice G, Della Polla G, Folcarelli L, Napoli A, Angelillo IF. Knowledge and attitudes of health care workers about monkeypox virus infection in Southern Italy. *Front Public Health.* (2023) 11:1091267. doi: 10.3389/fpubh.2023.1091267

25. Lounis M, Riad A. Monkeypox (MPOX)-related knowledge and vaccination hesitancy in non-endemic countries: concise literature review. *Vaccines.* (2023) 11:229. doi: 10.3390/vaccines11020229

26. Harapan H, Alleta A, Anwar S, Setiawan AM, Maulana R, Wahyuniati N, et al. Attitudes towards Zika virus infection among medical doctors in Aceh province, Indonesia. *J Infect Public Health.* (2018) 11:99–104. doi: 10.1016/j.jiph.2017.06.013

27. Harapan H, Rajamoorthy Y, Utomo PS, Anwar S, Setiawan AM, Alleta A, et al. Knowledge and attitude towards pregnancy-related issues of Zika virus infection among general practitioners in Indonesia. *BMC Inf Dis BioMed Central Ltd.* (2019) 19:1–12. doi: 10.1186/s12879-019-4297-4

28. Harapan H, Aletta A, Anwar S, Setiawan AM, Maulana R, Wahyuniati N, et al. Healthcare workers' knowledge towards Zika virus infection in Indonesia: a survey in Aceh. *Asian Pacific J Trop Med.* (2017) 10:189–94. doi: 10.1016/j.apjtm.2017.01.018

29. StopBlaBlaCam. *Cameroon's Health System is Short of 55,000 Staff*. (2023). Available online at: https://www.stopblablacam.com/politics/0312-7852-cameroon-s-health-system-is-short-of-55-000-staff (accessed December 27, 2023).

30. Mpox, Poxvirus, CDC. (2023). Available online at: https://www.cdc.gov/poxvirus/monkeypox/index.html (accessed February 17, 2023).

31. Eysenbach G. Improving the quality of web surveys: the checklist for reporting results of internet e-surveys (CHERRIES). *J Med Internet Res.* (2004) 6:e34. doi: 10.2196/jmir.6.3.e34

32. Wang L, Abualfoul M, Oduor H, Acharya P, Cui M, Murray A, et al. A cross-sectional study of knowledge, attitude, and practice toward COVID-19 in solid organ transplant recipients at a transplant center in the United States. *Front Pub Health.* (2022) 10:880774. doi: 10.3389/fpubh.2022.880774

33. *Cameroon Baptist Convention Health Services – Quality Care to All*. (2023). Available online at: https://cbchealthservices.org/ (accessed February 19, 2023).

34. Harapan H, Setiawan AM, Yufika A, Anwar S, Wahyuni S, Asrizal FW, et al. Knowledge of human monkeypox viral infection among general practitioners: a cross-sectional study in Indonesia. *Pathog Glob Health.* (2020) 114:68. doi: 10.1080/20477724.2020.1743037

35. Jalil A, Mahmood QK, Fischer F. Young medical doctors' perspectives on professionalism: a qualitative study conducted in public hospitals in Pakistan. *BMC Health Services Research BioMed Central.* (2020) 10:20. doi: 10.1186/s12913-020-05681-w

36. Kapoor KK, Tamilmani K, Rana NP, Patil P, Dwivedi YK, Nerur S. Advances in social media research: past, present and future. *Inf Syst Front.* (2017) 20:531–58. doi: 10.1007/s10796-017-9810-y

37. Tajudeen YA, Oladipo HJ, Muili AO, Ikebuaso JG. Monkeypox: A review of a zoonotic disease of global public health concern. *Health Promot Perspect.* (2023) 13:1–9. doi: 10.34172/hpp.2023.01

38. Najimudeen M, Chen HWJ, Jamaluddin NA, Myint MH, Marzo RR. Monkeypox in pregnancy: susceptibility, maternal and fetal outcomes, and one health concept. *Int J MCH AIDS.* (2022) 11:e594. doi: 10.21106/ijma.594

39. Chirwa M. Access and use of internet in teaching and learning at two selected teachers' colleges in Tanzania. *IJEDICT.* (2018) 14:4—16.

Appendix

Questions used to measure the knowledge of monkeypox among general practitioners in Indonesia

Questions used to measure knowledge

No. Question Yes No

1 Monkeypox is prevalent in Southeast Asia countries

2 Monkeypox is prevalent in Western and Central Africa

3 There are many human monkeypox cases in Cameroon (greater than 10 cases)

4 There is an outbreak of human monkeypox in the center region of Cameroon

5 Monkeypox is a viral disease infection

6 Monkeypox is a bacterial disease infection

7 Monkeypox is easily transmitted human-to-human

8 Monkeypox could be transmitted through a bite of an infected monkey

9 Travelers from America continent are the main source of imported cases of monkeypox

10 Monkeypox and smallpox have similar signs and symptoms

11 Monkeypox and smallpox have the same signs and symptoms

12 Flu-like syndrome is one of the early signs or symptoms of human monkeypox

13 Rashes on the skin are one of the signs or symptoms of human monkeypox

14 Papules on the skin are one of the signs or symptoms of human monkeypox

15 Vesicles on the skin are one of the signs or symptoms of human monkeypox

16 Pustules on the skin are one of the signs or symptoms of human monkeypox

17 Lymphadenopathy (swollen lymph nodes) is one clinical sign or symptom that could be used to differentiate monkeypox and smallpox cases

18 One management option for monkeypox patients who are symptomatic is to use paracetamol

19 Antivirals are required in the management of human monkeypox patients

20 Antibiotics are required in the management of human monkeypox patients

21 Diarrhea is one of the signs or symptoms of human monkeypox

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Diagnostic performance of rapid antigen testing for SARS-CoV-2: the COVid-19 AntiGen (COVAG) extension study

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Background: This study is the extension of the COVAG study. We compared two RATs, the Panbio COVID-19 Ag Rapid Test (Abbott) and the SD Biosensor Q SARS-CoV-2 Rapid Antigen Test (Roche), against RT-PCR on the foil of new variants.

Methods: We included 888 all-comers at a diagnostic center between October 20, 2021, and March 18, 2022. RT-PCR-positive samples with a Ct value ≤32 were examined for SARS-CoV-2 variants.

Findings: The sensitivity of the Abbott-RAT and Roche-RAT were 65 and 67%, respectively. For both RATs, lower Ct values were significantly correlated with higher sensitivity. For samples with Ct values ≤25, the sensitivities of the Roche-RAT and of the Abbott-RAT were 96 and 95%, for Ct values 25–30 both were 19%, and for Ct values ≥30 they were 6 and 2%, respectively. The RATs had substantially higher sensitivities in symptomatic than asymptomatic participants (76, 77%, vs. 29, 31%, for Abbott-RAT, Roche-RAT, respectively) and in participants referred to testing by their primary care physician (84, 85%) compared to participants who sought testing due to referral by the health department (55, 58%) or a warning by the Corona-Warn-App (49, 49%). In persons with self-reported previous COVID-19 sensitivities were markedly lower than in patients without previous COVID-19: 27% vs. 75% for Roche-RAT and 27% vs. 73% for Abbott-RAT. We did not find significant correlation between vaccination status and sensitivity. The Omicron variant was detected with a sensitivity of 94 and 92%, the delta variant with a sensitivity of 80 and 80% for Abbott-RAT and Roche-RAT, respectively. This difference is attributable to the lower Ct values of the Omicron samples compared to the Delta samples. When adjusted for the Ct value, a multivariate logistic regression did not show a significant difference between Omicron and Delta. In terms of sensitivity, we found no significant difference between the wild-type and the Omicron and Delta variants, but a significantly lower sensitivity to the alpha variant compared to the other variants. The specificities were > 99% overall.

KEYWORDS

SARS-CoV-2, rapid antigen detection test, antigen test, variants, omicron, delta, COVID-19, diagnostic

1 Introduction

Severe acute respiratory syndrome Coronavirus type 2 (SARS-CoV-2) is the causative agent of Coronavirus disease 19 (COVID-19). COVID-19 emerged in late 2019, quickly spread around the world and was declared a global pandemic on March 11, 2021, by the World Health Organization (WHO) (1). Since its emergence multiple SARS-CoV-2 variants developed which mostly were characterized by mutations in the Spike protein but also within the Nucleocapsid protein (2–5). Variants showing a decrease in the effectiveness of available diagnostic tests among other criteria are termed Variants of Concern (VOC) by the WHO (6). To date the WHO has listed 5 VOCs, namely: B.1.1.7 (alpha), B.1.351 (beta), P.1 (gamma), B.1.617.2 (delta) and the currently prevailing B.1.1.529 (Omicron) (6). For Omicron several sub-lineages have been identified with BA.5 being the currently dominant one in Europe (3).

The clinical presentation of COVID-19 ranges from asymptomatic to prolonged illness requiring intensive care treatment and death (7, 8). As SARS-CoV-2 can be transmitted by symptomatic as well as asymptomatic persons the identification of infectious carriers is crucial to contain COVID-19 by means of contact tracing and isolation of infectious patients (8). This requires effective testing and an early diagnosis of COVID-19. Detection of acute SARS-CoV-2 infection can be achieved by direct testing including nucleic acid amplification tests (NAATs) or through rapid antigen tests (RATs). NAATs identify viral RNA in specimens from the respiratory tract while RATs recognize viral proteins, mostly the Nucleocapsid protein (9). To date NAAT-based assays such as reverse transcription-polymerase chain reaction (RT-PCR) are the gold standard in detecting acute SARS-CoV-2 infection. RATs are widely employed as well as they can be conducted at the point of care, provide fast results within 15–30 min, and can be used for self-testing. Positive RAT results need to be verified by RT-PCR testing (10). Indirect tests including assays detecting antibodies against the Spike-or the Nucleocapsid protein are not useful in the diagnosis of acute infection as they only become positive after 3 days and more or may be already positive from an earlier infection (Nucleocapsid-and Spike antibodies) or vaccination (Spike-Antibodies) (11, 12).

This study is the extension of the COVAG study originally performed from February 1, 2021, to March 31, 2021. During the first data collection period we saw that the alpha variant decreased the effectiveness of the RATs compared to the wild-type (13). As new SARS-CoV-2 variants emerged afterwards, the COVAG study was continued to comprehensively examine two of the most sensitive RATs in a real-world, prospective, head-to-head study, placing specific emphasis on clinical characteristics and the presence of SARS-CoV-2 variant genotypes (9).

2 Methods

2.1 Setting and participants

This prospective study was conducted at the Corona Test Centre Cannstatter Wasen in Stuttgart, Germany as an extension of the COVAG study (13). Individuals scheduled for RT-PCR testing of nasopharyngeal swabs were advised of the study orally and in writing. Participants had to be aged ≥18 years and capable of understanding the nature, significance, and implications of the study. Children and adolescents <18 years of age and patients obviously suffering from clinical conditions requiring emergency hospitalization were excluded. All participants provided written and informed consent. The study was approved by Ethics Committee II (Mannheim) of the University of Heidelberg (reference number 2020-417MF) and the German Institute for Drugs and Medical Devices.

We recorded demographic characteristics, reasons for testing, medical history including SARS-CoV-2 vaccination history, clinical symptoms, and vital signs (heart rate, blood pressure, body temperature, and oxygen saturation) and we stratified the reasons for testing into four major categories: participants referred by their primary care physicians, by the Health Department, participants seeking RT-PCR testing to confirm a positive antigen test and participants who received a warning in their digital contact-tracing app (Corona-Warn-App). We grouped the participants based on their COVID-19 vaccination status into non-vaccinated (0 or 1 vaccination), vaccinated (2 vaccinations), boostered (3 or more vaccinations) and with unknown vaccination status.

In addition to collecting the oro-and nasopharyngeal swabs for RT-PCR testing, we collected two completely independent nasopharyngeal swab specimens to run two commercially available and widely used RATs. The swabs were collected by medically educated personnel of the test center by rotating teams with strict adherence to the instructions issued by the manufacturers. We used the Abbott Panbio™ COVID-19 Ag Rapid Test (Abbott Rapid Diagnostics Jena GmbH, Jena, Germany www.abbott.com/poct) and the Roche-SD Biosensor SARS-CoV-2 Rapid Antigen Test (identical to SD BIOSENSOR Standard Q COVID-19 Ag www.sdbiosensor.com; Roche Diagnostics; Mannheim, Germany www.roche.com). We chose those two tests in continuation of our first data collection period and because they were among the most sensitive tests according to a Cochrane analysis (13, 14).

Hereafter, we refer to the tests as Abbott-RAT and Roche-RAT, respectively. We randomly assigned the participants to three sampling groups according to the sequence of collecting the nasopharyngeal swabs (group 1: RT-PCR, RAT-Roche, RAT-Abbott; group 2: RAT-Roche, RAT-Abbott, RT-PCR; and group 3: RAT-Abbott, RT-PCR, RAT-Roche) to reduce bias based on the order of test performance.

2.2 Analytical procedures

Both the Abbott-RAT and the Roche-RAT were carried out by medically educated staff according to the manufacturers' instructions on-site at the Corona Test Centre, immediately after sampling the nasopharyngeal swabs. The nasopharyngeal swabs for real-time RT-PCR (rRT-PCR) testing were placed in 2 mL of a phosphate-buffered saline solution (ISOTON™ II Diluent, Becton Dickinson, Galway, Ireland) and delivered to the SYNLAB Medical Care Centre

Leinfelden-Echterdingen. PCR was always performed after onsite interpretation of the RATs, excluding that the interpretation of RATs was affected by the PCR results.

SARS-CoV-2 RNA was extracted from the nasopharyngeal swab samples and purified using the PurePrep Pathogens kit and a PurePrep 96 instrument (Molgen, Veenendaal, the Netherlands) within 6 h after sampling to limit degradation. The integrity of the RNA was monitored by co-amplification of a control RNA included in the solution for the lysis of the swabs. In cases in which neither SARS-CoV-2 RNA nor the control RNA were detected, the RNA preparation was repeated. The rRT-PCR assay was performed using either the RIDA®GENE SARS-CoV-2 test kit (R-Biopharm, Darmstadt, Germany) or the Allplex SARS-CoV2 (Seegene, Seoul, Korea) or the Virella SARS-CoV-2 seqc (Gerbion, Kornwestheim, Germany) on the CFX96 Touch Real-Time PCR detection device (Bio-Rad, Feldkirchen, Germany) or the CFX-96 IVD Real-Time PCR detection device (Bio-Rad, Feldkirchen, Germany) according to the manufacturers' instructions. The RIDA®GENE SARS-CoV-2 test kit targets the SARS-CoV-2 envelope (E) gene, the Allplex SARS-CoV2 targets the N-gene, S-gene/RdRP and the E-gene (pan Sarbecovirus) and the Virella Seqc SARS-CoV2 targets the RdRp/S-gene and the E-gene (pan Sarbecovirus). Samples producing a cycle threshold (Ct) ≤ 35 were considered positive by RT-PCR.

We screened RT-PCR-positive samples with a Ct ≤ 32 for SARS-CoV-2 variants of concern (VOC). Until November 8th 2021 this analysis was performed at SYNLAB Medical Care Center Leinfelden-Echterdingen using the Kits Seegene Allplex Variant I (Seegene, Seoul, Korea) and Virella SARS-CoV2 Mut 3 (Gerbion, Kornwestheim, Germany) according to the supplier's instructions. Afterwards the VOC analysis was performed at SYNLAB Medical Care Center Weiden using the Novaplex SARS-CoV-2 Variants I Assay, Novaplex SARS-CoV-2 Variants IV Assay and Novaplex SARS-CoV-2 Variants VII Assay (Seegene, Seoul, Korea) according to the supplier's instructions.

Samples were screened for B.1.617.2 (delta), B.1.617.2.1 (delta plus), B.1.1.529/BA.1 (omicron) and BA.2 (omicron stealth). Samples with positive results for L452R and P681R and absence of K417N were assigned to the delta variant. Positive results for L452R, P681R and K417N were considered as Delta plus. Presence of N501Y, E484A and HV69/70del were considered as Omikron BA.1 and occurrence of N501Y, E484A with absence of HV69/70del as Omikron BA.2.

2.3 Statistical analysis

Continuous data are presented as means, standard deviations (SD), medians, and 25th and 75th percentiles. Categorical data are presented as absolute numbers and percentages (Table 1).

In our analysis, the performance indicators for the two RATs in relation to RT-PCR (chosen as the gold standard for having COVID-19) are given by sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), and diagnostic efficacy (number of correct test results divided by the total number of test results).

In Table 2, the *p*-values apply to two-sided testing of the null hypothesis that the difference between the Abbott-RAT and the Roche-RAT performance indicators is equal to zero. The probability densities underlying the two-sided testing are estimated by means of 5,000 bootstrap iterations.

The risk of having COVID-19 according to baseline anthropometric and anamnestic characteristics was expressed in terms of crude odds ratios (ORs) and ORs adjusted for age and sex as calculated by logistic regression (Supplementary Table S1).

We also visualized the sensitivities of both RDTs relative to the rRT-PCR-derived Ct values (Figure 1) and the PPVs and NPVs according to hypothetical disease prevalence rates in the range of 0–0·05 (Figure 1). To compare the PPV and NPV of the RDTs with standardized criteria on performance, we also used the following hypothetical sensitivity and specificity levels (tiers 1–3) recommended by Kost et al. (15): tier 1, 90, 95%; tier 2, 95, 97.5%; and tier 3, 100%, ≥99% (Figure 1).

Finally, we investigated whether the sensitivities of the two RDTs were related to the reason for testing, comorbidities, clinical symptoms, vital signs, or SARS-CoV-2 genotypes using univariate (Table 2) and multivariate logistic regression (Table 3).

The statistical tests were two-sided and $p < 0{\cdot}05$ was considered significant. The analyses were carried out using R v4.0.2.[1]

3 Results

3.1 Clinical characteristics of participants

The extension of the COVAG study was conducted from October 20, 2021 to March 18, 2022. Figure 1 shows the data collection period and the emergence of variants framed within the course of the COVID-19 pandemic in Germany. A total of 1,508 persons agreed to participate in this study. 21 persons were disregarded from further evaluation because at least one of the three tests was not available. This resulted in 1487 persons enrolled in the COVAG Extension study (Figure 3; Supplementary Table S1) including 801 (53.9%) women, 685 (46.1%) men and one diverse person (0.1%). Adverse effects from performing any of the tests were not experienced.

Within the period of data collection, self-testing with RATs and RT-PCR confirmation in the case of a positive RAT was performed very frequently in Germany which in many participants who already had a positive self-test beforehand. To reduce selection bias, we excluded these 591 (39.7%) participants from the further analyses (Figure 2). 888 participants were tested for other reasons. Those included a warning by the Corona-Warn-App in 419 (28.2%) participants, a referral from the health department in 236 (15.9%) participants, and a referral from the primary care physician in 233 (15.7%) participants. For 8 (0.5%) participants no information regarding the reason for testing was available (Table 1). The anthropometric and anamnestic characteristics of all 1,487 participants can be found in Supplementary Table S1. Further data analysis was performed for the 888 participants with reasons for testing other than to confirm a positive RAT.

Of 888 participants, 497 (56%) were women and 390 (43.9%) were men, one person (0.1%) is assigned neither to women nor to men. 665 (74.9%) participants self-reported having no comorbidities, while 223

1 http://www.r-project.org

TABLE 1 Clinical characteristics of participants of the COVAG Extension study with reasons for testing other than to confirm a positive RAT.

	All*	Male	Female	*p***
Age, years, mean ± SD, median (25, 75%)	39.34 ± 13.8 36 (28–49)	39.26 ± 13.94 36 (28–49.75)	39.37 ± 13.71 37 (28–49)	0.902
Reason for testing				
Referral from physician	232 (26.16%)	103 (26.41%)	129 (25.96%)	0.878
Referral from health departments (mostly contact persons of infected patients)	236 (26.61%)	96 (24.62%)	140 (28.17%)	0.2512
Warning by Corona-Warn-App	419 (47.24%)	191 (48.97%)	228 (45.88%)	0.3787
Comorbidities				
Any comorbidity	223 (25.14%)	100 (25.64%)	123 (24.75%)	0.8151
No comorbidity	664 (74.86%)	290 (74.36%)	374 (75.25%)	0.8151
Hypertension	84 (9.47%)	43 (11.03%)	41 (8.25%)	0.1673
Dyslipoproteinaemia	42 (4.74%)	16 (4.1%)	26 (5.23%)	0.5247
Diabetes mellitus	19 (2.14%)	9 (2.31%)	10 (2.01%)	0.8177
COPD	8 (0.9%)	3 (0.77%)	5 (1.01%)	1
Ischemic heart disease	7 (0.79%)	2 (0.51%)	5 (1.01%)	0.475
Previous COVID-19	101 (11.39%)	41 (10.51%)	60 (12.07%)	0.5232
Clinical symptoms				
Any clinical symptom	446 (50.28%)	171 (43.85%)	275 (55.33%)	0.0007
No clinical symptom	441 (49.72%)	219 (56.15%)	222 (44.67%)	0.0007
Malaise	325 (36.64%)	125 (32.05%)	200 (40.24%)	0.014
Shortness of breath	68 (7.67%)	22 (5.64%)	46 (9.26%)	0.0559
Cough	268 (30.21%)	101 (25.9%)	167 (33.6%)	0.015
Fever	54 (6.09%)	25 (6.41%)	29 (5.84%)	0.7778
Diarrhea	43 (4.85%)	16 (4.1%)	27 (5.43%)	0.4318
Musculoskeletal pain	145 (16.35%)	56 (14.36%)	89 (17.91%)	0.1704
Headache	266 (29.99%)	92 (23.59%)	174 (35.01%)	0.0002
Nausea	37 (4.17%)	11 (2.82%)	26 (5.23%)	0.0904
Vaccination status				
Not vaccinated	98 (11.04%)	40 (10.26%)	58 (11.65%)	0.5897
Primary vaccinated	321 (36.15%)	138 (35.38%)	183 (36.75%)	0.725
Boostered	468 (52.7%)	211 (54.1%)	257 (51.61%)	0.4983
Vital signs (binary)				
SysBP>130 mmHg and/or DiaBP>90 mmHg	233 (26.27%)	132 (33.85%)	101 (20.32%)	<0.0001
Other blood pressures	638 (71.93%)	251 (64.36%)	387 (77.87%)	<0.0001
Body temperature > 37°C	39 (4.4%)	14 (3.59%)	25 (5.03%)	0.3262
Body temperature ≤ 37°C	848 (95.6%)	376 (96.41%)	472 (94.97%)	0.3262
Oxygen saturation > median	200 (22.55%)	52 (13.33%)	148 (29.78%)	<0.0001
Oxygen saturation ≤ median	673 (75.87%)	331 (84.87%)	342 (68.81%)	<0.0001
Vital signs (metric)				
Systolic blood pressure, mmHg, mean ± SD, median (25, 75%)	125.15 ± 16.64120 (115–135)	129.38 ± 15.44130 (120–140)	121.81 ± 16.8120 (110–130)	<0.0001
Diastolic blood pressure, mmHg, mean ± SD, median (25, 75%)	80.93 ± 9.85 80 (75–90)	82.75 ± 9.65 80 (80–90)	79.51 ± 9.78 80 (70–85)	<0.0001
Body temperature, °C, mean ± SD, median (25, 75%)	36.31 ± 0.65 36.3 (36.1–36.5)	36.22 ± 0.85 36.3 (36.1–36.5)	36.38 ± 0.41 36.4 (36.1–36.6)	0.0008
Oxygen saturation, %, mmHg, mean ± SD, median (25, 75%)	97.65 ± 1.95 98 (97–98)	97.53 ± 1.29 98 (97–98)	97.75 ± 2.33 98 (98–99)	0.0812

*Female or male. **Two-sided *t* test and Fisher test on equality of means for metric and categorical data, respectively.

TABLE 2 Diagnostic performance of 2 commercial RATs for SARS-Cov-2 antigens in participants of the COVAG extension study with reasons for testing other than to confirm a positive RAT.

	N (%)	CT median (25th, 75th percentile)	Sensitivity (%)	Sensitivity (%)	P*	Specificity (%)	Specificity (%)	P*	PPV (%)	PPV (%)	P*	NPV (%)	NPV (%)	P*	EFF (%)	EFF (%)	P*
		In positives	Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT	
All probands	888 (100%)	20.36 (16.98, 29.97)	67.02 (62.39, 71.79)	65.43 (60.67, 70.18)	0.2091	99.71 (99.56, 100)	99.57 (99.35, 100)	0.6947	98.44 (97.44, 100)	97.62 (96.15, 100)	0.1057	91.84 (90.51, 93.28)	91.47 (90.1, 92.91)	0.1419	92.79 (91.55, 94.09)	92.34 (91.22, 93.58)	0.1159
Age > median	442 (49.77%)	19.96 (17.37, 29.54)	70 (63.5, 76.56)	68 (61.67, 74.62)	0.3479	99.71 (99.55, 100)	99.42 (99.1, 100)	0.6507	98.59 (97.56, 100)	97.14 (95.12, 100)	0.1797	91.91 (90, 93.98)	91.4 (89.47, 93.52)	0.2285	92.99 (91.19, 94.58)	92.31 (90.51, 94.24)	0.1797
Age ≤ median	446 (50.23%)	20.81 (16.61, 29.99)	63.64 (56.6, 70.97)	62.5 (55.56, 70)	0.4781	99.72 (99.57, 100)	99.72 (99.57, 100)	1	98.25 (96.88, 100)	98.21 (96.88, 100)	0.6566	91.77 (89.92, 93.73)	91.54 (89.66, 93.46)	0.4781	92.6 (90.91, 94.28)	92.38 (90.91, 93.94)	0.4781
Women	497 (55.97%)	19.96 (17.36, 28.04)	68.42 (62.34, 74.67)	66.67 (60.53, 72.91)	0.2095	99.74 (99.6, 100)	99.48 (99.19, 100)	0.6711	98.73 (97.83, 100)	97.44 (95.74, 100)	0.1597	91.39 (89.49, 93.29)	90.93 (89.01, 92.88)	0.0977	92.56 (90.94, 94.26)	91.95 (90.33, 93.66)	0.0839
Men	390 (43.92%)	20.57 (16.57, 30.71)	64.38 (56.82, 72.34)	63.01 (55.32, 71.11)	0.6065	99.68 (99.51, 100)	99.68 (99.51, 100)	1	97.92 (96.3, 100)	97.87 (96.15, 100)	0.7324	92.4 (90.52, 94.37)	92.13 (90.22, 94.25)	0.6065	93.08 (91.54, 95)	92.82 (91.15, 94.62)	0.6065
Reason for testing																	
Referral from physician	233 (26.24%)	18.55 (16.31, 21.45)	84.81 (79.17, 90.57)	83.54 (77.78, 89.8)	0.4839	98.7 (97.94, 100)	98.7 (97.94, 100)	1	97.1 (95.12, 100)	97.06 (95.12, 100)	0.5407	92.68 (89.91, 95.5)	92.12 (89.29, 95.33)	0.4839	93.99 (92.26, 96.13)	93.56 (91.61, 96.13)	0.4839
Referral from health departments (mostly contact persons of infected patients)	236 (26.58%)	23.2 (17.21, 31.55)	57.58 (48.84, 66.67)	54.55 (45.95, 63.64)	0.2811	99.71 (99.07, 100)	99.41 (99.07, 100)	0.9822	98.7 (95.45, 100)	97.3 (95, 100)	0.6937	85.82 (82.31, 89.36)	84.92 (81.36, 88.64)	0.2831	87.92 (84.71, 90.45)	86.86 (84.08, 89.81)	0.3473
Warning by Corona-Warn-App	419 (47.18%)	22.77 (18.23, 31.2)	48.84 (37.93, 60)	48.84 (37.93, 60)	0.9944	99.87 (99.59, 100)	99.87 (99.59, 100)	1	97.67 (90.91, 100)	97.67 (90.91, 100)	1	94.47 (92.86, 96.2)	94.47 (92.87, 96.2)	0.9944	94.63 (93.19, 96.06)	94.63 (93.19, 96.06)	0.9944

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TABLE 2 (Continued)

	N (%)	CT median (25th, 75th percentile)	Sensitivity (%)	Sensitivity (%)	P*	Specificity (%)	Specificity (%)	P*	PPV (%)	PPV (%)	P*	NPV (%)	NPV (%)	P*	EFF (%)	EFF (%)	P*
		In positives	Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT	
Comorbidities																	
Any comorbidity	223 (25.11%)	25.44 (18.16, 32.15)	52.83 (43.24, 62.5)	49.06 (39.39, 58.82)	0.2099	99.41 (99.08, 100)	98.82 (98.17, 100)	0.6535	96.55 (93.75, 100)	92.86 (87.5, 100)	0.1555	87.11 (83.85, 90.6)	86.15 (82.81, 89.76)	0.1049	88.34 (85.23, 91.28)	87 (83.89, 89.93)	0.0881
No comorbidity	665 (74.89%)	19.71 (16.56, 26.71)	72.59 (67.37, 77.91)	71.85 (66.46, 77.27)	0.6075	99.81 (99.71, 100)	99.81 (99.71, 100)	1	98.99 (98.28, 100)	98.98 (98.28, 100)	0.7316	93.46 (92.05, 94.93)	93.3 (91.86, 94.74)	0.6075	94.29 (93, 95.49)	94.14 (93, 95.49)	0.6075
Hypertension	84 (9.46%)	18.64 (16.69, 26.21)	76.47 (62.5, 91.67)	64.71 (50, 81.82)	0.2139	98.51 (97.62, 100)	98.51 (97.62, 100)	1	92.86 (85.71, 100)	91.67 (83.33, 100)	0.8299	94.29 (91.11, 97.92)	91.67 (87.5, 95.92)	0.2139	94.05 (**)	91.67 (**)	0.2139
Dyslipoproteinaemia	42 (4.73%)	18.5 (17.87, 21.74)	83.33	83.33	Not defined	98.61 (95.45, 100)	98.61 (95.45, 100)	1	90.91	90.91	Not defined	97.26 (95.45, 100)	97.26 (95.45, 100)	1	96.43 (**)	96.43 (**)	1
Diabetes mellitus	19 (2.14%)	19.62 (18.38, 20.35)	80	80	Not defined	96.43 (**)	96.43 (**)	1	88.89	88.89	Not defined	93.1 (**)	93.1 (**)	1	92.11 (**)	92.11 (**)	1
COPD	8 (0.9%)	15.35 (14.37, 24.82)	66.67	66.67	Not defined	90 (100, 100)	90 (100, 100)	1	80	80	Not defined	81.82 (**)	81.82 (**)	1	81.25 (**)	81.25 (**)	1
Ischemic heart disease	7 (0.79%)	**	**	**	Not defined	92.86 (100, 100)	92.86 (100, 100)	1	0	0	Not defined	100 (100, 100)	100 (100, 100)	1	92.86 (100, 100)	92.86 (100, 100)	1
Previous COVID-19	101 (11.37%)	31.19 (25.75, 33.19)	26.67 (15, 38.1)	26.67 (15, 38.1)	0.996	99.3 (97.67, 100)	98.59 (97.67, 100)	0.9886	94.12 (75, 100)	88.89 (75, 100)	0.9898	76.22 (70, 82.26)	76.09 (70, 82.26)	0.9922	77.72 (71.64, 83.58)	77.23 (71.64, 83.58)	0.9908

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TABLE 2 (Continued)

	N (%)	CT median (25th, 75th percentile)	Sensitivity (%)	Sensitivity (%)	P*	Specificity (%)	Specificity (%)	P*	PPV (%)	PPV (%)	P*	NPV (%)	NPV (%)	P*	EFF (%)	EFF (%)	P*
		In positives	Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT	
No previous COVID-19	787 (88.63%)	19.23 (16.53, 25.2)	74.68 (70, 79.46)	72.78 (67.96, 77.78)	0.1855	99.68 (99.51, 100)	99.68 (99.51, 100)	1	98.33 (97.26, 100)	98.29 (97.18, 100)	0.2761	94 (92.76, 95.28)	93.58 (92.33, 94.9)	0.1855	94.66 (93.52, 95.81)	94.28 (93.14, 95.43)	0.1855
Clinical symptoms																	
Any clinical symptom	447 (50.34%)	18.68 (16.48, 23.3)	77.4 (72.55, 82.33)	76.03 (71.13, 81.05)	0.3965	99.34 (98.96, 100)	99.34 (98.96, 100)	1	98.26 (97.14, 100)	98.23 (97.1, 100)	0.4667	90.06 (87.78, 92.41)	89.52 (87.21, 91.86)	0.3965	92.17 (90.4, 93.96)	91.72 (89.93, 93.62)	0.3965
No clinical symptom	441 (49.66%)	30.81 (23.24, 32.69)	30.95 (20.69, 40.91)	28.57 (18.52, 38.46)	0.6631	99.87 (99.62, 100)	99.75 (99.62, 100)	0.9982	96.3 (85.71, 100)	92.31 (83.33, 100)	0.7025	93.22 (91.55, 95.02)	92.99 (91.26, 94.74)	0.3331	93.31 (91.5, 94.9)	92.97 (91.5, 94.56)	0.4841
Malaise	325 (36.6%)	18.55 (16.37, 21.68)	80.56 (75.36, 86.11)	78.7 (73.24, 84.42)	0.2797	99.54 (99.28, 100)	99.54 (99.28, 100)	1	98.86 (98.08, 100)	98.84 (98.04, 100)	0.5205	91.14 (88.61, 93.75)	90.38 (87.8, 93.12)	0.2797	93.23 (91.24, 95.39)	92.62 (90.78, 94.47)	0.2797
Shortness of breath	68 (7.66%)	18.32 (15.5, 21.02)	85 (75, 100)	80 (66.67, 92.86)	0.6851	97.92 (96.55, 100)	97.92 (96.55, 100)	1	94.44 (88.89, 100)	94.12 (88.89, 100)	0.8463	94 (90.32, 100)	92.16 (87.5, 97.14)	0.6851	94.12 (**)	92.65 (**)	0.6851
Cough	269 (30.29%)	18.56 (16.41, 21.94)	80.91 (75.68, 86.3)	77.27 (71.83, 83.08)	0.0323	98.74 (98, 100)	98.74 (98, 100)	1	97.8 (96.36, 100)	97.7 (96.23, 100)	0.1395	88.2 (84.87, 91.67)	86.26 (82.79, 89.92)	0.0323	91.45 (89.39, 93.85)	89.96 (87.71, 92.74)	0.0323
Fever	54 (6.08%)	18.25 (15.8, 21.1)	87.5 (77.78, 100)	81.25 (70, 100)	0.6607	98.68 (95.65, 100)	98.68 (95.65, 100)	1	96.55 (87.5, 100)	96.3 (85.71, 100)	0.8855	94.94 (91.67, 100)	92.59 (88, 100)	0.6607	95.37 (**)	93.52 (**)	0.6607
Diarrhea	44 (4.95%)	18.64 (18.36, 29.02)	72.73 (50, 100)	72.73 (50, 100)	1	98.48 (94.74, 100)	98.48 (94.74, 100)	1	94.12 (75, 100)	94.12 (75, 100)	1	91.55 (86.36, 100)	91.55 (86.36, 100)	1	92.05 (**)	92.05 (**)	1

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TABLE 2 (Continued)

	N (%)	CT median (25th, 75th percentile)	Sensitivity (%)	Sensitivity (%)	P*	Specificity (%)	Specificity (%)	P*	PPV (%)	PPV (%)	P*	NPV (%)	NPV (%)	P*	EFF (%)	EFF (%)	P*
		In positives	Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT	
Musculoskeletal pain	145 (16.33%)	19.85 (16.28, 23.13)	77.78 (70.45, 85.37)	74.6 (66.67, 82.5)	0.2155	99.39 (98, 100)	99.39 (98, 100)	1	98.99 (96.55, 100)	98.95 (96.3, 100)	0.4675	85.34 (80.3, 90.48)	83.59 (78.29, 88.89)	0.2155	90 (86.6, 92.78)	88.62 (84.54, 91.75)	0.2155
Headache	266 (29.95%)	18.42 (16.35, 21.32)	82.56 (77.05, 88.46)	81.4 (75.86, 87.5)	0.4799	99.44 (99.12, 100)	99.44 (99.12, 100)	1	98.61 (97.62, 100)	98.59 (97.56, 100)	0.647	92.27 (89.78, 95.16)	91.79 (89.23, 94.62)	0.4799	93.98 (92.09, 96.05)	93.61 (91.53, 96.05)	0.4799
Nausea	37 (4.17%)	20.52 (17.85, 21.43)	80 (60, 100)	80 (60, 100)	1	98.15 (93.75, 100)	98.15 (93.75, 100)	1	94.12 (75, 100)	94.12 (75, 100)	1	92.98 (88.24, 100)	92.98 (88.24, 100)	1	93.24 (**)	93.24 (**)	1
Vaccination status																	
Non-vaccinated (0 or 1 vaccination)	98 (11.04%)	19.55 (16.35, 29.25)	67.57 (57.14, 79.17)	64.86 (54.17, 76.19)	0.4831	99.18 (97.3, 100)	98.36 (97.3, 100)	0.9858	98.04 (92.86, 100)	96 (92.31, 100)	0.7928	83.45 (77.55, 89.58)	82.19 (76.09, 88.89)	0.4887	87.24 (82.13, 92.31)	85.71 (81.54, 90.77)	0.5729
Vaccinated (2 vaccinations)	321 (36.15%)	20.9 (17.73, 31.55)	61.54 (52.78, 70.45)	60 (51.28, 68.89)	0.5333	99.22 (98.8, 100)	99.22 (98.8, 100)	1	95.24 (91.67, 100)	95.12 (91.67, 100)	0.5919	91.04 (88.79, 93.48)	90.71 (88.42, 93.09)	0.5333	91.59 (89.72, 93.93)	91.28 (89.25, 93.46)	0.5333
Boostered (3 or more vaccinations)	463 (52.14%)	20.08 (16.78, 25.42)	70.59 (63.96, 77.42)	70.59 (63.79, 77.5)	0.999	99.87 (99.59, 100)	99.87 (99.59, 100)	1	99.17 (97.14, 100)	99.17 (97.14, 100)	0.9928	93.79 (92.18, 95.52)	93.79 (92.18, 95.51)	0.999	94.49 (92.88, 95.79)	94.49 (92.88, 95.79)	0.999
Unknown vaccination status	6 (0.68%)	26.21 (26.21, 26.21)	100	0	**	90 (100, 100)	90 (100, 100)	1	66.67	0	not defined	100 (100, 100)	81.82 (**)	0.6491	91.67 (100, 100)	75 (**)	0.6491
Vital signs																	
SysBP>130 mmHg and/or DiaBP>90 mmHg	234 (26.35%)	20.46 (16.65, 29.94)	65.22 (55.17, 75.76)	63.04 (53.01, 73.08)	0.4745	99.73 (99.17, 100)	99.73 (99.17, 100)	1	98.36 (93.75, 100)	98.31 (93.75, 100)	0.6362	92.14 (89.55, 94.81)	91.69 (88.97, 94.24)	0.4745	92.95 (90.38, 94.87)	92.52 (89.74, 94.87)	0.4745

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TABLE 2 (Continued)

	N (%)	CT median (25th, 75th percentile)	Sensitivity (%)	Sensitivity (%)	p*	Specificity (%)	Specificity (%)	p*	PPV (%)	PPV (%)	p*	NPV (%)	NPV (%)	p*	EFF (%)	EFF (%)	p*
		In positives	Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT		Roche-RAT	Abbott-RAT	
Other blood pressures	638 (71.85%)	20.2 (17.02, 29.8)	68.84 (63.44, 74.44)	67.39 (61.8, 73.12)	0.3581	99.6 (99.39, 100)	99.4 (99.08, 100)	0.6543	97.94 (96.55, 100)	96.88 (94.92, 100)	0.1471	92.05 (90.48, 93.68)	91.7 (90.06, 93.39)	0.2311	92.95 (91.53, 94.35)	92.48 (91.06, 93.88)	0.1847
Body temperature > 37°C	39 (4.39%)	17.35 (16.27, 18.57)	77.78 (60, 100)	88.89 (75, 100)	0.6895	98.33 (94.44, 100)	98.33 (94.44, 100)	1	93.33	94.12	not defined	93.65 (89.47, 100)	96.72 (94.44, 100)	0.6895	93.59 (**)	96.15 (**)	0.6895
Body temperature ≤ 37°C	849 (95.61%)	20.46 (17.03, 30.16)	66.48 (61.4, 71.54)	64.25 (59.35, 69.34)	0.0907	99.7 (99.54, 100)	99.55 (99.32, 100)	0.6571	98.35 (97.26, 100)	97.46 (95.89, 100)	0.0623	91.76 (90.41, 93.24)	91.24 (89.84, 92.76)	0.0477	92.7 (91.52, 93.99)	92.11 (90.81, 93.46)	0.0377
Oxygen saturation > median	200 (22.52%)	20.59 (17.6, 28.94)	60 (50, 71.43)	62.5 (52, 74.07)	0.6807	99.69 (99.02, 100)	99.69 (99.02, 100)	1	97.96 (92.31, 100)	98.04 (92.86, 100)	0.8879	90.88 (87.93, 94.02)	91.4 (88.5, 94.69)	0.6807	91.75 (88.72, 93.98)	92.25 (89.47, 94.74)	0.6807
Oxygen saturation ≤ median	674 (75.9%)	20.3 (16.74, 30.02)	69.39 (64.08, 74.74)	65.99 (60.42, 71.72)	0.0291	99.62 (99.42, 100)	99.43 (99.13, 100)	0.6551	98.08 (96.83, 100)	97 (95.08, 100)	0.0445	92.11 (90.56, 93.67)	91.29 (89.69, 92.97)	0.0139	93.03 (91.76, 94.43)	92.14 (90.65, 93.54)	0.0117
SARS-CoV-2 Genotype (for CT ≤ 32)																	
Delta variant	41 (4.62%)	19.55 (16.27, 23)	80.49 (70.37, 88.89)	80.49 (70.37, 88.89)	0.9928	not defined	not defined	not defined	100 (100, 100)	100 (100, 100)	1	0 (0, 0)	0 (0, 0)	1	80.49 (70.37, 88.89)	80.49 (70.37, 88.89)	0.9928
Omikron variant	62 (6.98%)	17.62 (15.71, 19.82)	91.94 (**)	93.55 (**)	0.6759	not defined	not defined	not defined	100 (100, 100)	100 (100, 100)	1	0 (0, 0)	0 (0, 0)	1	91.94 (**)	93.55 (**)	0.6759

*p-value Roche versus Abbott (test on equality, based 5,000 bootstrap iterations). **Too low number of patients with negative/positive rapid test for calculation.

10.3389/fmed.2024.1352633

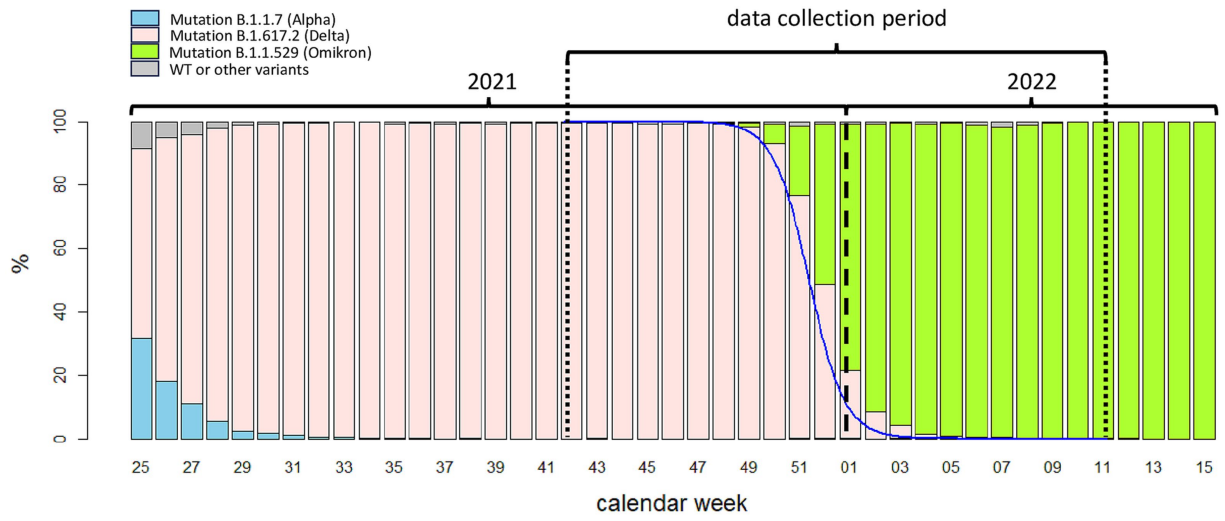

FIGURE 1
Framing of the COVAG extension study (October 20, 2021 to March 18, 2022) into the time course of the COVID-19 pandemic in Germany. *Abszissa:* calendar week within 2021 and 2022; *bars:* Germany-wide weekly proportions of variants of concern (VOC) in percent. *Blue solid line:* estimated proportion of variant B.1.617.2 (Delta) in the COVAG extension study to Germany (based on logistic regression with the categories 'Delta' vs. 'Omikron').

(25.1%) reported having any comorbidities. The most common comorbidities were hypertension (9.5%) and dyslipoproteinemia (4.7%). Other comorbidities were low in frequency. 101 (11.4%) participants self-reported having had a previous COVID-19 infection (Table 1).

98 (11.0%) participants are non-vaccinated (0 or 1 vaccination against COVID-19), 321 (36.2%) participants are 'vaccinated' (2 vaccinations against COVID-19), 463 participants (52.1%) have received a booster vaccination (3 or more vaccinations against COVID-19). For six persons (0.7%), the vaccination status is unknown.

447 (50.3%) participants reported having clinical symptoms while 441 (49.7%) reported none. The most common symptoms were malaise, cough, headache, and musculoskeletal pain at frequencies of 36.6, 30.3, 30.0, and 16.3%, respectively (Table 1).

188 (21.2%) participants were tested positive for SARS-CoV-2 by RT-PCR. 126 (14.2%) were tested positive by the Abbott-RAT and 128 (14.4%) by the Roche-RAT. 125 (14.1%) samples had a Ct value ≤25, 16 (1.8%) a Ct value 25–30, 47 (5.3%) ≥ 30. 155 (17.5%) RT-PCR samples had a Ct value ≤32. 52 RT-PCR positive samples with a Ct value ≤32 could not examined be for variants. Of the remaining 103 samples, the Omicron variant was found in 41 and the Delta variant was found in 62.

3.2 Diagnostic performance of RATs

Sensitivity. The Abbott-RAT and the Roche-RAT had overall sensitivities of 65.4% (95% CI 60.7–70.2%) and 67.0% (95% CI 62.4–71.8%) respectively (Table 2). The sensitivities of both RATs were significantly associated with the Ct-value derived from RT-PCR (Figure 1A).

The Abbott-RAT and Roche-RAT did not show a significant difference in sensitivity ($p = 0.2091$; Table 2). Due to higher power in the total study cohort ($n = 1{,}487$) the Roche-RAT had a significantly higher sensitivity than the Abbott-RAT ($p = 0.0093$, Supplementary Table S2). Among participants seeking testing due to a referral by their primary care physician, the sensitivities for the Abbot-RAT and Roche-RAT were 83.5 and 84.8%, for participants with a referral by the health department they were 54.6 and 57.6% and following a warning by their Corona-Warn-App the sensitivities were 48.8% for both tests (Table 2), respectively. In the participants excluded because they were tested to confirm a positive antigen test the sensitivities of the Abbott-RAT and the Roche-RAT were 93.0 and 94.5%.

Participants with previous COVID-19 showed significantly lower sensitivities of only 26.7% for both RATs (OR 0.12 (95%CI: 0.05,0.3), $p < 0.0001$). This finding is attributable to Ct values being markedly higher (Median 31.2) in patients with previous COVID-19 and not consistent anymore when adjusted for the Ct value (Table 3).

For participants without previous COVID-19, significantly higher sensitivities (72.8 and 74.7%, Abbott-RAT and Roche-RAT, respectively) were found in line with markedly lower Ct values (Median 19.2).

In symptomatic participants, the sensitivities were significantly higher (76.0 and 77.4%%, Abbott-RAT and Roche-RAT, respectively) than in asymptomatic participants (28.6 and 31.0%, Abbott-RAT and Roche-RAT, respectively). This finding is in line with Ct-values being lower in symptomatic patients than in asymptomatic patients (Ct Median 18.7 vs. 30.8, Table 2).

We further analyzed the diagnostic performance of RATs according to the vaccination status. The sensitivities of the RATs in non-vaccinated participants (0 or 1 vaccination) were 64.9 and 67.7% for Abbott-RAT and Roche-RAT, respectively. For participants with two vaccinations, the sensitivities were 59.4 and 60.9%. For participants with at least one booster vaccination, we find sensitivities equal to 70.6% for both RATs.

We also investigated whether the SARS-CoV-2 variants Delta and Omicron affected the sensitivity of the RATs. Both variants had similar sensitivities compared to the wild-type from the first wave of the Covag study. Compared to the alpha variant the alpha variant had significantly lower sensitivities than the wild-type, delta and omicron (Figure 4) (13).

TABLE 3 Predictors of positive RATs among SARS-CoV-2 positive samples in a multivariate model (CT ≤ 32, $N = 155$).

Covariable		Roche-RDT	Roche-RDT1	Abbott-RDT	Abbott-RDT1
		OR (95% CI)	*p*-value	OR (95% CI)	*p*-value
Age and sex	Age, per year	0.95 (0.89,1.02)	0.1691	0.99 (0.92,1.07)	0.8437
	Men	1.0 (reference)		1.0 (reference)	
	Women	0.66 (0.12,3.47)	0.6215	0.2 (0.02,1.67)	0.1384
Ct value	Ct value on rRT-PCR, per unit	0.54 (0.42,0.7)	<0.0001	0.49 (0.36,0.67)	<0.0001
Reason for testing	Referral from physician	1.0 (reference)		1.0 (reference)	
	Referral from health department	0.21 (0.02,1.91)	0.1656	1.12 (0.08,15.04)	0.9308
	Warning by Corona app	0.05 (0,0.51)	0.0117	0.16 (0.02,1.6)	0.1192
Comorbidities	No comorbidity	1.0 (reference)		1.0 (reference)	
	Any comorbidity	1.9 (0.11,31.79)	0.6562	0.28 (0.02,4.66)	0.3752
	Previous Covid	0.24 (0.03,2.1)	0.1980	1.24 (0.08,18.98)	0.8772
Clinical symptom	No clinical symptoms	1.0 (reference)		1.0 (reference)	
	At least one clinical symptom	0.38 (0.05,2.91)	0.3501	1.06 (0.11,9.82)	0.9598
Vaccination	Not vaccinated	1.0 (reference)		1.0 (reference)	
	Vaccinated	1.8 (0.16,20.32)	0.6344	6.91 (0.46,103.9)	0.1624
	Boostered	0.92 (0.1,8.89)	0.9423	4.53 (0.38,53.99)	0.2321
SARS-CoV-2 genotype	Delta	1.0 (reference)		1.0 (reference)	
	Omikron	0.51 (0.07,3.51)	0.4967	2.78 (0.26,29.58)	0.3972

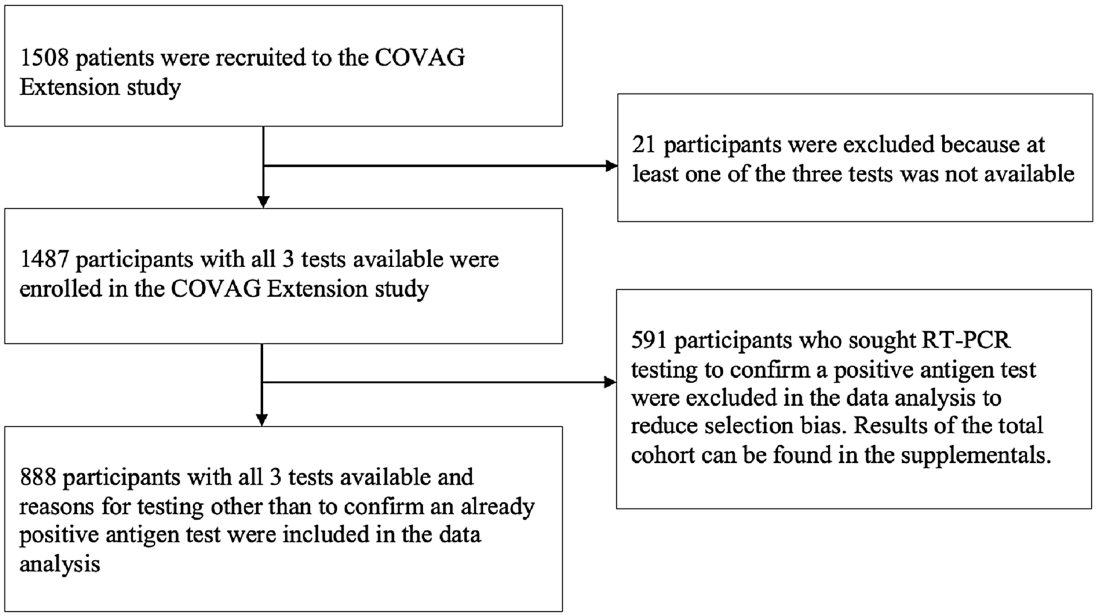

FIGURE 2
Flowchart illustrating the inclusion of participants into the COVAG extension study and data analysis.

To firmly establish independent predictors of sensitivity, we calculated ORs for having a positive RAT according to subgroups by multivariate logistic regression (Table 3). Covariables were age, sex, Ct value, reason for testing, presence or absence of any comorbidity and previous COVID-19, COVID-19 vaccination status, presence or absence of any clinical symptom, and the SARS-CoV-2 genotype. As expected, Ct values were strongly associated with the sensitivities of both tests. The sensitivities of the Abbott-RAT and Roche-RAT were

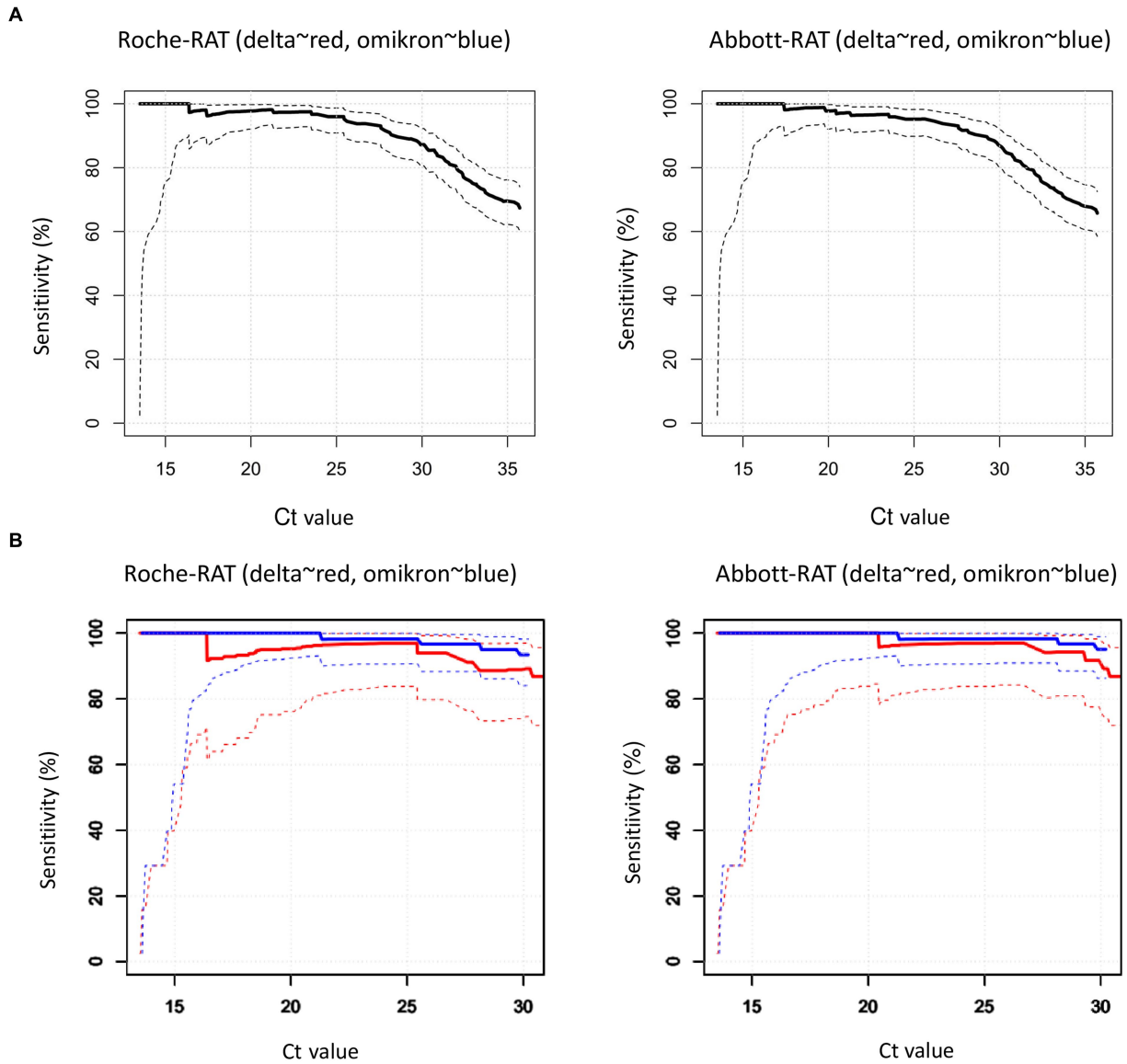

FIGURE 3
Relationships between sensitivities of RDTs vs. rRT-PCR cycle threshold (Ct) values. The solid lines indicate sensitivities, the dotted lines represent the upper, and the lower bounds the corresponding 95% confidence intervals. **(A)** Left: Roche-RDT; right: Abbott-RDT. **(B)** Sensitivities according to SARS-CoV-2 genotypes. Left: Roche-RDT; right: Abbott-RDT; *red*: Delta variant; *blue*: Omikron variant.

lower in participants who sought testing due to a warning in the Corona Warn App.

When excluding the Ct value from the multivariate logistic regression symptomatic participants were detected with a significantly higher sensitivity than asymptomatic participants (Abbott-RAT: OR 4.35, $p = 0.0081$; Roche-RAT: OR 3.46, $p = 0.0216$). However, when adjusting for the Ct value this finding was not significant anymore (Table 3). The vaccination status was not associated with a change in sensitivity of the RATs.

As the Ct value is the strongest predictor for the sensitivity of the RATs, we calculated the sensitivity of the RATs separately for different Ct values. For a Ct value ≤25 the sensitivities were 95.2 and 96.0% for the Abbott-RAT and the Roche-RAT, respectively (Table 4). For a Ct value of 25–30 both RATs had a sensitivity of 18.8%. For a Ct value of 30–32, the sensitivities were 0.0 and 7.1% respectively, for Ct values ≥32 the sensitivities were 3.0 and 6.0% for Abbott-RAT and Roche-RAT, respectively.

Specificity. The specificity exceeded 99% overall and in mostly all participant strata (Table 2; Supplementary Table S2).

PPV, NPV, and diagnostic performance. The rate of true negatives in our study cohort ($n = 888$) was 697 of 700 (99.6%) and 698 of 700 (99.7%), the rate of false negatives was 65 of 188 (34.6%) and 62 of 188 (33.0%) for the Abbott-RAT and the Roche-RAT, respectively. The rate of true positives was 123 of 188 (65.4%) and 126 of 700 (67%). The rate for false positives was 3 of 700 (0.4%) and 2 of 700 (0.3%) for Abbott-RAT and Roche-RAT, respectively.

When also including the participants who already had a positive self-test beforehand (total of $n = 1{,}487$) the rate of false negatives

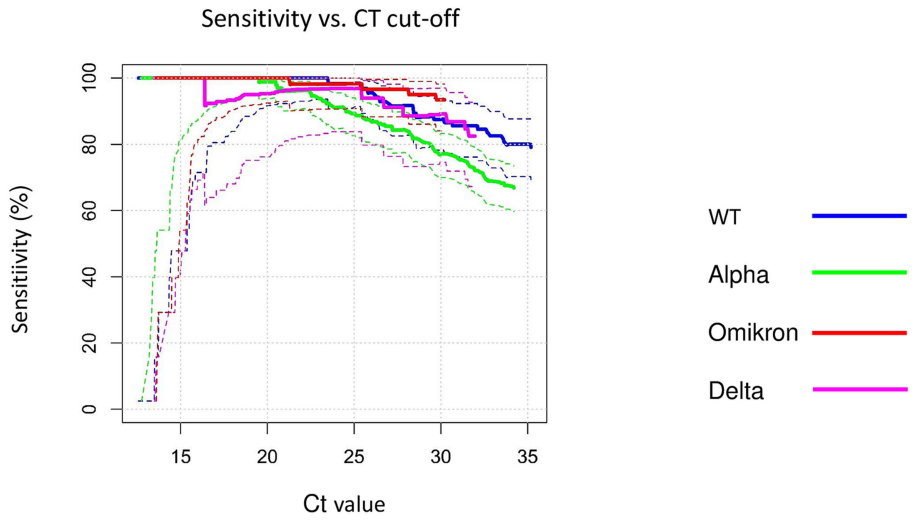

FIGURE 4
rRT-PCR cycle thresholds (Ct) values on rRT-PCR for SARS-Cov-2 RNA of different variants versus sensitivities of the Roche-RDT. The *solid lines* indicate sensitivities, the *dotted lines* represent the upper and the lower bounds the corresponding 95% confidence intervals. *Magenta*: Delta; *red*: Omikron; *green*: Alpha; *blue*: WT.

TABLE 4 Sensitivities of Abbott-RAT and Roche-RAT versus Ct values.

All variants	*N* (%)	CT median (25th, 75th percentile)	Sensitivity (%)	Sensitivity (%)
Ct value		In positives	Roche-RDT	Abbott-RDT
≤ 25	125 (67%)	17.95 (15.98, 20.35)	96.00	95.20
25–30	16 (8%)	27.96 (26.56, 29.27)	18.75	18.75
30–32	14 (7%)	30.88 (30.47, 31.35)	7.14	0.00
≥ 32	33 (18%)	33.62 (32.73, 34.67)	6.06	3.03

decreased to 101 of 704 (14.4%) and 90 of 704 (12.8%) for the Abbott-RAT and the Roche-RAT, respectively. The rate of false positives was also overall very low with 4 of 783 (0.5%) and 2 of 783 (0.3%) for the Abbott-RAT and the Roche-RAT. Of the 591 participants who sought RT-PCR testing to confirm a positive self-test, 511 (86.5%) were confirmed positive by RT-PCR while 80 (13.5%) were tested negative by RT-PCR.

The SARS-CoV-2 prevalence in our study cohort was 78.8% ($n = 888$). At this prevalence the PPV was at 97.6 and 98.4% for Abbott-RAT and Roche-RAT ($n = 888$, Table 2). For symptomatic participants the PPV was higher (98.2 and 98.3%, Abbott-RAT and Roche-RAT, respectively) than for asymptomatic participants (92.3 and 96.3%, Abbott-RAT and Roche-RAT, respectively). The NPV was 91.5 and 91.8% for Abbott-RAT and Roche-RAT. The NPV was higher for asymptomatic (93.0 and 93.2%, Abbott-RAT and Roche-RAT, respectively) than for symptomatic participants (89.5 and 90.1%, Abbott-RAT and Roche-RAT, respectively).

Because patients with SARS-CoV-2 infections were enriched in our study population compared to the general population, we examined the PPVs and NPVs at assumed prevalence rates up to 0.05 (Figure 5). To compare the PPV and NPV of the RATs with standardized performance criteria, we also used the following hypothetical sensitivity and specificity levels (tiers 1–3) recommended by Kost et al. (15): tier 1, 90, 95%; tier 2, 95, 97.5%; and tier 3, 100%, ≥99% (Figure 5). At this prevalence rate, our results suggest a PPV and NPV of 88.9 and 98.2% for Abbott-RAT, and 92.5 and 98.3% for the Roche-RAT, the Roche-RAT displaying a higher PPV than the Abbott-RAT and both scoring higher than the hypothetical tiers 1 through 3, reflecting increases in NPV in the order of Abbott-RAT < Roche-RAT < tier 1 < tier 2 < tier 3. The NPVs ranged in the order of tier 3 > tier 2 > tier 1 > Roche-RAT > Abbott-RAT.

4 Discussion

This study is an extension of the COVAG study which is one of the largest prospective, real-world evaluations of RATs to date (13). We compared two of the most sensitive RATs provided by Abbott Diagnostics and Roche Diagnostics, especially in the light of newly emerged variants (9). We found that the sensitivities of RATs for asymptomatic patients was as low as 30%. We found that the Omicron and Delta variant were detected with not significantly different sensitivities compared to the wild-type at Ct values >25 (13).

In contrast to the first wave of our study, there was no significant difference in sensitivity between the Abbott-RAT and Roche-RAT (13). However, with an extended sample size ($n = 1{,}487$) after including participants seeking RT-PCR testing to confirm a positive antigen test, the Roche-RAT had a significantly higher sensitivity than the Abbott-RAT ($p = 0.0093$; Supplementary Table S2), attributable to the

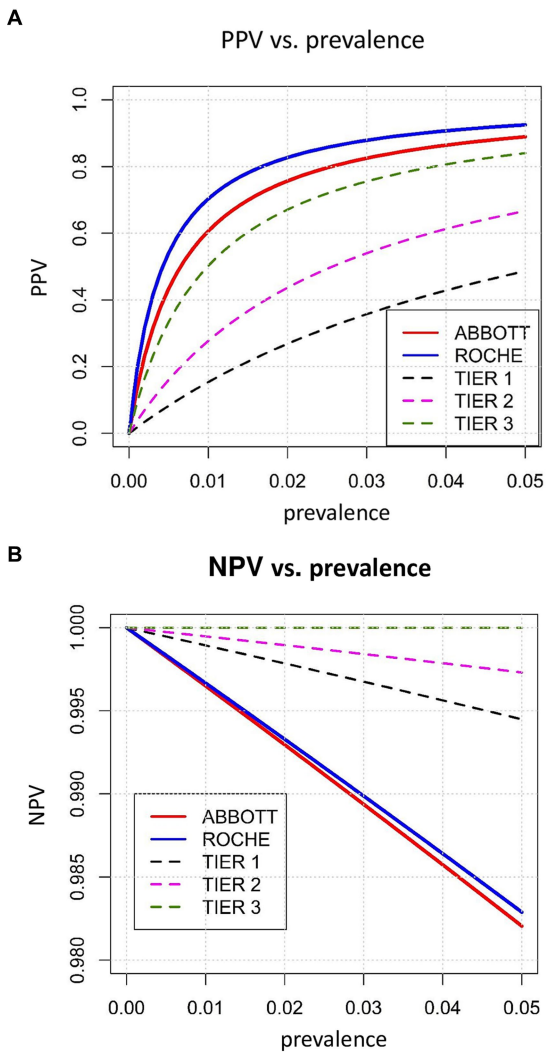

FIGURE 5
(A) Prevalence versus PPV for tier 1–3 [for hypothetical sensitivities and specificities (15)] and both rapid tests. **(B)** Prevalence versus NPV for tier 1–3 [for hypothetical sensitivities and specificities (15)] and both rapid tests.

participants with an age ≤ median. This finding is in good agreement with the results of the first wave of our study.

The sensitivities were substantially higher among participants referred by their primary care physician (84–85%, Table 2). As primary care physicians refer patients to RT-PCR testing based on their clinical presentation and history, the pretest probability is higher and patients with higher symptom burden sent for testing, also reflected by lower Ct values in these participants. This shows that the sensitivity of the RATs can be increased by considering the clinical background. The PPVs of RATs was overall very good (88–92%). Compared to the tiers recommend by Kost et al. the NPV occur to be lower than the recommend values of tier 1–3. However, due to the smallness of the discrepancies between the measured NPVs and the recommended NPV ranges (<2%) and the small number of false positives, the last statement made about the NPVs should be taken with caution.

4.1 Diagnostic performance of the RATs

The WHO formulated minimum performance requirements of ≥80% sensitivity and ≥97% specificity for RATs (16). The European Centre for Disease Prevention and Control (ECDC) agreed to the performance requirements set by the WHO (17). In our study both RATs did not meet the sensitivity performance requirements while meeting the specificity requirements (Abbott-RAT: sensitivity 65.4%, specificity 99.6%; Roche-RAT: sensitivity 67.0%, specificity 99.7%). Similar results were reported by a Cochrane Analysis which reported sensitivities of 56.7% (95% CI 44.3–68.3%) and 64.4% (95% CI 52.2–75.0%) for the Abbott-RAT and the Roche-RAT, respectively (9). In a large comparative *in vitro* evaluation of 122 RATs reported the Paul-Ehrlich-Institut (PEI), the overall sensitivity of the Abbott-RAT and the Roche-RAT were 64.0 and 46.0%, respectively (18). While the Abbott-RAT showed a comparable sensitivity of 65.4% in our study, the Roche-RAT yielded a better sensitivity of 67.0%. However, also in the study by the PEI both RATs failed to meet the sensitivity requirement set by the WHO. This is in large contrast to the sensitivities of 97.6 and 95.5%, respectively, reported by the providers Abbott and Roche. for samples with Ct values ≤30 (19, 20).

During our study comparable sensitivities (95.2 and 96% for Abbott-RAT and Roche-RAT, respectively) were reported only for Ct values ≤25. For Ct values of 25–30 the sensitivities were only 18.8% for both tests.

The RATs´ performance strongly relates to Ct values. The study by the Paul-Ehrlich-Institut showed sensitivities for the Abbott-RAT of 100% for Ct-values ≤25, 60.9% for Ct values between 25–30 and 0% for Ct values ≥30 (18). The Roche-RAT in comparison yielded a sensitivity of 88.9% for Ct values ≤25, 30.4% for Ct values between 25–30 and also 0% for Ct values ≥30 (18). Evidently thus, the performance of the RATs in our study is worse than in the *in vitro* study by the Paul-Ehrlich-Institut, suggesting that challengeable information will only be obtained under real world conditions. This notwithstanding the common denominator of the results from Paul-Ehrlich-Institut and of ours is that the performance requirements are only met for samples with a Ct ≤ 25. Hence, patients with a high viral load are well detected while patients with a lower viral load are missed (21).

An important clinical distinction is whether symptoms are present or not. The sensitivity of the RATs is markedly lower for asymptomatic than for symptomatic patients. With a sensitivity of around 30%, asymptomatic and infected patients were detected at very low sensitivity in our study. Symptomatic patients on the other hand are detected with a sensitivity of around 77%. A Cochrane analysis by Dinnes et al. reported similar results for symptomatic (Abbott-RAT: 74.8%; Roche-RAT: 78.8%) and higher results for asymptomatic (Abbott-RAT: 56.9%; Roche-RAT: 59.4%) patients compared to our study (9). Although slightly below the performance requirements of the WHO RATs may be considered useful in symptomatic patients while they are not in asymptomatic patients. These differences in sensitivity are clearly attributable to the lower Ct values of symptomatic patients. In Germany RATs have been used for screening of asymptomatic persons (10). Yet, in these patients RATs are clearly insufficient for screening.

The RNA viral load determined by RT-PCR is only a proxy for the infectiousness of patients as also non-infectious viral RNA is detected

by RT-PCR. To reliably determine the infectiousness of a patient, viral growth can be examined in culture. In a study from the UK, contacts of SARS-CoV-2 infected patients were recruited, and RT-PCR and virus culture were performed daily. Additionally, a RAT different from the ones used in our study was performed in RT-PCR positive samples as well as in samples one day before and after a positive RT-PCR. The sensitivity of the RATs was higher for samples with positive viral cultures (79%) than for samples with only positive RT-PCR (47%). Positive viral cultures were detected for a median of 5 days (IQR 3–7 days) and the peak viral load determined by viral cultures and RT-PCR was at a median of 3 days after symptom onset (IQR 3–5/6 days). Interestingly the sensitivity of the RATs was lower before and during the peak viral load (sensitivity: 67%) than after the peak viral load (sensitivity: 92%) (22). This shows that RATs have reduced sensitivity during the beginning of infection possibly leading to delayed diagnosis (22). In a study from Germany the Roche-RAT was compared to RT-PCR and viral culture. Although the Roche-RAT reached a sensitivity of only 42.8%, none of the samples with positive viral cultures was missed (23). Hence and accordance to the current study, RATs appear to have a low overall sensitivity, while highly infectious participants may reliably be detected.

The specificity of the RATs was overall very good and met the specificity requirements of the WHO and ECDC (16, 17, 24).

True positivity of the RATs approaches 100 percent and false positive results do virtually not occur. This places into question whether the general recommendation to confirm a positive RAT by PCR should always be followed. However, copy numbers determined upon disease onset may be useful to monitor the progression of and recovery from COVID-19.

4.2 Influence of the SARS-CoV-2 genotype on the diagnostic performance of RATs

During the first data collection period from February 1 to March 31, 2021, the dominant variants were the wild-type and the alpha variant. The sensitivities of the RATs for the alpha variant were significantly lower than for the wild-type also when adjusted for the Ct-value (13). In the current wave of our study (October 20, 2021 to March 18, 2022), the prevailing variants were Delta followed by Omicron. Omicron was detected with a high sensitivity of 92–94%, while Delta was detected with a lower sensitivity of 80%. This difference can solely be explained by the lower Ct values of Omicron compared to Delta (Median 17.6, IQR 15.7–19.8 vs. Median 19.6, IQR 16.3–23). Consistently, in a multivariate logistic regression adjusted for the Ct values there was no significant difference between Omicron and Delta anymore. Also, when compared at set Ct values of ≤25, 25–30, ≥30 there was no significant difference in sensitivity for Delta and Omicron, respectively. While it has been argued that that Omicron produces a higher viral load leading to better detection by RATs in general, recent findings do not confirm this assumption (25, 26). Another study from the USA also found that the sensitivities for Omicron compared to the Delta variant are not significantly different (27).

We further examined the sensitivities for Omicron and Delta compared to the wild-type data coming from the first data collection period.

4.3 Influence of the COVID-19 vaccination and previous infection on the diagnostic performance of RATs

For patients with previous COVID-19 the sensitivities for Abbott-RAT and Roche-RAT were very low (26.7%). These low sensitivities are attributable to the high Ct values in these patients (Median 30, IQR 25–33). This is plausible because patients with a previous COVID-19 infection may have lower viral loads due to mucous IgA built in response to the previous infection (28). As in the first data collection period, we also found in the COVAG Extension study that both the sensitivities and the viral load of patients with comorbidities are low. This is unexpected and may reflect a referral bias in the sense that the indication for testing is more frequent and earlier in patients at high risk for severe COVID-19. There was no significant difference in the sensitivities between vaccinated and unvaccinated participants (65–66% vs. 66–70%, Table 2), perhaps since mucous IgA is formed to a lesser extent after vaccination (29). This could explain why the vaccination status does not seem to influence the sensitivity of RATs, while a previous COVID-19 infection could. Another explanation would be that after vaccination antibodies are formed only against the Spike protein whereas after a previous infection antibodies against the Spike protein and the Nucleocapsid protein are formed (30). As RATs detect the Nucleocapsid antigen Nucleocapsid antibodies could reduce available antigens for detection.

4.4 Limitations

Among the limitations of this study is that the reference method RT-PCR does not indicate the infectiousness of patients, because RT-PCR can also detect non-viable virus particles, also there is a certain correlation between the Ct value and infectivity (31). Another limitation is that we used three different PCR kits targeting different genes. This may have produced small differences in the PCR performance characteristics.

Furthermore, we performed RATs once only and not in series. Serial testing for SARS-CoV-2 with RATs may substantially increase their diagnostic performance (32).

4.5 Conclusion

The diagnostic performance of RATs is highly associated with the viral load. The sensitivity of RATs is substantially higher in symptomatic than in asymptomatic patients and in patients referred by primary care physicians compared to other reasons for testing. Hence, RATs are significantly more useful in a clinical setting than for screening purposes. Our study does not suggest that the vaccination status influences the sensitivity of RATs.

Data availability statement

Data will be made available to researchers upon justified request and formal agreement to make sure that rules of good scientific

practice are obeyed and that credit is given to the people who have been in charge of the design and the organization of the study. Interested researchers are invited to address their request or proposal to WM (winfried.maerz@synlab.com). The authors confirm that they accessed and validated these data and that all other researchers can access the data in the same manner the authors did.

Ethics statement

The studies involving humans were approved by Ethik-Kommission II der Universität Heidelberg, Medizinische Fakultät Mannheim. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CW: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Supervision, Visualization, Writing – original draft, Writing – review & editing. AD: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. EW: Formal analysis, Investigation, Project administration, Writing – review & editing. H-JW: Writing – review & editing, Project administration. HB: Investigation, Writing – review & editing. AS: Investigation, Writing – review & editing. SH: Writing – review & editing. NL: Investigation, Methodology, Writing – review & editing. WM: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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Conflict of interest

CW, EW, HB, AS, NL, SH, and WM were employed by SYNLAB Holding Germany GmbH or its regional subsidiaries. AD is the owner of Company Dr. Dressel Consulting.

The remaining author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmed.2024.1352633/full#supplementary-material

References

1. World Health Organization. (2020). WHO characterizes COVID-19 as a pandemic. Available at: https://www.who.int/emergencies/diseases/novel-coronavirus-2019/events-as-they-happen (Accessed September 24, 2022)

2. Zhang Y, Zhang H, Zhang W. SARS-CoV-2 variants, immune escape, and countermeasures. *Front Med*. (2022) 16:196–207. doi: 10.1007/s11684-021-0906-x

3. European Centre for Disease Prevention and Control (ECDC). (2022). SARS-CoV-2 variants of concern as of 22 September 2022. Available at: https://www.ecdc.europa.eu/en/covid-19/variants-concern (Accessed September 26, 2022)

4. Wu H, Xing N, Meng K, Fu B, Xue W, Dong P, et al. Nucleocapsid mutations R203K/G204R increase the infectivity, fitness, and virulence of SARS-CoV-2. *Cell Host Microbe*. (2021) 29:1788–1801.e6. doi: 10.1016/j.chom.2021.11.005

5. Osterman A, Badell I, Basara E, Stern M, Kriesel F, Eletreby M, et al. Impaired detection of omicron by SARS-CoV-2 rapid antigen tests. *Med Microbiol Immunol*. (2022) 211:105–17. doi: 10.1007/s00430-022-00730-z

6. World Health Organization. (2022) Tracking SARS-CoV-2 variants. Available at: https://www.who.int/activities/tracking-SARS-CoV-2-variants (Accessed September 26, 2022)

7. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. (2020) 395:1054–62. doi: 10.1016/s0140-6736(20)30566-3

8. Glenet M, Lebreil AL, Heng L, N'Guyen Y, Meyer I, Andreoletti L. Asymptomatic COVID-19 adult outpatients identified as significant viable SARS-CoV-2 shedders. *Sci Rep*. (2021) 11:20615. doi: 10.1038/s41598-021-00142-8

9. Dinnes J, Sharma P, Berhane S, van Wyk SS, Nyaaba N, Domen J, et al. Rapid, point-of-care antigen tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst Rev*. (2022) 2022:CD013705. doi: 10.1002/14651858.cd013705.pub3

10. Bundesministerium für Gesundheit. (2022) Fragen und Antworten zu COVID-19 Tests. Bundesministerium für Gesundheit. Available at: https://www.bundesgesundheitsministerium.de/coronavirus/nationale-teststrategie/faq-covid-19-tests.html (Accessed December 14, 2022)

11. Deeks JJ, Dinnes J, Takwoingi Y, Davenport C, Spijker R, Taylor-Phillips S, et al. Antibody tests for identification of current and past infection with SARS-CoV-2. *Cochrane Libr*. (2020) 2020:cd013652. doi: 10.1002/14651858.cd013652

12. Stocking C, de Miguel L, Suteu G, Dressel A, Soricelli A, Roskos M, et al. Evaluation of five widely used serologic assays for antibodies to SARS-CoV-2. *Diagn Microbiol Infect Dis*. (2022) 102:115587. doi: 10.1016/j.diagmicrobio.2021.115587

13. Wertenauer C, Brenner Michael G, Dressel A, Pfeifer C, Hauser U, Wieland E, et al. Diagnostic performance of rapid antigen testing for SARS-CoV-2: the COVid-19 antigen (COVAG) study. *Front Med*. (2022) 9:9. doi: 10.3389/fmed.2022.774550

14. Dinnes J, Deeks JJ, Berhane S, Taylor M, Adriano A, Davenport C, et al. Rapid, point-of-care antigen tests for diagnosis of SARS-CoV-2 infection. *Cochrane Database Syst Rev*. (2021) 2022:CD013705. doi: 10.1002/14651858.cd013705.pub2

15. Kost GJ. The impact of increasing disease prevalence, false omissions, and diagnostic uncertainty on coronavirus disease 2019 (COVID-19) test performance. *Arch Pathol Lab Med*. (2021) 145:797–813. doi: 10.5858/arpa.2020-0716-sa

16. World Health Organization. (2021). Antigen-detection in the diagnosis of SARS-CoV-2 infection: Interim guidance, 6 October 2021. Available at: https://apps.who.int/iris/handle/10665/345948

17. European Centre for Disease Prevention and Control. (2021). Options for the use of rapid antigen detection tests for COVID-19 in the EU/EEA – first update. Available at: https://www.ecdc.europa.eu/en/publications-data/options-use-rapid-antigen-tests-covid-19-eueea-first-update

18. Scheiblauer H, Filomena A, Nitsche A, Puyskens A, Corman VM, Drosten C, et al. Comparative sensitivity evaluation for 122 CE-marked rapid diagnostic tests for SARS-CoV-2 antigen, Germany, September 2020 to April 2021. *Euro Surveill*. (2021) 26:2100441. doi: 10.2807/1560-7917.es.2021.26.44.2100441

19. Abbott. (2022) PANBIO™ COVID-19 Ag rapid test device. Available at: https://www.globalpointofcare.abbott/de/product-details/panbio-covid-19-ag-antigen-test.html (Accessed October 26, 2022)

20. Roche. (2022) SARS-CoV-2 rapid antigen test instructions to use. Available at: https://assets.cwp.roche.com/f/94122/x/074742adcc/packungsbeilage_sars-cov-2rapidantigentest_023_de_202104.pdf (Accessed October 26, 2022)

21. Singanayagam A, Patel M, Charlett A, Bernal JL, Saliba V, Ellis J, et al. Duration of infectiousness and correlation with RT-PCR cycle threshold values in cases of COVID-19, England, January to May 2020. *Euro Surveill*. (2020) 25:2001483. doi: 10.2807/1560-7917.es.2020.25.32.2001483

22. Hakki S, Zhou J, Jonnerby J, Singanayagam A, Barnett JL, Madon KJ, et al. Onset and window of SARS-CoV-2 infectiousness and temporal correlation with symptom onset: a prospective, longitudinal, community cohort study. *Lancet Respir Med*. (2022) 10:1061–73. doi: 10.1016/s2213-2600(22)00226-0

23. Korenkov M, Poopalasingam N, Madler M, Vanshylla K, Eggeling R, Wirtz M, et al. Evaluation of a rapid antigen test to detect SARS-CoV-2 infection and identify potentially infectious individuals. *J Clin Microbiol*. (2021) 59:e0089621. doi: 10.1128/jcm.00896-21

24. World Health Organization. Antigen-detection in the diagnosis of SARS-CoV-2 infection. *Interim Guidance*. (2021) 6:2021.

25. Loacker L, Dlaska M, Griesmacher A, Anliker M. Ct values of different SARS CoV2 variants: a single center observational study from Innsbruck. *Austria Clin Chem Lab Med*. (2022) 60:e225–7. doi: 10.1515/cclm-2022-0542

26. Yuasa S, Nakajima J, Takatsuki Y, Takahashi Y, Tani-Sassa C, Iwasaki Y, et al. Viral load of SARS-CoV-2 omicron is not high despite its high infectivity. *J Med Virol*. (2022) 94:5543–6. doi: 10.1002/jmv.27974

27. Soni A, Herbert C, Filippaios A, Broach J, Colubri A, Fahey N, et al. Comparison of rapid antigen tests' performance between delta and omicron variants of SARS-CoV-2: a secondary analysis from a serial home self-testing study. *Ann Intern Med*. (2022) 175:1685–92. doi: 10.7326/m22-0760

28. Sterlin D, Mathian A, Miyara M, Mohr A, Anna F, Claër L, et al. IgA dominates the early neutralizing antibody response to SARS-CoV-2. *Sci Transl Med*. (2021) 13:2223. doi: 10.1126/scitranslmed.abd2223

29. Sheikh-Mohamed S, Isho B, Chao GYC, Zuo M, Cohen C, Lustig Y, et al. Systemic and mucosal IgA responses are variably induced in response to SARS-CoV-2 mRNA vaccination and are associated with protection against subsequent infection. *Mucosal Immunol*. (2022) 15:799–808. doi: 10.1038/s41385-022-00511-0

30. Montague BT, Wipperman MF, Chio E, Crow R, Hooper AT, O'Brien MP, et al. Elevated serum IgA following vaccination against SARS-CoV-2 in a cohort of high-risk first responders. *Sci Rep*. (2022) 12:14932. doi: 10.1038/s41598-022-19095-7

31. Platten M, Hoffmann D, Grosser R, Wisplinghoff F, Wisplinghoff H, Wiesmüller G, et al. SARS-CoV-2, CT-values, and infectivity—conclusions to be drawn from side observations. *Viruses*. (2021) 13:1459. doi: 10.3390/v13081459

32. Wu S, Archuleta S, Ming LS, Somani J, Chye QS, Fisher D. Serial antigen rapid testing in staff of a large acute hospital. *Lancet Infect Dis*. (2022) 22:14–5. doi: 10.1016/s1473-3099(21)00723-4

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Algorithms for predicting COVID outcome using ready-to-use laboratorial and clinical data

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The pandemic caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is an emerging crisis affecting the public health system. The clinical features of COVID-19 can range from an asymptomatic state to acute respiratory syndrome and multiple organ dysfunction. Although some hematological and biochemical parameters are altered during moderate and severe COVID-19, there is still a lack of tools to combine these parameters to predict the clinical outcome of a patient with COVID-19. Thus, this study aimed at employing hematological and biochemical parameters of patients diagnosed with COVID-19 in order to build machine learning algorithms for predicting COVID mortality or survival. Patients included in the study had a diagnosis of SARS-CoV-2 infection confirmed by RT-PCR and biochemical and hematological measurements were performed in three different time points upon hospital admission. Among the parameters evaluated, the ones that stand out the most are the important features of the T1 time point (urea, lymphocytes, glucose, basophils and age), which could be possible biomarkers for the severity of COVID-19 patients. This study shows that urea is the parameter that best classifies patient severity and rises over time, making it a crucial analyte to be used in machine learning algorithms to predict patient outcome. In this study optimal and medically interpretable machine learning algorithms for outcome prediction are presented for each time point. It was found that urea is the most paramount variable for outcome prediction over all three time points. However, the order of importance of other variables changes for each time point, demonstrating the importance of a dynamic approach for an effective patient's outcome prediction. All in all, the use of machine learning algorithms can be a defining tool for laboratory monitoring and clinical outcome prediction, which may bring benefits to public health in future pandemics with newly emerging and reemerging SARS-CoV-2 variants of concern.

KEYWORDS

SARS-CoV-2, COVID-19, hematological and biochemical parameters, predictive biomarkers, machine learning

1 Introduction

The global panorama was abruptly reshaped at the end of 2019, when a new coronavirus, SARS-CoV-2, emerged, heralding the beginning of the COVID-19 pandemic. The virus quickly crossed borders and redefined the way biosciences worked. As nations faced the unprecedented challenges posed by this highly contagious and often serious respiratory disease, a collective call to action to control the infection through the production of an effective vaccine reverberated around the world with the greater aim of containing the virus. From frontline healthcare workers to research laboratories, the pandemic has demanded a comprehensive response.

Several countries started vaccination programs against SARS-CoV-2, totalizing over 13 billion doses of vaccines administered by 2023. Nonetheless, despite many efforts to improve vaccine coverage, less than 70% of the world population received at least one dose of these vaccines (1). Moreover, such rates are heterogeneous and may be under 30% in low-income regions. Overall, nearly 5.7 million new cases of COVID-19 were reported at the beginning of 2023 (2).

While most COVID-19 cases may remain asymptomatic or with mild symptoms, patients with severe COVID-19 may present cardiovascular problems, liver, neurological, gastrointestinal, kidney and hematological outcomes (3). In addition, the rate of mortality of critically ill COVID-19 patients without vaccination is high and post-acute sequelae are common in patients who survive the disease. Therefore, it is essential to study and understand the mechanisms involved in mortality and survival of severe COVID-19 as well as developing tools based on ready-to-use laboratorial and clinical data.

Currently, there is no definite tool to predict mortality by COVID-19, although several biomarkers have been proposed for such purpose (4). Tests such as blood count, creatine kinase (CK), D-dimer, lactate dehydrogenase (LDH), C-reactive protein (CRP), aspartate aminotransferase (AST), ferritin, prothrombin, glycemia, ferritin, cardiac biomarkers (troponin, CK-MB, Pro-BNP), 25 OH-Vitamin D, ions (Na/K/Ca/Mg) and others should be taken into consideration in the diagnosis (5). However, there is no standard protocol, thresholds defined nor algorithms using those parameters to predict clinical outcome.

In this sense, the field of artificial intelligence (AI) and, more precisely, machine learning (ML) has been making remarkable strides in several sectors, demonstrating its potential to revolutionize various aspects of modern medicine. This convergence between the COVID-19 pandemic and the power of AI and ML underlines the importance of interdisciplinary collaboration in tackling complex challenges, offering diverse possibilities in a future when technology and healthcare interconnect to create more resilient, adaptable and efficient global systems.

The World Health Organization has recently called attention to the importance of AI as an aid to the healthcare system and has issued regulatory considerations on artificial intelligence for health (6), which highlights the importance of systems being efficient and safe, as well as being made quickly available to persons in need. In fact, the speed with which this technology is deployed and the possibility of errors during this process must be considered in order to prevent causing any high-scale harm to healthcare professionals and, consequently, patients. Therefore, the regulation of artificial intelligence in health is essential and could bring safe benefits to the population, as an important tool for health promotion and care.

The use of AI and ML in the context of the pandemic is centered on pattern detections that can be obtained from medical images to laboratory parameters. However, AI is not limited to this, since it can be equally used in therapy, prognosis and also extremely useful in public health management (7–9). These tools are invaluable for understanding, predicting, and responding to the spread of COVID-19, demonstrating their ability to provide data-based information and facilitate evidence-based decision-making. In fact, our detection approach meets most of the World Health Organization (WHO) guidelines for point-of-care bioanalysis, including sensitivity, accessibility, ease of use, speed of delivery and rapidity (10, 11). This advantage contributes significantly to the creation of new diagnostic concepts. It should be noted that this strategy is promising for large-scale individual testing, which is essential for an effective response to the pandemic and the gradual restoration of social circulation (12).

This study, therefore, uses machine learning (ML) to predict the clinical outcomes of severe COVID-19 patients, taking advantage of readily available laboratory parameters and clinical data. To achieve this goal, we adopted a new methodological approach, using data from patients in the intensive care unit (ICU) of a central hospital of the metropolitan area of Belo Horizonte, (Minas Gerais state), one of the largest cities in Brazil, which was one of the most affected countries by COVID-19 worldwide. Using conventional and unconventional statistical analysis, survival versus deceased groups were compared by constructing Receiver Operating-Characteristics (ROC) curves to assess the performance and accuracy of each parameter evaluated. Finally, we used cutting-edge strategies based on the Python programming language to develop a prediction solution based on machine learning, a pioneering approach never before applied to this data set. With this, we underline the importance of routine hospital laboratory tests and their integration with appropriate machine learning models, offering another avenue for the early identification of patients in need of immediate intervention.

In the present study, we presented ML-based methods to define and predict the clinical outcome of patients and the importance of using it to classify the severity of COVID-19 patients. Optimal and medically interpretable machine learning algorithms for outcome prediction are presented for each time point. It was found that urea is the most paramount variable for outcome prediction over all three time points. However, the order of importance of other variables changes for each time point, demonstrating the importance of a dynamic approach for an effective patient's outcome prediction.

The article is organized as follows: in section 2, we present the laboratory parameters we evaluated in the three different times and their correlation shown as heatmaps. We also define which methods we used to analyze the data. In section 3 we present the results obtained from the different methods for analyzing the parameters. In section 4 we discuss the best method for predicting the clinical outcome of patients and the importance of using it to classify the severity of COVID-19 patients. Section 5 shows the limitations of our work.

2 Methods

2.1 Patient data

This study was carried out using data from patients admitted to the ICU of the Risoleta Neves hospital in Belo Horizonte, Minas Gerais state, Brazil, which is a referral unit for clinical and surgical emergencies managed by the Federal University of Minas Gerais. The study was approved by the Institutional's Ethics Committee (CAAE: 45086721.1.0000.5149 - opinion number 4.751.423).

The patients were admitted by the hospital between May 2020 and March 2021 and their inclusion in the study was dependent on the confirmation of SARS-CoV-2 infection by RT-PCR. Patients were over 18 years old (median age range = 64) and had hematological and biochemical data accessed at three time points: time 1 (T1–0 to 6 days of hospitalization), time 2 (T2–7 to 14 days of hospitalization) and time 3 (T3 – >14 days of hospitalization).

The COVID patients (total $n = 81$) were further classified according to the outcome of the disease and referred to as: "Discharge" ($n = 28$) or "Death" ($n = 53$). Serum samples were collected in tubes containing gel and in the absence of anticoagulant by venipuncture during the morning routine of the ICU visit, aliquoted and stored at -80°C until processing.

2.2 Statistical analysis

GraphPadPrism 8.0 software (GraphPad Software Inc.) was used for the conventional statistical analysis of the data to compare the groups. The Analysis of Variance (ANOVA) test, followed by Tukey's post-test for parametric data and the Kruskal-Wallis test, followed by Dunn's post-test for non-parametric data were used to compare the groups. For the comparative analysis between two groups, the Student's t-test was used for parametric data and the Mann–Whitney test for non-parametric data.

The groups of COVID-19 patients were compared and contrasted in the three time periods evaluated in this study. For that, the Receiver Operating-Characteristics curves or ROC curves were constructed to assess the performance and accuracy of each parameter evaluated, with values of the Area Under the Receiver Operating-Characteristics Curve (AUROC) less than 0.70 showing poor performance, values between 0.70 and 0.80 showing moderate performance, values between 0.80 and 0.90 showing good performance, and values greater than or equal to 0.90 showing excellent performance. For the analyses, the patient's results were evaluated according to clinical and laboratory factors. In all cases, statistically significant differences were considered when the p-value was less than 0.05.

We also used GraphPadPrism 8.0 (GraphPad Software Inc.) to build the correlation amongst all parameters, which were visualized by heatmaps that were built to underscore putative and prospective clusters of parameters with predictive potential. Spearman r correlation indices were the basis to create the heatmaps. The data under scrutiny in the heatmaps were age, outcome (discharge or death), hospital stay, red blood cells, hemoglobin, hematocrit, mean corpuscular volume, global leukocyte count, neutrophils, neutrophil/lymphocyte ratio, eosinophils, basophils, monocytes, lymphocytes, platelets, pH, pCO2, pO2, HCO3, SatO2, BE, potassium, sodium, calcium, chlorine, glycemia, lactate, creatinine, urea, and gender.

2.3 Machine learning analysis

In this work, we use the Python language to build a machine learning-based prediction solution. Five different ML models were trained to be able to predict patient's outcome (discharge or death) with the same data used for statistical analyses for the three time points. These models were Decision Tree Classifier (DT) (13), eXtreme Gradient Boosting (XGBoost) (14), K-Nearest Neighbors (KNN) (15), Logistic Regression (LR) (16), and Support Vector Machine (SVM) (17). With the training of machine learning models, we seek to obtain better results than those obtained by ANOVA test.

The five machine learning models were trained using one to five features. The selection of features was made based on the results of the χ^2 test, implemented in the Scikit-learn library (18), which evaluates the relationship between random variables, allowing us to identify and exclude the features that are most likely to be unrelated to the class, making them unimportant for the classification. To carry out the categorical data analysis based on χ^2 test, the missing data was imputed with the median of each feature and scaled in such a way that each feature is in the range from 0 to 1.

The probabilities of patients progressing to death were obtained in a leave-one-out cross-validation (LOOCV) process in which all available samples in the data set are used, one by one, as test data, while the rest of the samples are used as training data. Therefore, in each LOOCV cycle, we have n-1 samples in the training base and 1 test sample, where n is the total number of samples. There are plenty of available cross-validation (CV) techniques. To choose the optimal CV technique, the bias-variance trade-off should be considered, as well as the signal-to-noise ratio of the data, the computational complexity, and the final user's preferences (19). However, LOOCV is particularly suited for small data sets with high signal-to-noise ratio over CV set or other CV techniques because it provides a model performance estimate that is less susceptible to bias, it tends not to overestimate the test error rate, and there is no randomness in the training/validation database splits (19, 20). LOOCV is computationally expensive but a very powerful and versatile technique, suitable for any kind of predictive model (20). In each LOOCV cycle, we calculate the probabilities of the "death" outcome for the training base and the test sample. Then, with the training base probabilities, we determine the training AUROC in each cross-validation cycle as well as the probability of the "death" outcome of the test sample. At the end of the LOOCV process, we have n training AUROCs and n test sample probabilities. The average training AUROC is the average of the "n" training AUROCs and the test is obtained with the probabilities of each of the test samples. In each cycle of the LOOCV process, the missing data for each feature present in the training base is imputed with its median, and in addition, we balance it using the Synthetic Minority Oversampling Technique (SMOTE) (21). After balancing the training database, we scale it using the Robust Scaler technique implemented in the Scikit-learn library (18).

For the optimization process of the hyperparameters of the models the Optuna library (22) was used in such a way as to maximize the average training AUROC and the AUROC test. This optimization is known as multi-objective since it considers two objective functions. This was done so that the optimized hyperparameters of the models are such that the training AUROC is always greater than the test AUROC to avoid and monitor underfitting and overfitting models.

3 Results

3.1 Divergent snapshot of clinical, biochemical and hematological parameters according to disease outcome during severe COVID-19

In order to provide an overview of the parameters and possibly pinpoint differences between discharge and death outcomes, a

comprehensive analysis using heatmap strategy was performed displaying the whole dataset generated by the study. Results are shown in Figure 1 which displays correlations amongst parameters for COVID patients (Heatmap A - Figure 1) as well as the same correlations of patients whose outcomes were either discharge (Heatmap B - Figure 1) and death (Heatmap C - Figure 1). Data analysis at the time of admission (T1) demonstrated that stronger inverse and direct correlations were observed when patients were subdivided by outcome as compared to the COVID-19 group, which displayed less significant correlations in the heatmaps as compared to the subgroups.

Furthermore, the data analysis carried out at the three time points were able to distinguish discharge and death mostly at late time points, starting at T2 for the following parameters: neutrophils, overall leukocytes, sodium and urea. Conversely, urea was the sole parameter able to distinguish patients at an early time point (T1), as shown in Table 1 and Figure 2. The significant *p*-values in Table 1 were obtained by ANOVA followed by Tukey's post-test when comparing the three times and Student's *t*-test when comparing two groups (discharge and death).

Figure 2 shows the longitudinal analysis of laboratory parameters considered more important in COVID-19 patients with a discharge ($n = 28$; blue circle) and death ($n = 53$; red circle) outcome. In Figure 2A, the parameters of the two (discharge vs. death) groups are compared at the three time points. In Figure 2B, we observed the same parameters only at T1. Urea (p-value = 0.0102) was the only parameter that showed a statistical difference in distinguishing individuals who survived and did not survive COVID-19.

Sodium ion appears below the cut-off point (level considered as normal for healthy individuals) at time points T1, T2 and T3 of discharged patients but only at T1 of patients who progressed to death, with the maximum value for sodium being 146 mmol/L.

Regarding the hemogram analysis, we noticed that the neutrophils of the patients who were discharged are significantly lower than those from patients who progressed to death. The same is observed from T2 time point onwards in the overall leukocyte counts; patients who were discharged had lower overall leukocyte counts than the patients who progressed to death.

3.2 Performance of urea to distinguish disease outcome during severe COVID-19

Considering the interesting results of urea observed, the performance of this parameter in segregating discharge versus death was evaluated using ROC curve analysis. Figure 3 shows the longitudinal analysis for urea using the absolute urea dosage (mg/dL). Individual data analysis demonstrates that urea levels increased over time in patients with COVID-19 regardless of clinical outcome (Figures 3A,B). However, the results confirmed the different pattern between discharge versus death at T1, which is also observed for the late time points (T2 and T3) (Figure 3C). Furthermore, the ROC curves and AUROC values in Figure 3D demonstrate the moderate but always increasing performance of urea as a biomarker of clinical outcome. Therefore, these results indicate that urea should be taken into consideration while building algorithms for prognostics and prediction purposes.

3.3 Performance of laboratory parameters to distinguish disease outcome during severe COVID-19 using machine learning approaches

To improve the potency and accuracy of performance analysis of laboratory and clinical parameters of critically ill COVID-19 patients with different clinical outcomes, we performed a feature importance analysis using the same dataset to pinpoint additional biomarkers to discriminate discharge and death. In Figure 4, we show the five more important features for each time point, as selected using the χ^2 analysis.

In agreement with the above presented results, urea dosage resulted in the most important feature of all parameters considered here, with increasing values as time progressed. However, the remaining four more important features completely changed as time progressed.

Changes in different parameters are directly related to the physiological changes that accompany the development and evolution of the disease and the body's attempt to recover from the resulting changes. Thus, the increase in creatine in T2 follows the increase in urea concentration already observed in T1 and both are related to the evolution of the patients' renal failure. The increase in lactate concentration in T2 occurs due to the reduction in oxygen supply in the tissues, showing the advance of cellular dysfunction, which in turn may result from failure of renal functions revealed already in T1 with the increase in urea concentration. The increase in sodium (hypernatremia) is a common effect in the intensive care environment, which justifies the increase in its concentration at T3. Hypernatremia may also be directly associated with the increase in urea concentration (observed in T1 and T2) due to changes in osmotic diuresis that worsen over the period of hospitalization, as well as the increase in lactate concentration (observed in T2).

As described above, we trained and optimized the five different ML models using an increasing number of the most important features, starting from urea, for T1, T2, and T3. Table 2 contains the AUROCs obtained by this procedure. In this table, the highlighted values in gray are the highest results using only the first most important feature (Urea). These results obtained from models trained only with Urea are fundamental because they make a direct comparison with the results obtained by the longitudinal statistical analysis presented previously. The performance of the best ML model considering just urea was moderate for T1 and T2 but reaching a good performance in T3. However, the obtained AUROC values by the best ML model were always higher than by the longitudinal statistical analysis. It is also important to note that the best ML model varies from T1 to T3. While DT resulted in the model with higher performance for T1 [AUROC = 0.78 (0.65–0.91)], it was replaced by SVC in T2 [AUROC = 0.77 (0.64–0.89)], and T3 [AUROC = 0.871 (0.77–0.97)]. The performance of the best ML increased from moderate in T1 and T2 to good in T3.

Increasing the number of features considered in each ML model brings a modest performance gain for 3 features at T1, AUROC = 0.78 (0.68–0.90). However, the gain increases for T2, AUROC = 0.87 (0.76–0.98) and T3, AUROC = 0.91 (0.81–1.00). Once again, the best model for the multi-features scenario changed from the moderate performance of XGBoost at T1, to the good performance of DT at T2, and to the excellent performance of SVC at T3.

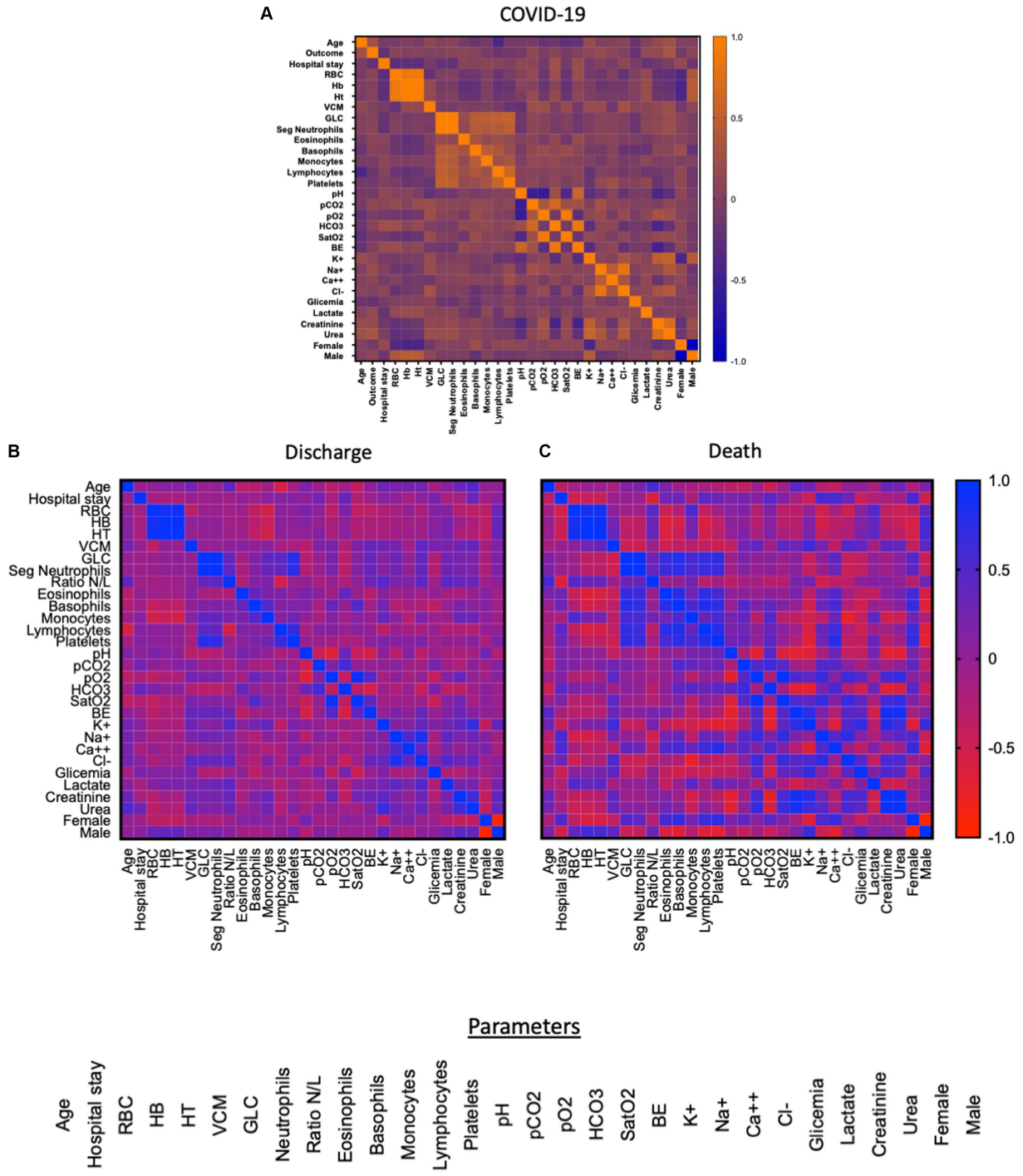

FIGURE 1
Heatmap of the correlation between the parameters of T1 (0 to 7 days) of the patients' hospitalization. Red indicates low correlation and blue indicates high correlation. Figure **(A)** shows the correlation between 31 parameters: age, clinical outcome, length of stay, CBR, HB, HT, VCM, GLC, neutrophils, neutrophil/lymphocyte ratio, eosinophils, basophils, monocytes, lymphocytes, platelets, pH, pCO2, pO2, HCO3, SatO2, BE, K+, Na+, Ca++, Cl-, glycemia, lactate, creatinine, urea, gender (female, male) while figure **(B)** refers to the group of patients who were discharged ($n = 28$) and **(C)** refers to the patients who died ($n = 53$), both figures highlight the correlation of 30 parameters: age, length of stay, RBC, HB, HT, VCM, GLC, neutrophils, neutrophil/lymphocyte ratio, eosinophils, basophils, monocytes, lymphocytes, platelets, pH, pCO2, pO2, HCO3, SatO2, BE, K+, Na+, Ca++, Cl-, glycemia, lactate, creatinine, urea, sex (female, male).

An important aspect to be considered is the impact of the number of features. At all time points, we observed that the increase in the number of features tends to improve the performance of the models, especially for XGBoost, which benefited most from this expansion. However, this increase in feature complexity may also have led to an increase in variance, as evidenced by the greater variation in results. On the other hand, the use of the 3 most important features stood out in all time points, suggesting that a careful selection of features may be more beneficial in some cases than including all available features.

From the ROC curve of each of the best models at each moment, we determine the optimal threshold values that separates both classes. The optimal threshold is obtained from the point on the ROC curve closest to the coordinate [0,1] (23). Figure 5 displays a histogram of the probability of death for each patient. The histogram bars from the true discharge class are represented in blue, while the histogram bars for the true death class are represented in red. The solid lines are obtained from the Kernel Density Estimation (KDE) technique (24).

Based on the probability (of being in the death outcome) returned by the best ML model and the optimal thresholds calculated at each time point, it is possible to classify all patients into both classes and calculate different performance metrics such as accuracy, specificity and sensitivity.

TABLE 1 Selected laboratory parameters with potential to discriminate disease outcome during severe COVID-19.

Parameters	Time point	Value *p*
Seg. Neutrophils	2	0.0373
Seg. Neutrophils	3	0.0004
Global leukocyte count	2	0.0407
Global leukocyte count	3	0.0029
Na+	3	0.0438
Urea	1	0.0102
Urea	2	0.0135
Urea	3	<0.0001

The resulting values of these performance metrics are summarized in Table 3. In addition to these metrics, Table 3 shows the mean AUROC of the training data set, obtained as the average of the AUROC of the trained data set in each LOOCV cycle. The mean AUROC of the training data set spans over all samples in the database and, in that sense, can be directly compared with the AUROC of the test data set.

At T1, the XGBoost model demonstrated an accuracy of 73%, with a specificity of 85% and a sensitivity of 66%. It is observed that, although the specificity is relatively high, indicating the model's ability to identify true negatives, the sensitivity is relatively low, suggesting a limitation in the ability to identify true positives. This could be an indication that the model is inclined to classify more samples as negative, sacrificing the ability to detect positive cases.

At T2, the DT model exhibited a notable increase in accuracy, reaching 85%. Specificity also increased considerably to 95%, indicating an improvement in identifying true negatives. Additionally, sensitivity rose to 79%, demonstrating an improved ability to identify true positives. This suggests that the DT model achieved a better balance between the classification accuracy of the two classes, making it more robust at T2.

At T3, SVC achieved a remarkable accuracy of 89%. Specificity remained high, at 94%, while sensitivity increased further, reaching 86%. These results indicate that the SVC model can maintain a good ability to identify both true negative and true positive results, which is crucial for applications where the balance between these metrics is fundamental.

By analyzing AUROC results for training and testing at each time, it is possible to observe a positive and promising progression in the

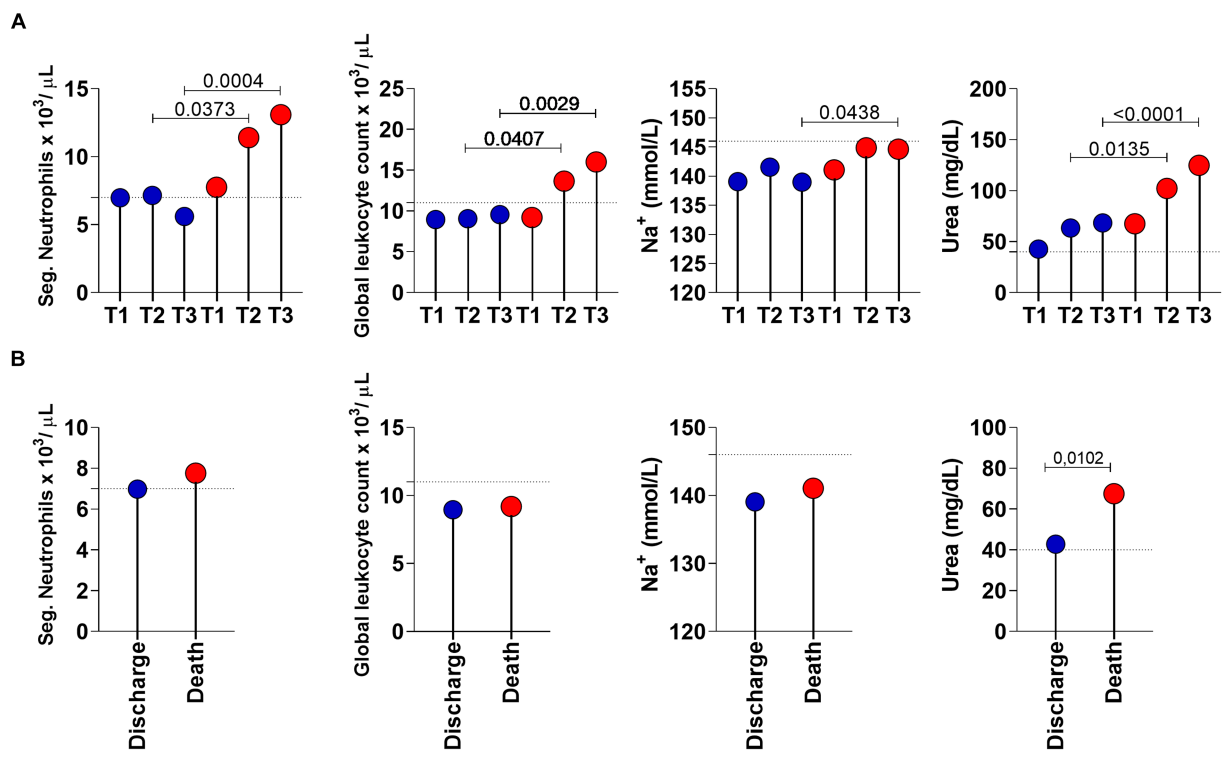

FIGURE 2
Longitudinal analysis of the selected laboratory parameters in COVID-19 patients with a discharge ($n = 28$; blue circle) and death ($n = 53$; red circle) outcome. **(A)** Lolipop graphs at time point T1 (0 to 6 days of hospitalization), T2 (7 to 13 days of hospitalization) and T3 (greater than 14 days of hospitalization). **(B)** Lolipop graphs at T1. Values of $p < 0.05$ were considered significant and are expressed by * or connector bars. The horizontal traced line represent the Cut-off points: Neutrophils: 7 $\times 10^3$/ µL; Global leukocytes: 11 $\times 10^3$ µL; Na^+: 146 mmol/L and Urea: 40 mg/dL.

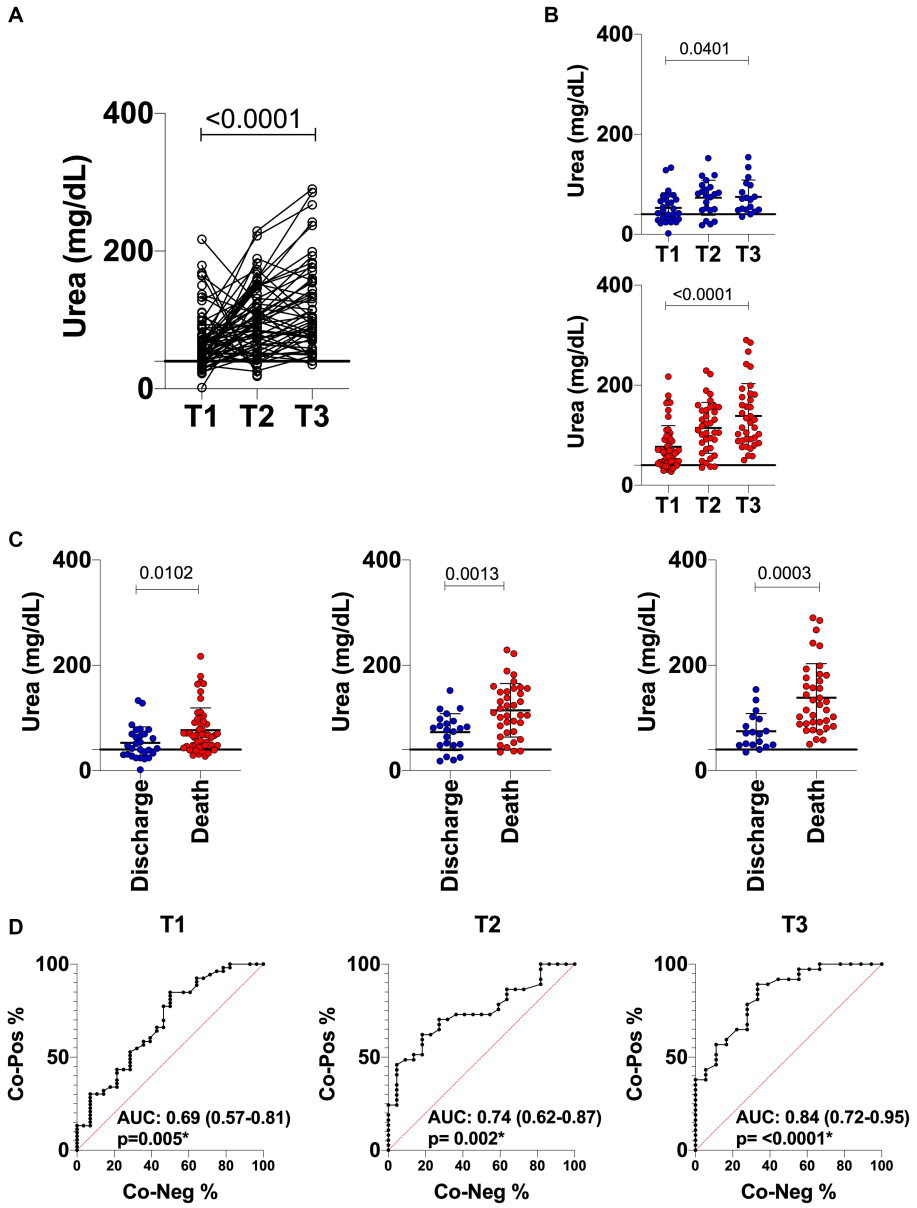

FIGURE 3
Longitudinal analysis of urea in COVID-19 patients with a discharge ($n = 26$; blue circle) and death ($n = 43$; red circle) outcome. **(A)** Line scatter plots of all COVID-19 patients being followed up ($n = 69$) at time points T1, T2 and T3 (greater than 14 days). **(B)** Scatter plots with individual urea values of COVID-19 patients in collections at time points T1, T2 and T3 with outcome of discharge ($n = 26$; blue circle) and death ($n = 43$; red circle). **(C)** Comparison of discharge versus death for each of the time points evaluated. **(D)** ROC curve analyses showing the performance of urea dosage at each time point of the study. The horizontal traced line represents the Cut-off point of 40 mg/dL for urea. The AUROC in the graph represents the performance of the biochemical parameter in distinguishing discharge and death. Values in brackets in the AUROC correspond to the 95% confidence interval (95%CI).

ability of machine learning models to effectively generalize their learnings to unseen data. This analysis reflects the continued evolution and advancement in the effectiveness of machine learning models, especially in medical diagnostic applications in which accuracy and the ability to distinguish between classes are critical.

The classification process carried out by ML models is usually difficult to interpret due to the mathematical complexity of the models. However, interpretable models are desirable to help physicians in the diagnosis process. To present a more intuitive and comprehensive view of the classification process, Figure 6 illustrates the DT model for 3 different scenarios. The DT model exhibits good performance in all time points, as well as a relatively straightforward interpretation. The first scenario (a) refers to time point T1 using only one feature (Urea). The second scenario (b) refers to the T2 time point using 3 features (Urea, Creatine, and Lactate). The third scenario (c) refers to the T3 time point, and 3 features were also used (Global, Urea, and neutrophil/lymphocyte sodium ratio).

For scenario (a), the decision tree begins its analysis, checking whether the value of Urea is less than or equal to 25.93. If this condition is true, the example is classified as Discharge. Then, if Urea is greater than 25.93, the tree continues the analysis. Within the range of 25.93 to 42.50 for Urea, the tree checks the value of Urea again. If

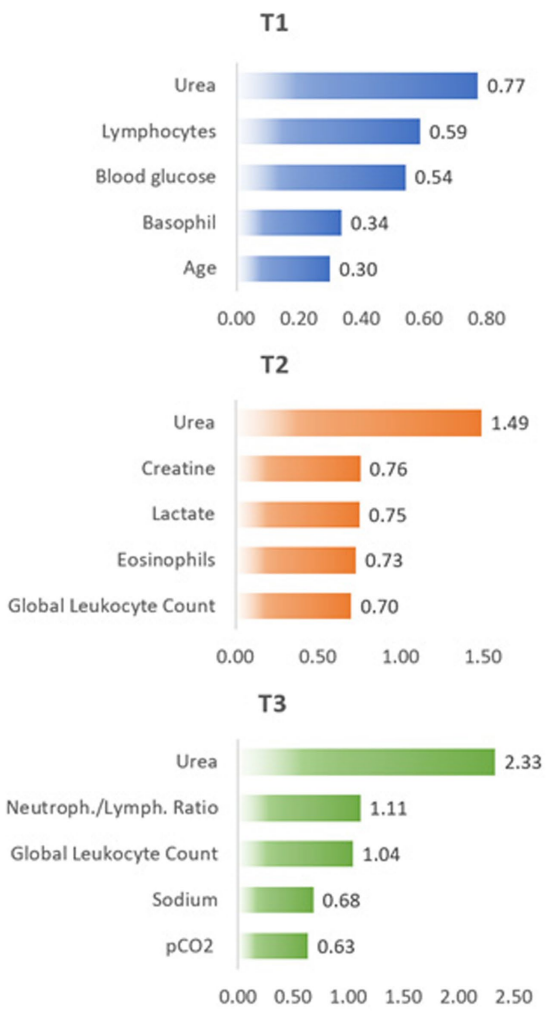

FIGURE 4
Feature importance results based on χ^2 test at different time points T1, T2, and T3.

Urea is within the range of 25.93 to 39.50, the example is classified as Death. On the other hand, if Urea is between 39.50 and 42.50, the classification will be Discharge. For Urea values greater than 42.50, the decision tree continues its analysis. It checks whether Urea is within the range of 42.50 to 87.50 and makes additional decisions. Within this range, if Urea is less than or equal to 73.06, the example is classified as Death. On the other hand, if Urea is between 73.06 and 87.50, the rating will be Discharge. Finally, for Urea values greater than 87.50, the decision tree classifies the example as Death. Note that the DT model finds intervals of Urea values 39,50–49,50 with a Discharge outcome. This island of Discharge outcome in the middle of a Death outcome region could be interpreted as a necessity for the model to consider more features, i.e., more processes represented by other features are influencing the outcome path of the patients at this time point. A similar description can be made for the other scenarios (b) and (c) following a similar reasoning to that made for scenario (a). Therefore, decision trees offer a clear and direct method to classify data based on feature values, thus contributing to the decision-making in a specific context, where classes (in our case Discharge and Death) represent different clinical results.

TABLE 2 AUROC results for the five optimized machine learning models.

Times	N° features	DT	XGBoost	KNN	LR	SVC
T1	1	0.78	0.74	0.69	0.69	0.72
	2	0.75	0.76	0.66	0.69	0.76
	3	0.76	**0.79**	0.64	0.68	0.66
	4	0.70	0.78	0.58	0.71	0.54
	5	0.71	0.73	0.55	0.68	0.59
T2	1	0.74	0.75	0.71	0.74	0.77
	2	0.84	0.78	0.71	0.71	0.68
	3	**0.87**	0.79	0.76	0.73	0.80
	4	0.76	0.82	0.68	0.75	0.77
	5	0.85	0.78	0.71	0.76	0.76
T3	1	0.80	0.85	0.76	0.83	0.87
	2	0.83	0.86	0.86	0.90	0.90
	3	0.87	0.87	0.86	0.89	**0.91**
	4	0.87	0.90	0.87	0.89	0.85
	5	0.86	0.90	0.85	0.89	0.91

4 Discussion

Laboratory parameters are essential for monitoring diseases. COVID-19 is a disease that also alters several laboratory parameters and early investigation of these alterations can allow for the correct and effective treatment of the patient and maybe even prevent post-acute sequelae from the disease. Previous studies demonstrate the importance of laboratory parameters in the diagnosis of COVID-19 and that these parameters can be used to stratify patients in order to plan the appropriate treatment (25). Dwivedi et al. (26) showed in their study that crucial biomarkers such as urea, creatinine, uric acid, ferritin, C-reactive protein, LDL, fibrinogen, bilirubin, albumin and procalcitonin, as well as IL-6 were able to indicate the severity of patients with severe COVID-19. Also in this study, the authors were able to compare the biomarkers in 2 waves of COVID-19, so the parameters analyzed were higher in the second wave, while our study shows how the length of hospitalization can quickly change the hematological and biochemical parameters, which confirms the importance of using laboratory parameters to anticipate a probable outcome. The work of Chávez-Ocaña et al. (27), in addition to analyzing laboratory parameters, also analyzed interleukins. This study shows albumin, lymphocytes, platelets and ferritin as factors that may correlate with the severity of COVID-19, and with regard to pro-inflammatory cytokines, the authors found IL-6, IL-10, IL-2 and IL-17 to be elevated in severe patients. The evaluation of interleukins is interesting, however, it is a costly test, so we focused on evaluating parameters that are common in the emergency hospital routine, in addition to being more accessible and with agile results.

Many studies have addressed the importance of using artificial intelligence to diagnose or monitor patients infected with SARS-CoV-2, (28–33) using laboratory data to predict the mortality risk of patients with COVID-19. Likewise, recent reports revealed that laboratory parameters such as neutrophils, urea and respiratory indices have great unique importance in predicting patient mortality.

De Souza et al. (32) shows that machine learning methods using demographic and clinical data along with comorbidities of the patients can assist in the prognostic prediction and physician decision-making. The outcome prediction in that work focuses more on individual variables such as age, symptoms, and comorbidities. Chung et al. (33) focusses the outcome prediction on the analysis of different scores. Each score involves a set of variables, with the best performance related to age, coronary heart disease, and the level of lymphocyte, procalcitonin and D-dimer. Aljame et al. (34), implemented a complex machine learning ensemble method for COVID-19 diagnosis that shows the importance of monocytes in determining positive cases of COVID-19, in addition to patients having other parameters that can diagnose the disease. Bahceci et al. (35) shows that hematological and biochemical parameters can be used to determine the patient's treatment, as they are of low cost and accessible. Routine laboratory tests available in hospitals can be an important ally in stratifying patient severity using Machine Learning (ML) tools. ML techniques can help doctors diagnose COVID-19, complementing the results of tests such as RT-PCR and increasing the possibility of a favorable clinical outcome for the patient. The use of AI in the field of medical diagnostics fills the gap in hospitals that have limited diagnostic methods, and also speeds up medical decision-making. In addition, the use of ML allows for the analysis of various parameters, including the diversity of data, which is important in terms of the representativeness of the population studied (36–38).

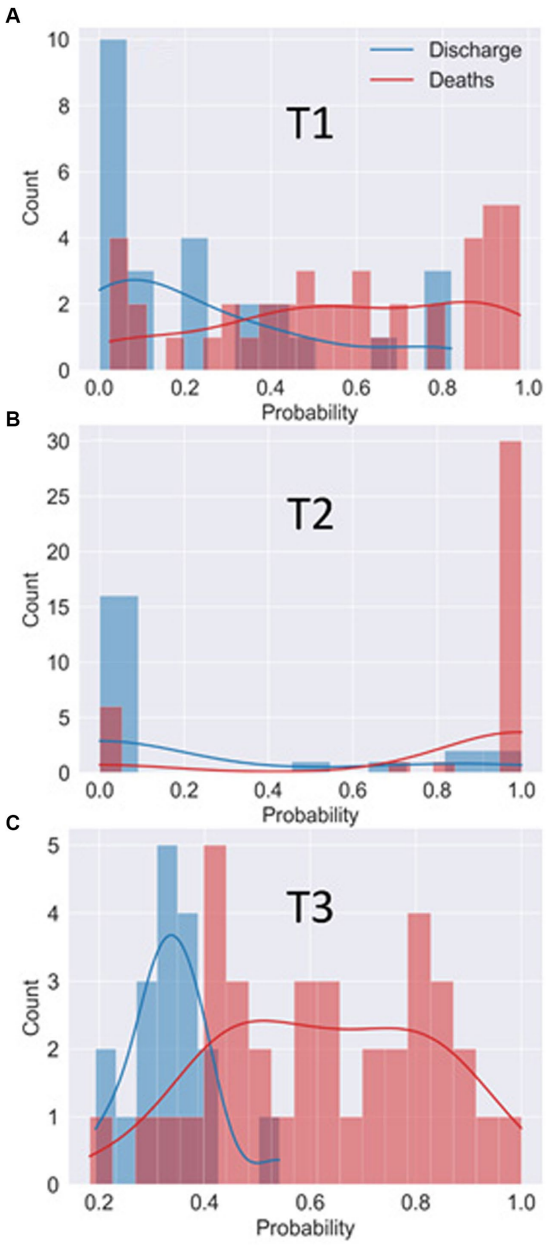

FIGURE 5
Histogram of the probability of patients being classified in the "death" class by the best model in T1 **(A)**, T2 **(B)** and T3 **(C)**. The histogram bars from the true discharge class are represented in blue while the histogram bars for the true death class are represented in red. The solid lines represent the estimated probability distribution for each class.

Our study shows that some laboratory parameters present early changes, such as urea, for example, demonstrating that a routine hospital laboratory test can help characterize the patient who may have an unfavorable clinical evolution. Using AI tools to identify, diagnose, analyze medical images, and collect hundreds of data points quickly in hospitals could have a positive impact on the medical field. AI is also important when the diagnostic possibilities depend on many other diagnostic tools, such as sepsis, for example, which needs to combine clinical and laboratory criteria. Nevertheless, the use of IA requires care, especially in the interpretation of the results, requiring a multidisciplinary team to obtain a reliable result (38, 39). Therefore, our study highlights the importance of using tests that are already part of the laboratory routine combined with machine learning.

Predicting the clinical progression of patients with severe COVID-19 is very important because patients can present post-acute sequelae such as kidney and heart infections, liver failure and compromised lung function (40). Long COVID is tightly associated with the severe cases of COVID-19 as well as the clinical management of patients during the acute phase of disease. Considering this, improving the clinical management of acute phase patients in future waves of the disease may help in halting the Long COVID epidemics the world is experiencing in these remaining years of the pandemic. The results of our work show the parameters that are important to evaluate in patients admitted to hospitals with COVID-19, being urea and lymphocytes at early time points of acute phase taken as categorical parameters in the classification of patients who have died.

TABLE 3 AUROC training, AUROC training, accuracy, specificity, and sensitivity of the best models at each time point.

Time point - model	AUROC training	AUROC test	Accuracy (%)	Specificity (%)	Sensitivity (%)
T1 - XGBoost	0.98	0.79	73	85	66
T2 - DT	0.99	0.87	85	95	79
T3 - SVC	0.92	0.91	89	94	86

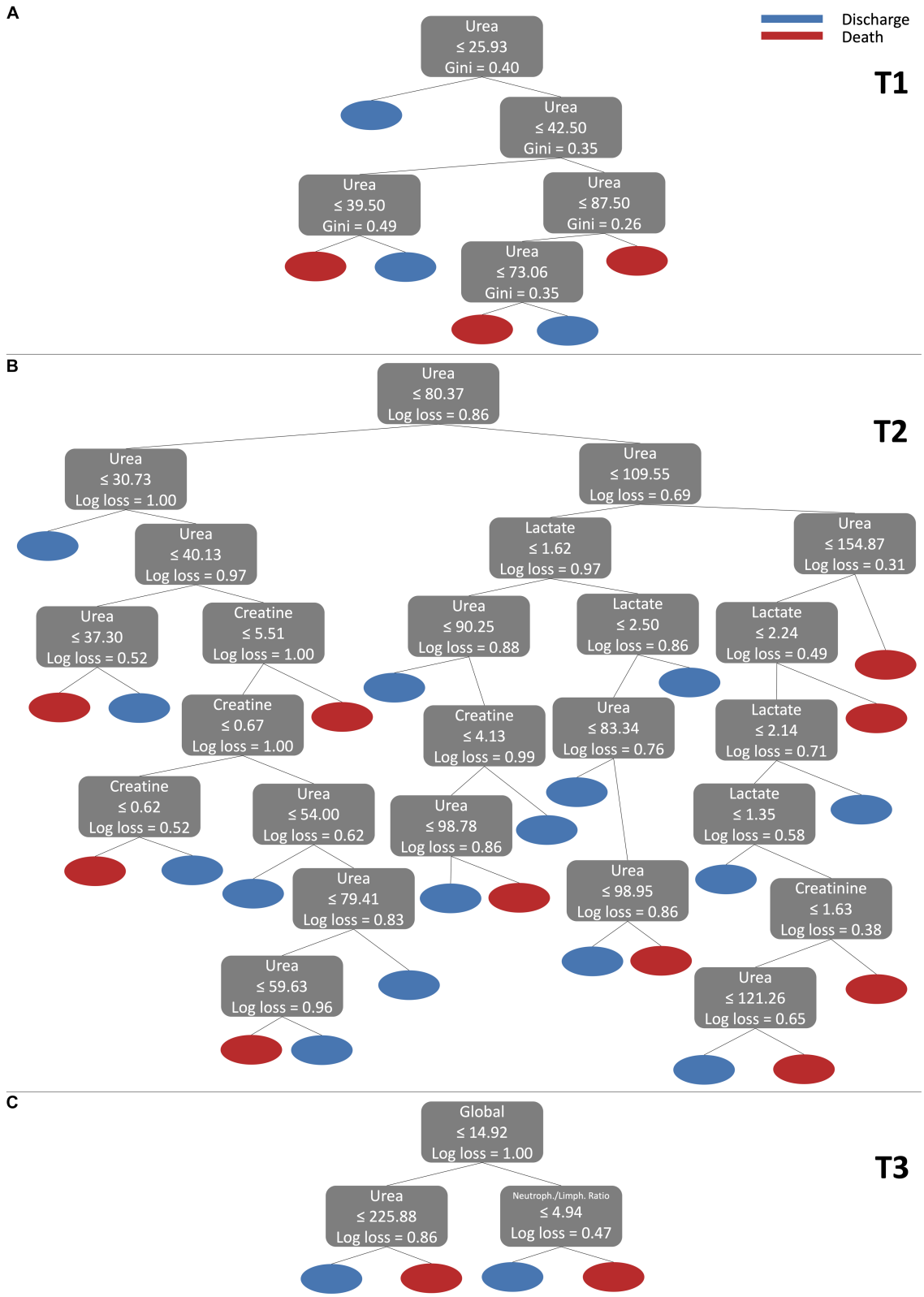

FIGURE 6
Visualization of DT model for 3 different scenarios. **(A)** refers to T1 using only one feature (Urea). **(B)** refers to T2 using 3 features (Urea, Creatine, and Lactate). **(C)** refers to T3 using 3 (Global, Urea, and neutrophil/lymphocyte sodium ratio).

Urea, the parameter classified as the most important in the outcome of COVID-19 patients, is closely linked to the amount of protein the individual eats, i.e., the richer the protein diet, the greater the excretion of urea. The protein ingested in the diet is metabolized

into essential and non-essential amino acids or into waste products and ions. In addition, amino acids are metabolized by the liver into urea, which is then excreted in the urine. The body's protein stores can be converted into essential and non-essential amino acids or they can be metabolized to form waste products and ions, which will also be excreted in the urine. Urea is synthesized in the liver by protein catabolism and blood urea is filtered by the glomerulus and undergoes tubular reabsorption, so urea is directly related to nutritional status, protein metabolism and kidney condition. SARS-CoV-2 can activate the renin-angiotensin-aldosterone system causing renal vasoconstriction, decreased glomerular filtration and decreased urea excretion, increased absorption of water as well as sodium and passive reabsorption of urea (41, 42).

Since urea is the end product of protein metabolism, it can be used as a marker of kidney function. A study by Cheng et al. (43) tested blood urea levels combined with D-dimer as predictors of hospital mortality in COVID-19 patients. High urea levels are associated with a worse outcome in patients with heart failure. One of the reasons involved in this process is moderate to severe dehydration due to fever that ICU stay may cause, so the blood flow reaches the kidneys with less pressure, triggering damage to the renal structures. Patients undergoing mechanical ventilation have high internal pressures, which reduces venous return. This increase in pressure in the lungs reduces cardiac pressure, so if the heart cannot pump blood effectively to the kidneys and other organs, it compromises their functioning. This explains why patients with heart failure have high levels of urea, due to the inefficient functioning of the kidneys, the organs responsible for excreting urea. The study by Shaikh et al. (44) shows significant associations of biomarkers such as urea, ferritin, glucose and creatinine with mortality and ICU admission, just as our data show how urea can be a good biomarker of severity in COVID-19.

Our data shows that more severe patients with death outcome had higher concentrations of lactate and urea than patients who were discharged. We observed that these concentrations tended to increase even more in later stages. Henry et al. (45) showed in their study that high lactate values are related to a worse prognosis. Lactate dehydrogenase is an intracellular enzyme that catalyzes the interconversion of pyruvate and lactate. Severe infections can cause tissue damage mediated by cytokines and the release of lactate dehydrogenase. As this enzyme is present in lung tissues, patients with severe COVID-19 tend to release a greater amount of lactate. Thus, lactate is a predictor of worse outcomes in hospitalized patients and reflects the putative multiple organ damage and failure, that play an important role in COVID-19 patients who progress to death.

Glucose is another decisive parameter in the clinical outcome of patients with COVID-19. It is known that patients with type 2 diabetes have an increased risk of developing severe COVID-19 and according to a previous study (46), these patients have increased levels of angiotensin-converting enzyme-2 (ACE2) the receptor for SARS-CoV-2, which favors the entry of the virus and decreases its clearance. Thus, an increase in glucose is related to an increase in viral replication, a probable serious complication due to deregulation of the immune system and an increase in the inflammatory response.

The innate immune system is of great importance in viral infections, especially in respiratory infections, in which the lung is the target organ. A differential and divergent cytokine storm both systemic and in the airways will also be crucial to define immune responses and outcome of critically ill COVID-19 patients (47). This inflamed milieu also allows for improved binding to surface antigens and can influence the secretion of other cytokines as interferons and interleukins, as well as regulatory factors. In COVID-19, lymphocytes are decreased, which may suggest an inefficient IgG response and a hampered leukocyte activation (48). In this study, we found that patients who died had higher overall leukocyte counts as well as higher neutrophil percentages than patients who were discharged in the onset of acute phase. Conversely, lymphopenia was observed in COVID patients regardless of outcome. As expected, neutrophils have been abundantly studied in COVID-19 and are, therefore, expected to be a hallmark of severity. However, AI models reveal that the order of importance of these parameters diverge amongst time points, which was unexpected. At T1, only lymphocyte counts ranked second and basophils ranked in the fourth position of importance, demonstrating that leukocytes other than neutrophils need further scrutiny and may contribute for the establishment of biomarkers at early time points of disease progression. In this regard, basophils are also cells of the innate immune system that migrate to inflammatory sites during allergic inflammation and infection that triggers the production of IL-4, which stimulates the proliferation of B and T cells. The promptness of these cells to respond to an allergen may explain their order of importance in the refined AI models used here. On the other hand, at T3, neutrophil/lymphocyte ratio contributes as the second most important biomarker for assessing COVID-19 outcome, demonstrating the importance of neutrophils at late stages of disease. The study by Kaur et al. (49) reinforces our findings, by showing that lymphopenia is common in patients with COVID-19 and that severe cases of the disease at late stages in the ICU had neutrophilia. In addition, Kılıc (50) et al. shows that patients with a lower lymphocyte count associated with depletion of CD4 and CD8 T cells had an increased risk of developing a severe COVID-19 outcome. The potential mechanism for explaining this phenomena is virus-induced lysis of the lymphocytes, since these cells express ACE2 and are therefore permissive to SARS-CoV-2 (50). Cytokine-induced atrophy of lymphatic organs can also occur, which impacts on lymphocyte renewal, and another mechanism would be inflammatory pro-mediators that can induce direct lymphocyte apoptosis (49).

COVID-19 is a disease that can affect several organs and the way the host's body reacts to the disease is fundamental in determining the patient's outcome. Some factors are considered risk factors, such as age. Studies such as that by Chen et al. (51) show that age is the most significant risk factor for developing severe COVID-19. The results we found using ML reinforce the importance of age both in the development of the disease and in the clinical outcome of this patient. The study by Hu et al. (52) reinforces that older adult patients with comorbidities progressed to more serious illnesses, thus highlighting that the older adult were prone to developing severe acute respiratory syndrome and septic shock. Therefore, the correct diagnosis and treatment in older adult patients is crucial in order to improve survival rate and prevention of post-acute sequelae in those populational stratum.

Our study shows that the use of measurable biochemical and hematological variables (urea, lymphocytes, glucose, neutrophil/lymphocyte ratio and basophils) constitutes excellent biomarkers for the severity of COVID-19 patients and outcome prediction of hospitalized patients, with strong highlight to urea. This study shows that urea is the parameter that best classifies patient severity and rises over time, making it an important analyte to be used in machine learning algorithms to

predict patient outcomes. However, in contrast to the previous studies that show the importance of age during severe COVID (51), we observed that once a patient is under treatment at the ICU, other parameters such as urea, lymphocytes, glucose and basophils at T1 were more important than age. As the patients' hospitalization time progressed (T2 and T3), age did not appear as an important feature, as other laboratory parameters such as urea, creatinine, lactate, eosinophils, neutrophil/lymphocyte ratio and global leukocytes. Therefore, our study demonstrates the importance of machine learning algorithms in the clinical evolution of patients.

The use of ML in the clinical monitoring of patients can generate fast and efficient results, ML can also be used to predict new outbreaks, using epidemiological data (53, 54). Routine tests in the hospital environment are essential for predicting a patient's clinical outcome, and when coupled with artificial intelligence, predictions can contribute even further to the survival rates and clinical management of patients. This work shows that laboratory parameters can change early and late during COVID-19 at its severe form, and conventional statistical analyses are insufficient to promote predictive power and contribute to decision making and clinical management of patients. Therefore, we present ML algorithms as a tool for predicting the clinical outcome of COVID-19 patients, to improve our preparedness for the more assertive and early treatment in future pandemics of newly mutated immune-resistant SARS-CoV-2 variants.

4.1 Limitations

Our study evaluates laboratory parameters at different times in a longitudinal design performed with patients from admission until the outcome (discharge or death), which limits the sample size of the study. The machine learning method here developed focus not only in performance, but also interpretability and generalizability of the models. However, the relatively low number of patients remains an important limitation, as well as the difficulties in obtaining a full set of data for all patients at all time points. Due to the rapid evolution of this disease, a more frequent collection of laboratory analysis (more time points) is also desirable and should be considered for future investigations.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data contains the identification of the patients in the study. Requests to access these datasets should be directed to alicelourenco90@gmail.com.

Ethics statement

The studies involving humans were approved by Research Ethics Committee of the Universidade Federal de Minas Gerais - Ethics Committee (CAAE: 45086721.1.0000.5149 - opinion number 4.751.423). The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

AL: Writing – original draft, Writing – review & editing. PA: Writing – original draft, Writing – review & editing. AP: Writing – original draft. GM-F: Writing – original draft. LG-dP: Writing – review & editing. CM: Writing – original draft, Data curation. FF: Writing – review & editing. JP: Writing – original draft, Writing – review & editing. JC-dR: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Hopkins KL, Underwood T, Iddrisu I, Woldemeskel H, Bon HB, Brouwers S, et al. Community-based approaches to increase COVID-19 vaccine uptake and demand: lessons learned from four UNICEF-supported interventions. *Vaccine*. (2023) 11:1180. doi: 10.3390/vaccines11071180

2. World Health Organization (2023). Available at: https://www.who.int/publications/m/item/weekly-epidemiological-update-on-covid-19---1-september-2023 (Accessed October 02, 2023).

3. Mokhtari T, Hassani F, Ghaffari N, Ebrahimi B, Yarahmadi A, Hassanzadeh G. COVID-19 and multiorgan failure: a narrative review on potential mechanisms. *J Mol Hist*. (2020) 51:613–28. doi: 10.1007/s10735-020-09915-3

4. Semiz S. COVID19 biomarkers: what did we learn from systematic reviews? *Front Cell Infect Microbiol*. (2022) 12:1038908. doi: 10.3389/fcimb.2022.1038908

5. Oliveira BA, Oliveira LCD, Sabino EC, Okay TS. SARS-CoV-2 and the COVID-19 disease: a mini review on diagnostic methods. *Rev Inst Med Trop São Paulo*. (2020) 62:e44. doi: 10.1590/s1678-9946202062044

6. World Health Organization. (2023). Regulatory considerations on artificial intelligence for health. Available at: https://iris.who.int/handle/10665/373421 (Accessed October 23, 2023).

7. Barbosa VADF, Gomes JC, De Santana MA, De Lima CL, Calado RB, Bertoldo Júnior CR, et al. Covid-19 rapid test by combining a random Forest-based web system and blood tests. *J Biomol Struct Dyn*. (2022) 40:11948–67. doi: 10.1080/07391102.2021.1966509

8. Ceccon DM, Amaral PHR, Andrade LM, Da Silva MIN, Andrade LAF, Moraes TFS, et al. New, fast, and precise method of COVID-19 detection in nasopharyngeal and tracheal aspirate samples combining optical spectroscopy and machine learning. *Braz J Microbiol*. (2023) 54:769–77. doi: 10.1007/s42770-023-00923-5

9. Baygül Eden A, Bakir Kayi A, Erdem MG, Demirci M. COVID-19 studies involving machine learning methods: a bibliometric study. *Medicine*. (2023) 102:e35564. doi: 10.1097/MD.0000000000035564

10. Nicoliche CYN, De Oliveira RAG, Da Silva GS, Ferreira LF, Rodrigues IL, Faria RC, et al. Converging multidimensional sensor and machine learning toward high-throughput and biorecognition element-free multidetermination of extracellular vesicle biomarkers. *ACS Sens*. (2020) 5:1864–71. doi: 10.1021/acssensors.0c00599

11. Sabaté Del Río J, Henry OYF, Jolly P, Ingber DE. An antifouling coating that enables affinity-based electrochemical biosensing in complex biological fluids. *Nat Nanotechnol*. (2019) 14:1143–9. doi: 10.1038/s41565-019-0566-z

12. Studdert DM, Hall MA. Disease control, civil liberties, and mass testing — calibrating restrictions during the Covid-19 pandemic. *N Engl J Med*. (2020) 383:102–4. doi: 10.1056/NEJMp2007637

13. Quinlan JR. Induction of decision trees. *Mach Learn*. (1986) 1:81–106. doi: 10.1007/BF00116251

14. Chen T, Guestrin C. XGBoost: a scalable tree boosting system. International Conference on Knowledge Discovery and Data Mining. San Francisco California USA: ACM (2016). p. 785–794.

15. Cover T, Hart P. Nearest neighbor pattern classification. *IEEE Trans Inform Theory*. (1967) 13:21–7. doi: 10.1109/TIT.1967.1053964

16. Cox DR. The regression analysis of binary sequences. *J R Stat Soc Ser B*. (1958) 20:215–32. doi: 10.1111/j.2517-6161.1958.tb00292.x

17. Cortes C, Vapnik V. Support-vector networks. *Mach Learn*. (1995) 20:273–97. doi: 10.1007/BF00994018

18. Pedregosa F, Varoquaux G, Gramfort A, Michel V, Thirion B, Grisel O, et al. Scikit-learn: machine learning in Python. *J Mach Learn Res*. (2011) 12:2825–30. doi: 10.5555/1953048.2078195

19. Arlot S, Celisse A. A survey of cross-validation procedures for model selection. *Statist Surv*. (2010) 4:40–79. doi: 10.1214/09-SS054

20. James G, Witten D, Hastie T, Tibshirani R, Taylor JE. *An introduction to statistical learning: with applications in Python*. Cham: Springer (2023). 607 p.

21. Chawla NV, Bowyer KW, Hall LO, Kegelmeyer WP. SMOTE: synthetic minority over-sampling technique. *Jair*. (2002) 16:321–57. doi: 10.1613/jair.953

22. Akiba T, Sano S, Yanase T, Ohta T, Koyama M. Optuna: a next-generation Hyperparameter optimization framework. Proceedings of the 25th ACM SIGKDD International Conference on Knowledge Discovery & Data Mining. Anchorage, AK: ACM (2019). p. 2623–2631.

23. Unal I. Defining an optimal cut-point value in ROC analysis: an alternative approach. *Comput Math Methods Med*. (2017) 2017:1–14. doi: 10.1155/2017/3762651

24. Scott DW. *Multivariate density estimation: theory, practice, and visualization*. *2nd* ed. Hoboken, New Jersey: John Wiley & Sons, Inc (2015).

25. Wang Y, Zhao J, Yang L, Hu J, Yao Y. Value of the neutrophil-lymphocyte ratio in predicting COVID-19 severity: a Meta-analysis. *Dis Markers*. (2021) 2021:1–10. doi: 10.1155/2021/2571912

26. Dwivedi T, Raj A, Das N, Gupta R, Gupta N, Tiwari P, et al. The evaluation of laboratory parameters as predictors of disease severity and mortality in COVID-19 patients: a retrospective study from a tertiary Care Hospital in India. *Cureus*. (2023) 15:e40273. doi: 10.7759/cureus.40273

27. Chávez-Ocaña SDC, Bravata-Alcántara JC, Cortés-Ortiz IA, Reyes-Sandoval A, García-Machorro J, Herrera-Gonzalez NE. Parameters to predict the outcome of severe and critical COVID-19 patients when admitted to the hospital. *JCM*. (2023) 12:1323. doi: 10.3390/jcm12041323

28. Stachel A, Daniel K, Ding D, Francois F, Phillips M, Lighter J. Development and validation of a machine learning model to predict mortality risk in patients with COVID-19. *BMJ Health Care Inform*. (2021) 28:e100235. doi: 10.1136/bmjhci-2020-100235

29. Meng F, Kottlors J, Shahzad R, Liu H, Fervers P, Jin Y, et al. AI support for accurate and fast radiological diagnosis of COVID-19: an international multicenter, multivendor CT study. *Eur Radiol*. (2022) 33:4280–91. doi: 10.1007/s00330-022-09335-9

30. Aslani S, Jacob J. Utilisation of deep learning for COVID-19 diagnosis. *Clin Radiol*. (2023) 78:150–7. doi: 10.1016/j.crad.2022.11.006

31. Malheiro DT, Bernardez-Pereira S, Parreira KCJ, Kobayashi T, Marra AR. Prevalence, predictors, and patient-reported outcomes of long COVID in hospitalized and non-hospitalized patients from the city of São Paulo, Brazil. *Front Public Health*. (2024) 11:1302669. doi: 10.3389/fpubh.2023.1302669

32. De Souza FSH, Hojo-Souza NS, Dos Santos EB, Da Silva CM, Guidoni DL. Predicting the disease outcome in COVID-19 positive patients through machine learning: a retrospective cohort study with Brazilian data. *Front Artif Intell*. (2021) 4:579931. doi: 10.3389/frai.2021.579931

33. Chung H-P, Tang Y-H, Chen C-Y, Chen C-H, Chang W-K, Kuo K-C, et al. Outcome prediction in hospitalized COVID-19 patients: comparison of the performance of five severity scores. *Front Med*. (2023) 10:1121465. doi: 10.3389/fmed.2023.1121465

34. AlJame M, Ahmad I, Imtiaz A, Mohammed A. Ensemble learning model for diagnosing COVID-19 from routine blood tests. *Inform Med Unlocked*. (2020) 21:100449. doi: 10.1016/j.imu.2020.100449

35. Bahceci I, Mercantepe F, Duran OFYildiz S, Sahin K. The relationship between laboratory findings and mortality in COVID-19 patients requiring intensive care. *Cureus*. (2023) 15:e41194. doi: 10.7759/cureus.41194

36. Goodman-Meza D, Rudas A, Chiang JN, Adamson PC, Ebinger J, Sun N, et al. A machine learning algorithm to increase COVID-19 inpatient diagnostic capacity. *PLoS One*. (2020) 15:e0239474. doi: 10.1371/journal.pone.0239474

37. Swapnarekha H, Behera HS, Nayak J, Naik B. Role of intelligent computing in COVID-19 prognosis: a state-of-the-art review. *Chaos Solitons Fractals*. (2020) 138:109947. doi: 10.1016/j.chaos.2020.109947

38. Chen W, Sá RC, Bai Y, Napel S, Gevaert O, Lauderdale DS, et al. Machine learning with multimodal data for COVID-19. *Heliyon*. (2023) 9:e17934. doi: 10.1016/j.heliyon.2023.e17934

39. Agnello L, Vidali M, Padoan A, Lucis R, Mancini A, Guerranti R, et al. Machine learning algorithms in sepsis. *Clin Chim Acta*. (2024) 553:117738. doi: 10.1016/j.cca.2023.117738

40. Alqaissi E, Alotaibi F, Ramzan MS. Graph data science and machine learning for the detection of COVID-19 infection from symptoms. *PeerJ Comput Sci*. (2023) 9:e1333. doi: 10.7717/peerj-cs.1333

41. Weiner ID, Mitch WE, Sands JM. Urea and Ammonia metabolism and the control of renal nitrogen excretion. *Clin J Am Soc Nephrol*. (2015) 10:1444–58. doi: 10.2215/CJN.10311013

42. Huang D, Yang H, Yu H, Wang T, Chen Z, Liang Z, et al. Blood urea nitrogen to serum albumin ratio (BAR) predicts critical illness in patients with coronavirus disease 2019 (COVID-19). *IJGM*. (2021) 14:4711–21. doi: 10.2147/IJGM.S326204

43. Cheng A, Hu L, Wang Y, Huang L, Zhao L, Zhang C, et al. Diagnostic performance of initial blood urea nitrogen combined with D-dimer levels for predicting in-hospital mortality in COVID-19 patients. *Int J Antimicrob Agents*. (2020) 56:106110. doi: 10.1016/j.ijantimicag.2020.106110

44. Shaikh AA, Mubasher TA, Makkawi MH, Alasmari SZ. Predictive value of ferritin, glucose, urea, and creatinine for COVID-19 severity and mortality in patients from Asir. *Saudi Arabia SMJ*. (2023) 44:773–81. doi: 10.15537/smj.2023.44.8.20230162

45. Henry BM, Aggarwal G, Wong J, Benoit S, Vikse J, Plebani M, et al. Lactate dehydrogenase levels predict coronavirus disease 2019 (COVID-19) severity and mortality: a pooled analysis. *Am J Emerg Med*. (2020) 38:1722–6. doi: 10.1016/j.ajem.2020.05.073

46. Pranata R, Henrina J, Raffaello WM, Lawrensia S, Huang I. Diabetes and COVID-19: the past, the present, and the future. *Metabolism*. (2021) 121:154814. doi: 10.1016/j.metabol.2021.154814

47. Gonçalves JJ, da Mata CPSM, Lourenço AA, Ribeiro ÁL, Ferreira GM, Fraga-Silva TFC, et al. Timeline kinetics of systemic and airway immune mediator storm for comprehensive analysis of disease outcome in critically ill COVID-19 patients. *Front Immunol*. (2022) 13:903903. doi: 10.3389/fimmu.2022.903903

48. Palladino M. Complete blood count alterations in COVID-19 patients: a narrative review. *Biochem Med*. (2021) 31:403–15. doi: 10.11613/BM.2021.030501

49. Kaur S, Bansal R, Kollimuttathuillam S, Gowda AM, Singh B, Mehta D, et al. The looming storm: blood and cytokines in COVID-19. *Blood Rev*. (2021) 46:100743. doi: 10.1016/j.blre.2020.100743

50. Kılıc J. Is lymphopenia a predictor of mortality in patients with COVID-19? *ACC*. (2023) 62:82–7. doi: 10.20471/acc.2023.62.01.10

51. Chen Y, Klein SL, Garibaldi BT, Li H, Wu C, Osevala NM, et al. Aging in COVID-19: vulnerability, immunity and intervention. *Ageing Res Rev*. (2021) 65:101205. doi: 10.1016/j.arr.2020.101205

52. Hu C, Li J, Xing X, Gao J, Zhao S, Xing L. The effect of age on the clinical and immune characteristics of critically ill patients with COVID-19: a preliminary report. *PLoS One*. (2021) 16:e0248675. doi: 10.1371/journal.pone.0248675

53. Udriștoiu AL, Ghenea AE, Udriștoiu Ș, Neaga M, Zlatian OM, Vasile CM, et al. COVID-19 and artificial intelligence: An approach to forecast the severity of diagnosis. *Life*. (2021) 11:1281. doi: 10.3390/life11111281

54. Cho G, Park JR, Choi Y, Ahn H, Lee H. Detection of COVID-19 epidemic outbreak using machine learning. *Front Public Health*. (2023) 11:1252357. doi: 10.3389/fpubh.2023.1252357

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Antimicrobial resistance in intensive care patients hospitalized with SEPSIS: a comparison between the COVID-19 pandemic and pre-pandemic era

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Introduction: Coronavirus disease 2019 (COVID-19) is a highly contagious viral illness caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has had a dramatic effect on the world, resulting in millions of deaths worldwide and causing drastic changes in daily life. A study reported that septic complications were associated with high mortality in COVID-19 patients. This study aimed to evaluate how the COVID-19 pandemic changed the pre-pandemic and post-pandemic prevalence of sepsis in ICUs and to evaluate the different risk factors associated with mortality and the different diffusion of microorganisms and their resistance.

Materials and methods: We conducted a single-center retrospective observational clinical study, observing all patients in the ICU of the SS Annunziata Hospital in Chieti (Italy) who were diagnosed with sepsis and had a bacterial isolate from their blood culture. Sepsis was diagnosed by SEPSIIS III criteria. We enrolled all in-patients in the ICU from January 2018 to December 2021. We divided the patients into three groups: (1) non-pandemic period (Np) hospitalized in 2018–2019, (2) pandemic period (Pp)-COVID hospitalized in 2020–2021 with a diagnosis of COVID-19, and (3) Pp-non-COVID patients hospitalized in 2020–2021 without a diagnosis of COVID-19.

Results: From January 2018 to December 2021, 1,559 patients were admitted to the ICU, of which 211 patients [36 (17.1%) in 2018, 52 (24.6%) in 2019, 73 (34.6%) in 2020, and 50 (23.7%) in 2021, respectively] met the selection criteria: 88 patients in period Np, 67 patients in Pp without COVID-19, and 56 patients Pp with COVID-19. The overall mortality of these patients was high (65.9% at 30 days in Np), but decreased during the Pp (60.9%): Pp-non-COVID was 56.7% vs. Pp-COVID 66.1%, with a statistically significant association with APACHE III score (OR 1.08, 95%CI 1.04–1.12, $p < 0.001$), SOFA score (OR 1.12, 95%CI 1.03–1.22,

$p = 0.004$), and age (OR 1.04, 95%CI 1.02–1.07, $p < 0.0001$). Between the Np vs. Pp periods, we observed an increase in a few Gram-positive bacteria such as *S. capitis* (1 pt. −0.9% vs. 14 pt. −7.65%- $p = 0.008$), *S. epidermidis*, *Streptococcus* spp., and *E. faecalis*, as well as a decrease in a case of blood culture positive for *S. aureus*, *S. hominis*, *and E. faecium.* In Gram-negative bacteria, we observed an increase in cases of *Acinetobacter* spp. (Np 6 pt. −5.1%- vs. Pp 20 pt. −10.9%, $p = 0.082$), and *Serratia* spp., while cases of sepsis decreased from *E. faecium* (Np 11 pt. −9.4%- vs. Pp 7 pt. −3.8%, $p = 0.047$), and *Enterobacter* spp., *S. haemolyticus*, *S. maltophilia*, *Proteus* spp., and *P. aeruginosa* have not changed. Finally, we found that resistance to OXA-48 ($p = 0.040$), ESBL ($p = 0.002$), carbapenems ($p = 0.050$), and colistin ($p = 0.003$) decreased with time from Np to Pp, particularly in Pp-COVID.

Conclusion: This study demonstrated how the COVID-19 pandemic changed the prevalence of sepsis in the ICU. It emerged that the risk factors associated with mortality were APACHE and SOFA scores, age, and, above all, the presence of ESBL-producing bacteria. Despite this, during the pandemic phase, we have observed a significant reduction in the emergence of resistant germs compared to the pre-pandemic phase.

KEYWORDS

COVID-19, sepsis, ICU, mortality, antimicrobial therapy

Introduction

Coronavirus disease 2019 (COVID-19) is a contagious viral disease caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). It has had a dramatic effect on the world, causing almost 7 million deaths worldwide and changing daily life. COVID-19 has many reports concerning different clinical manifestations and different risk factors and biomarkers associated with the worsening (1–3). According to a report from the end of May 2020, 1.3 million cases were reported to the United States Centers for Disease Control and Prevention (CDC), with 14% requiring hospitalization, 2% admitted to the intensive care unit (ICU), and 5% dying. The individual risk of severe illness varies by age, underlying comorbidities, and vaccination status. Sepsis is one of the leading causes of death associated with SARS-CoV-2 infection, accounting for 65% (4).

A 2016 SCCM/ESICM task force has defined sepsis as life-threatening organ dysfunction caused by a dysregulated host response to infection (Sepsis-3) as evidenced by organ dysfunction and infection (5).

The Global Burden of Disease Study in 2017 reported an estimated 48.9 million incident cases of sepsis (6). Approximately 11 million deaths were reported, representing 19.7% of all global deaths. Overall mortality decreased by almost 53% between 1990 and 2017.

The importance of identifying risk factors for sepsis was highlighted in one epidemiologic study, which found that septic shock was the fifth leading cause of years of lost productive life due to premature mortality (6). Sepsis risk factors include ICU admission (approximately 50% of ICU patients have a nosocomial infection), advanced age (≥65 years), bacteremia, immunosuppression, diabetes and obesity, cancer, previous hospitalization, genetic factors, community-acquired pneumonia, and severe acute respiratory illness from SARS-CoV-2. COVID-19 can predispose people to sepsis from secondary infections (7–10).

A study reported that sepsis, occurring as a complication of COVID-19, was associated with high mortality in COVID-19 patients (11).

Ventilated COVID-19 patients often receive multiple antibiotic courses. At the height of the pandemic, antibiotic stewardship policies were overridden (12), and ICU capacity was increased. A Spanish hospital reported increased antibiotic use (13). Such data raise concerns that resistance in hospitals may increase as a result of COVID-19 pressures, notwithstanding a lack of evidence that this has occurred.

This study aimed to evaluate how the COVID-19 pandemic changed the pre-pandemic and post-pandemic prevalence of sepsis in ICUs and to evaluate the different risk factors associated with mortality and different diffusions of microorganisms and their resistance.

Methods

Study design, setting, and population

We carried out a single-center retrospective observational clinical study that observed all the patients in the ICU of the SS Annunziata Hospital in Chieti (Italy) who were diagnosed with sepsis and who presented a bacterial isolate from blood culture.

We enrolled all in-patients in the ICU from January 2018 to December 2021. We divided the patients into three groups: (1) non-pandemic period (Np) hospitalized in 2018–2019, (2) pandemic period (Pp)-COVID hospitalized in 2020–2021 with a diagnosis of COVID-19, and (3) Pp-non-COVID patients hospitalized in 2020–2021 without a diagnosis of COVID-19.

Inclusion criteria:
- all patients admitted to intensive care for more than 48 h;
- age over 18 years old; and
- presence of two or more positive blood cultures in patients with clinical signs of active infection and sepsis.

Exclusion criteria:
- admission to the ICU for ongoing sepsis;
- the presence of only one positive blood culture kit; and
- pregnant women.

The study protocol was approved by the Ethics Internal Committee at the University "G. d'Annunzio" Chieti-Pescara (Ethics Committee Project No. 02 02/02/2022) and was performed according to the ethical standards established in the 1964 Declaration of Helsinki.

Variables, data sources, and measurement

Demographic data such as age and gender, as well as the presence of comorbidities such as diabetes, active malignancies, chronic kidney disease (CKD), drug addiction, and immunodeficiency were analyzed. Acute Physiologic Assessment and Chronic Health Evaluation (APACHE) score and Sequential Organ Failure Assessment (SOFA) score were calculated for all patients (14).

The days of hospitalization and the presence and type of the isolated germ were evaluated, with the characteristics of resistance and antibiotic therapy carried out empirically and after susceptibility testing.

Sepsis was diagnosed by SEPSIIS III criteria (15) and EUCAST guidelines.

Antimicrobial susceptibility testing

Every blood culture was placed in a BacT/ALERT® BPA (bioMérieux), which provided both a microbial detection system and a culture media with suitable nutritional and environmental conditions for organisms that might be present in the test sample. Inoculated bottles were incubated in the instrument and continuously monitored for the presence of microorganisms that would grow in the BacT/ALERT BPA bottles. The antimicrobial agents tested included ampicillin, amoxicillin/clavulanic acid, piperacillin/tazobactam, cefuroxime, cefotaxime, ceftriaxone, ceftazidime, cefepime, imipenem, ertapenem, gentamicin, amikacin, ciprofloxacin, levofloxacin, and trimethoprim/sulfamethoxazole. The results were interpreted by the EUCAST guidelines. Extended-spectrum beta-lactamase (ESBL) production was confirmed phenotypically using a combination disk test according to the EUCAST guidelines. Phenotypic screening for carbapenemase production in Enterobacteriaceae was performed using the Carba NP test. An antimicrobial sensitivity test was performed by Vitek 2 (bioMérieux).

Statistical analysis

Descriptive analysis was carried out using mean and standard deviation (±SD) or median and interquartile range (IQR) for the quantitative variables and percentage values for the qualitative ones. Normality distribution for quantitative variables was assessed by the Shapiro–Wilk test. The association between groups and explicative variables was investigated by Pearson $\chi 2$ test and Analysis of Variance (ANOVA) or analog test non-parametric Kruskal Wallis's test followed by the appropriate post-hoc test if significant. Bonferroni's correction

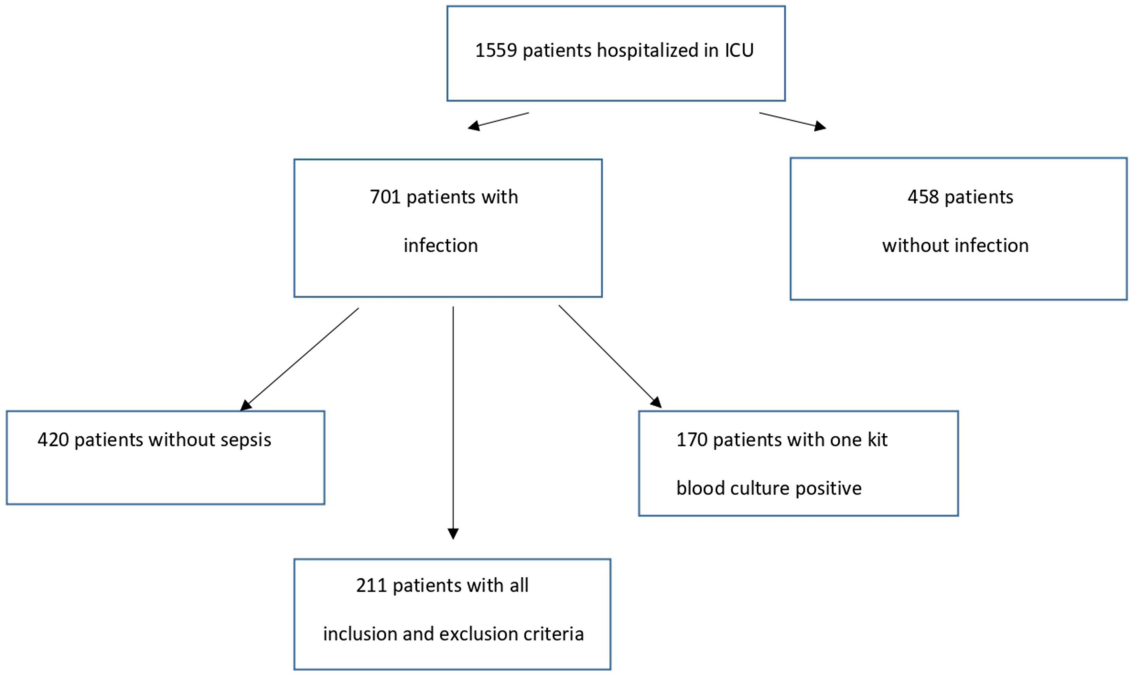

FIGURE 1
Study flow chart.

for multiple comparison tests was applied. The prevalence of patients admitted for infection per year with 95% confidence intervals (CIs) was calculated. In addition, the occurrence of mortality per year was also estimated. Crude odds ratios (ORs) and corresponding 95% CI were calculated to quantify the risk associated with mortality as an explicative variable using the Wald test. Statistical significance was set at a level of ≤0.05, unless adjustments for multiple comparisons were required. All analyses were performed using Stata software v17.1 (StataCorp, College Station, Texas, United States).

Results

From January 2018 to December 2021, 1,559 patients were admitted to the ICU, and 211 patients satisfied the selection criteria [36 (17.1%) in 2018, 52 (24.6%) in 2019, 73 (34.6%) in 2020, and 50 (23.7%) in 2021, respectively]: 88 patients in period Np, 67 patients in Pp without COVID-19, and 56 patients in Pp with COVID-19 (Figure 1). The demographic characteristics of the overall study population and their comorbidities are reported in Table 1. Briefly, the patient's median age was 70 (IQR 62–78) years, of which 65.6% were male. We found significant differences in the mean score of the SOFA score between the three groups ($p = 0.028$). Specifically, the SOFA score was higher in Pp-COVID 12.2 (±3.46) vs. Np 10.3 (±3.8) with $p = 0.008$.

The APACHE score remained unchanged significantly throughout the observation period. In the Pp group, patients remained longer in the ICU for 19 (9–38) days compared to 14 (3–37) days in the Np group ($p = 0.293$).

In the two study periods, with our strict criteria, Np and Pp sepsis showed an overall prevalence of 13.53%, with data of 9.33% in 2018, 13.40% in 2019, 18.02% in 2020, and 13.16% in 2021, respectively (Figure 2).

We found that among patients enrolled in the study, 135 (64%) had sepsis of medical origin and 76 (36%) had sepsis of surgical origin. However, the overall mortality rate for these patients was high, 65.9% in 30 days, but mortality decreased during the Pp to 60.9%: Pp-non-COVID was 56.7%, compared to Pp-COVID of 66.1%. Furthermore, in the overall population, ESBL microorganisms were associated with increased mortality (68% vs. 55%, with p = 0.028).

Crude OR indicates that the occurrence of mortality increased with the SOFA score (OR 1.12, 95%CI 1.03–1.22, $p = 0.004$), APACHE

TABLE 1 Characteristics of sample.

	Total	Np	Pp-non-COVID	Pp-COVID	
	N = 211	*N* = 88	*N* = 67	*N* = 56	*p*-value
Sex, n(%)					
Female	72 (34.1%)	32 (36.4%)	23 (34.3%)	17 (30.4%)	0.759
Male	139 (65.9%)	56 (63.6%)	44 (65.7%)	39 (69.6%)	
Age, years	70 (62–78)	71 (60.5–80.5)	70 (62–77)	69 (64–75)	0.845
Comorbidity, n(%)					
No	15 (7.1%)	3 (3.4%)	4 (6.0%)	8 (14.3%)	0.056
Yes	196 (92.9%)	85 (96.6%)	63 (94.0%)	48 (85.7%)	
Diabetes mellitus, n(%)					
No	163 (77.3%)	64 (72.7%)	53 (79.1%)	46 (82.1%)	0.563
Yes	48 (22.7%)	24 (26.3%)	14 (20.9%)	10 (17.9%)	
Cancer					
No	183 (86.7%)	75 (85.2%)	56 (83.6%)	52 (92.9%)	0.276
Yes	28 (13.3%)	13 (14.8%)	11 (16.4%)	4 (7.1%)	
Acute renal failure, n(%)					
No	172 (81.5%)	69 (78.4%)	54 (80.6%)	49 (87.5%)	0.381
Yes	39 (18.5%)	19 (21.6%)	13 (19.4%)	7 (12.5%)	
Drug addiction, n(%)					
No	203 (96.2%)	82 (93.2%)	65 (97.0%)	56 (100.0%)	0.103
Yes	8 (3.8%)	6 (6.8%)	2 (3.0%)	0 (0.0%)	
HIV, n(%)					
No	202 (95.7%)	83 (94.3%)	63 (94.0%)	56 (100.0%)	0.184
Yes	9 (4.3%)	5 (5.7%)	4 (6.0%)	0 (0.0%)	
SOFA Score	11.2 (3.7)	10.6 (3.8)*	11.2 (3.6)	12.2 (3.4)*	0.028
APACHE Score	25.9 (8.2)	27.3 (8.9)	25.1 (7.5)	24.9 (7.6)	0.122
ICU, days	18 (7–35)	14 (3–37)	19 (9–38)	18 (10–31)	0.293

N (%) mean and (sd) or median and interquartile range (IQR) are shown when appropriate. *p-value < α/3 for Bonferroni multiple testing correction vs. Np.

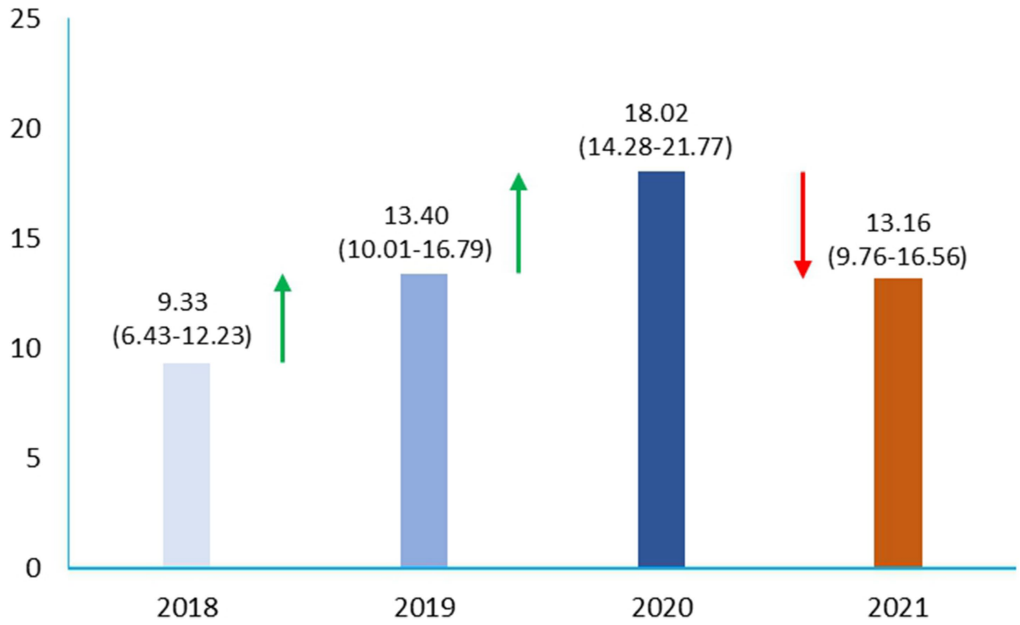

FIGURE 2
Prevalence of sepsis shock in pre-pandemic and pandemic era in ICU.

score (OR 1.08, 95%CI 1.04–1.12, $p<0.0001$), and age (OR 0.98, 95%CI 0.97–0.99, $p=0.023$; Table 2).

Characteristics of the overall germs in the ICU

The blood cultures from CVC were positive on 23.1% in Np and an increase in the Pp-noCOVID compared with Pp-COVID 67.3% vs. 72.4% ($p<0.0001$). In addition, we found that resistance to OXA-48 ($p=0.040$), ESBL ($p=0.002$), carbapenems ($p=0.050$), and colistin ($p=0.003$) decreased with time from Np to Pp, particularly in Pp-COVID (Table 3).

About germs, there was no significant difference in germ circulation between Gram-positive vs. Gram-negative among groups (Np 58.1%, Pp-noCOVID 59.8%, Pp-COVID 61.8% vs. Np 41.9%, Pp-noCOVID 40.2%, Pp-COVID 38.2%, $p=0.875$, respectively). Between the Np vs. Pp period, we observed an increase in a few Gram-positive bacteria, such as *S. capitis* (1 patient 0.9% vs. 14 patients 7.65%, $p=0.008$), *S. epidermidis, Streptococcus* spp., *and E. faecalis* and a decrease in cases of blood culture positive for *S. aureus, S. hominis, and E. faecium.*

Finally, in Gram-negative bacteria, we observed an increase in cases of *Acinetobacter* spp. (Np 6 pt. −5.1%- vs. Pp 20 pt. −10.9%, $p=0.082$) and *Serratia* spp., while the cases of sepsis decreased from *E. faecium* (Np 11 pt. −9.4%- vs. Pp 7 pt. −3.8%, $p=0.047$), and *Enterobacter* spp., *S. haemolyticus*, *S. maltophilia*, *Proteus* spp., and *P. aeruginosa* have not changed over the time of the study.

Discussion

We found a substantial increase in the prevalence of cases of sepsis and septic shock in patients admitted to the ICU from 2018 to 2020 and a reduction in 2021.

Sepsis is characterized by a high mortality rate. The rate estimates range from 10 to 52%, depending on how the data are collected (16–20). Mortality rates increase linearly according to the severity of the disease (19). In one study, the mortality rates of septic shock were 46% (21). In another study, the mortality associated with sepsis was ≥10%, while in the case of septic shock, it was ≥40% (22). We did not detect differences in the medical or surgical origin of sepsis in the study periods, probably due to the sample size. In addition, we know that patients with sepsis and positive blood cultures have a higher severity of illness and higher mortality (23) and this represents a particular group risk population.

There are different studies on patients in ICU with a mortality rate of 59% in COVID-19 sepsis vs. 29% in the same period without COVID-19, or 58.7% vs. 40%, respectively (24, 25). Our data show that in ICU patients, there was an increase in the 30-day mortality rate from 2018 to 2020, with a reduction in 2021 and a return to mortality values in the pre-COVID-19 era. This trend can probably be traced back to the different phases of the COVID-19 disease that impacted patient survival. Indeed, in 2020, there was a pandemic that caught healthcare systems unprepared with the absence of knowledge related to COVID-19 physiopathology and its treatment. Initially, the physiopathology of the SARS-CoV-2 infection was unknown. COVID-19 is classically divided into two phases: the first is characterized by a high viral load, while the second is associated with the activation of an inflammatory response, including the appearance of a cytokine storm, which is then responsible for the evolution of ARDS and MOF, and eventually death (3, 26, 27).

Numerous therapies were attempted in the first half of 2020 that proved largely ineffective. Only in the second half of 2020 did pathophysiological knowledge increase, and the discovery of effective therapeutic strategies made it possible to approach patients better, allowing for better survival even in patients with septic complications, as can be seen from the 2021 data (27–29).

The subsequent diffusion of the massive vaccination strategy resulted in the modification of the severity of COVID-19, allowing for the development of vaccine immunity, which changed the natural

TABLE 2 Crude OR and 95% CI for identifying risk associated with mortality.

	*ORc (95% CI)	*P*-value
Sex		
Female	1	
Male	0.86 (0.47–1.57)	0.631
Groups		
Np	1	
Pp-non-COVID	0.49 (0.25–0.96)	0.039
Pp-COVID	0.73 (0.35–1.50)	0.396
Age, years	1.04 (1.02–1.07)	<0.0001
Comorbidity		
No	1	
Yes	1.31 (0.44–3.84)	0.619
Diabetes mellitus		
No	1	
Yes	0.89 (0.45–1.77)	0.757
Cancer		
No	1	
Yes	0.65 (0.28–1.46)	0.298
Acute renal failure		
No	1	
Yes	1.92 (0.85–4.31)	0.111
Drug addiction		
No	1	
Yes	0.50 (0.12–2.07)	0.343
HIV		
No	1	
Yes	1.03 (0.25–4.27)	0.959
SOFA Score	1.12 (1.03–1.22)	0.004
APACHE Score	1.08 (1.04–1.12)	<0.0001
ICU, days	0.98 (0.97–0.99)	0.023

*ORc, crude odds ratio; 95% CI, 95% confidence interval.

history of the disease due to a more ready immune system response to infection. Finally, the greater availability and increasingly correct use of DPI have probably contributed to the reduction of the incidence of sepsis and mortality in patients observed in 2021.

There are no clear data on mortality rates in the COVID-19 era, but in particular, there are no data on the mortality rates of patients in the ICU who died from sepsis and SARS-CoV-2 infection.

In the general population, COVID-19-related mortality appears to be lower in younger patients (<44 years) without comorbidities (<10%) (30). Risk factors for mortality are known in COVID-19 and sepsis, such as advancing age, immunosuppression, and hospitalization (31, 32). This concordance of factors could help explain why we have seen an increase in sepsis in the first phase of COVID-19.

In this ICU population, age is a significant risk factor for mortality; data about this are widely available in the literature (33, 34). Another risk factor highlighted in our study is the correlation between mortality and days of stay in the ICU. Furthermore, as expected, other risk factors associated with mortality included comorbidities, which affected fewer COVID-19 patients than non-COVID-19 patients in this study. Patients with sepsis who were diagnosed with COVID-19 had fewer comorbidities. These data are attributable to the fact that patients with SARS-CoV-2 infection were mainly hospitalized for severe respiratory insufficiency, while, as is known, patients admitted to intensive care without a diagnosis of COVID-19 were hospitalized for the appearance of septic shock, which we know is linked to the presence of comorbidities. In this study with septic patients, the SOFA and APACHE scores were correlated with mortality, but the SOFA score is higher in COVID-19 patients vs. non-COVID-19 patients. These data can also be explained by the clinical conditions that were secondary to the cytokine storm that evolves in ARDS or MOF.

Neutrophils are the first immune cells recruited to the site of inflammation following stimulation by chemotactic factors released from damaged pulmonary tissues. Both exogenous and endogenous inflammatory stimuli can be recognized by specific receptors in human neutrophils. This further promotes the recruitment and activation of circulating neutrophils. These activated neutrophils produce several cytotoxic products and various proinflammatory cytokines. The overwhelming activation of neutrophils contributes to surrounding tissue damage and even lung dysfunction (35). Therefore, in COVID-19 ARDS patients, higher counts of neutrophils are observed and represent a predictor of poor outcome (36, 37).

A previous study on COVID-19 patients showed that neutrophils are an early marker in high-risk COVID-19 patients for acute respiratory failure and organ damage. Based on these results, we believe that classic inflammation markers such as CRP are not sufficient for stratification on COVID-19 patients. Instead, the dosing of factors among the relationship between neutrophils and lymphocytes (NRLs), IL-6, LDH, and ferritin could be useful for the early identification of patients at high risk of ARDS and death (3, 36).

Furthermore, in our study, it emerged that in the pre-pandemic era, the cases of sepsis associated with blood culture from a peripheral vein were statistically higher, while in the COVID-19 period, the cases of sepsis isolated from CVC increased. These data have never been found in the literature, and the reasons for this increased incidence of CVC-related infections could be associated with increased use of CVC and immunosuppression secondary to SARS-CoV-2 infection and to the cytokine storm phase that makes the immune system particularly dysregulated (27).

In the literature, we know that the types of sepsis-related microorganisms have changed over time. Gram-positive bacteria are mostly responsible for sepsis in the United States, although the number of cases of Gram-negative sepsis remains remarkable (32, 38). The incidence of fungal sepsis has increased over the past decade but remains lower than bacterial sepsis (16). In approximately one-half of cases of sepsis, the microorganism is not identified, so we have culture-negative sepsis (39). In our study, we have highlighted in the pandemic era a significant increase in cases of sepsis from the *CNS and Acinetobacter* spp. The epidemiological report of the European Centre for Disease Prevention and Control (ECDC) on hospital-acquired infections in the ICU, computed from 2017 data, showed a predominance of Gram-positive pathogens in bloodstream infections (40). Gram-negative bacilli cause approximately a quarter to a half of all bloodstream infections, and this depends on geographic region,

TABLE 3 Characteristics of the overall bacteria in ICU.

	Total	Np	Pp-non-COVID	Pp-COVID	
	N = 300	*N* = 117	*N* = 107	*N* = 76	*P*-value
S. aureus					
No	274 (91.3%)	105 (89.7%)	95 (88.8%)	74 (97.4%)	0.093
Yes	26 (8.7%)	12 (10.3%)	12 (11.2%)	2 (2.6%)	
S. hominis					
No	290 (96.7%)	112 (95.7%)	104 (97.2%)	74 (97.4%)	0.848
Yes	10 (3.3%)	5 (4.3%)	3 (2.8%)	2 (2.6%)	
S. capitis					
No	285 (95.0%)	116 (99.1%)	102 (95.3%)	67 (88.2%)	0.003
Yes	15 (5.0%)	1 (0.9%)*	5 (4.7%)	9 (11.8%)	
S. epidermidis					
No	217 (72.3%)	87 (74.4%)	76 (71.0%)	54 (71.1%)	0.821
Yes	83 (27.7%)	30 (25.6%)	31 (29.0%)	22 (28.9%)	
Streptococcus					
No	294 (98.0%)	117 (100.0%)	104 (97.2%)	73 (96.1%)	0.075
Yes	6 (2.0%)	0 (0.0%)	3 (2.8%)	3 (3.9%)	
E. faecalis					
No	286 (95.3%)	113 (96.6%)	103 (96.3%)	70 (92.1%)	0.325
Yes	14 (4.7%)	4 (3.4%)	4 (3.7%)	6 (7.9%)	
E. faecium					
No	282 (94.0%)	106 (90.6%)	101 (94.4%)	75 (98.7%)	0.064
Yes	18 (6.0%)	11 (9.4%)	6 (5.6%)	1 (1.3%)	
E. coli					
No	274 (91.3%)	102 (87.2%)	101 (94.4%)	71 (93.4%)	0.130
Yes	26 (8.7%)	15 (12.8%)	6 (5.6%)	5 (6.6%)	
K. pneumoniae					
No	269 (89.7%)	105 (89.7%)	96 (89.7%)	68 (89.5%)	0.998
Yes	31 (10.3%)	12 (10.3%)	11 (10.3%)	8 (10.5%)	
Proteus					
No	298 (99.3%)	116 (99.1%)	106 (99.1%)	76 (100.0%)	0.709
Yes	2 (0.7%)	1 (0.9%)	1 (0.9%)	0 (0.0%)	
Acinetobacter					
No	274 (91.3%)	111 (94.9%)	97 (90.7%)	66 (86.8%)	0.146
Yes	26 (8.7%)	6 (5.1%)	10 (9.3%)	10 (13.2%)	
S. maltophilia					
No	296 (98.7%)	115 (98.3%)	106 (99.1%)	75 (98.7%)	0.880
Yes	4 (1.3%)	2 (1.7%)	1 (0.9%)	1 (1.3%)	
P. aeruginosa					
No	284 (94.7%)	111 (94.9%)	98 (91.6%)	75 (98.7%)	0.108
Yes	16 (5.3%)	6 (5.1%)	9 (8.4%)	1 (1.3%)	
Enterobacter					
No	290 (96.7%)	112 (95.7%)	105 (98.1%)	73 (96.1%)	0.656
Yes	10 (3.3%)	5 (4.3%)	2 (1.9%)	3 (3.9%)	
S. haemolyticus					
No	293 (97.7%)	112 (95.7%)	107 (100.0%)	74 (97.4%)	0.080

(Continued)

TABLE 3 (Continued)

	Total	Np	Pp-non-COVID	Pp-COVID	
	N = 300	*N* = 117	*N* = 107	*N* = 76	*P*-value
Yes	7 (2.3%)	5 (4.3%)	0 (0.0%)	2 (2.6%)	
Serratia					
No	294 (98.0%)	115 (98.3%)	104 (97.2%)	75 (98.7%)	0.770
Yes	6 (2.0%)	2 (1.7%)	3 (2.8%)	1 (1.3%)	
Peripheral blood cultures					
No	41 (13.7%)	6 (5.1%)	17 (15.9%)	18 (23.7%)	0.001
Yes	259 (86.3%)	111 (94.9%)	90 (84.1%)*	58 (76.3%)*	
CVC blood cultures					
No	146 (48.7%)	90 (76.9%)	35 (32.7%)	21 (27.6%)	<0.0001
Yes	154 (51.3%)	27 (23.1%)	72 (67.3%)*	55 (72.4%)*	
Oxa R					
No	82 (27.3%)	23 (19.7%)	32 (29.9%)	27 (35.5%)	0.041
Yes	218 (72.7%)	94 (80.3%)	75 (70.1%)*	49 (64.5%)*	
ESBL					
No	111 (37.0%)	30 (25.6%)	43 (40.2%)	38 (50.0%)	0.002
Yes	189 (63.0%)	87 (74.4%)	64 (59.8%)	38 (50.0%)*	
R Carbapenemi					
No	133 (44.3%)	42 (35.9%)	51 (47.7%)	40 (52.6%)	0.050
Yes	167 (55.7%)	75 (64.1%)	56 (52.3%)	36 (47.4%)*	
R Aminoglicosidi					
No	162 (54.0%)	61 (52.1%)	58 (54.2%)	43 (56.6%)	0.832
Yes	138 (46.0%)	56 (47.9%)	49 (45.8%)	33 (43.4%)	
R Glycopentide					
No	149 (49.7%)	50 (42.7%)	59 (55.1%)	40 (52.6%)	0.150
Yes	151 (50.3%)	67 (57.3%)	48 (44.9%)	36 (47.4%)	
R Daptomicina					
No	170 (56.7%)	61 (52.1%)	64 (59.8%)	45 (59.2%)	0.447
Yes	130 (43.3%)	56 (47.9%)	43 (40.2%)	31 (40.8%)	
R Fluorochinoloni					
No	87 (29.0%)	33 (28.2%)	30 (28.0%)	24 (31.6%)	0.848
Yes	213 (71.0%)	84 (71.8%)	77 (72.0%)	52 (68.4%)	
R Fosfomicina					
No	236 (78.7%)	93 (79.5%)	80 (74.8%)	63 (82.9%)	0.401
Yes	64 (21.3%)	24 (20.5%)	27 (25.2%)	13 (17.1%)	
R Colistina					
No	132 (44.0%)	42 (35.9%)	44 (41.1%)	46 (60.5%)	0.003
Yes	168 (56.0%)	75 (64.1%)*	63 (58.9%)*	30 (39.5%)	
R Linezolid					
No	175 (58.3%)	60 (51.3%)	65 (60.7%)	50 (65.8%)	0.111
Yes	125 (41.7%)	57 (48.7%)	42 (39.3%)	26 (34.2%)	

N (%) are shown when appropriate. *p*-value < α for Pearson's chi-square test between couples: *S. capitis* (Np vs. Pp-COVID); peripheral blood cultures (Pp-non-COVID vs. Np and Pp-COVID vs. Np); CVC blood cultures (Pp-non-COVID vs. Np and Pp-COVID vs. Np); Oxa R (Pp-non-COVID vs. Np and Pp-COVID vs. Np); ESBL (Pp-COVID vs. Np); R Carbapenemi (Pp-COVID vs. Np); R Colistina (Np vs. Pp-COVID and Pp-non-COVID vs. Pp-COVID).

whether the onset of the infection is in the hospital or the community, and other patient risk factors (41). This study showed an increase in *S. capitis* and *S. epidermidis*, but also in Gram-negative bacteria such as *Acinetobacter* spp. and *Serratia* spp. These data agree with the data relating to germs usually circulating in the ICU, but they do not seem to agree with a recent study in Iraq that instead shows a high incidence

of Gram-positive sepsis mainly caused by *Streptococcus, Haemophilus,* and *Moraxella* (42). Probably, the different circulation of Gram-positive bacteria is related to the different characteristics of the patients and the countries.

We observed a significant reduction in the number of resistances of isolated germs, which may also be linked to a reduced selective pressure of antibiotic therapy for a better and more appropriate use of antibiotic therapy especially in the COVID-19 period. Patients in the ICU frequently are on or have recently been on antibiotics, which increases the risk of infections with *P. aeruginosa* and other non-fermenting Gram-negative *bacilli*, such as *Acinetobacter species*, that have intrinsic or acquired resistance to commonly used agents.

In our study, we particularly observed over time during the pandemic era a decreased resistance related to OXA-48, ESBL, carbapenems, and colistin. Our data contrast with unique but recently published data on non-ICU patients showing a higher incidence of ESBL-producing *E. coli* in COVID-19 patients than in non-COVID-19 patients. ESBL infections are associated with longer hospital stays and higher mortality rates in different population situations (43); these data are probably linked to a decrease in the number of Gram-negative bacteria that also led to the reduction of ESBLs.

In agreement with the literature data, we found a significant correlation between mortality from sepsis or septic shock and the presence of an ESBL germ. Neither was associated with the APACHE score and therefore with the patient's clinical severity or with the diagnosis of COVID-19 (44, 45).

We know that the limitations of this study are the lack of information on colonization rates and molecular analysis for clusters of bacteria isolates. Additionally, our study is a single-center study, and therefore, our results cannot be generalized to all conditions. Perhaps during the pandemic phase, there was a more appropriate use of antibiotic therapies, but above all, PPE was used more correctly in ICUs.

Conclusion

This study demonstrated how the COVID-19 pandemic changed the prevalence of sepsis in the ICU. It emerged that the risk factors associated with mortality were SOFA and APACHE scores, age, days in the ICU, and, above all, the presence of ESBL-producing bacteria. Despite this, during the pandemic phase, we have observed a significant reduction in the emergence of resistant germs compared to the pre-pandemic phase. The COVID-19 pandemic revealed the critical need for effective infection-control policies and the correct use of antibiotic stewardship, along with a number of interventions to reduce sepsis and other co-infections in COVID-19 units. Compliance with guidelines for infection control and standards of antibiotic care is imperative.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

Ethics statement

The study protocol was approved by the Ethics Internal Committee at the University "G. d'Annunzio" ChietiPescara (Ethics Committee Project No. 02 the 02/02/2022) and was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Written informed consent from the patients was not required to participate in this study in accordance with the national legislation and the institutional requirements.

Author contributions

KF: Conceptualization, Writing – original draft. LV: Data curation, Validation, Writing – original draft. PB: Data curation, Formal analysis, Writing – review & editing. MN: Data curation, Formal analysis, Writing – review & editing. CU: Conceptualization, Funding acquisition, Writing – review & editing. AG: Funding acquisition, Methodology, Writing – review & editing. MB: Investigation, Methodology, Writing – review & editing. GT: Data curation, Methodology, Resources, Writing – review & editing. JV: Conceptualization, Supervision, Writing – original draft. SM: Conceptualization, Supervision, Writing – original draft.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Dufrusine B, Valentinuzzi S, Bibbo S, Damiani V, Lanuti P, Pieragostino D, et al. Iron Dyshomeostasis in COVID-19: biomarkers reveal a functional link to 5-lipoxygenase activation. *Int J Mol Sci*. (2022) 24:15. doi: 10.3390/ijms24010015

2. Ucciferri C, Di Gasbarro A, Borrelli P, Di Nicola M, Vecchiet J, Falasca K. New therapeutic options in mild moderate COVID-19 outpatients. *Microorganisms*. (2022) 10:131. doi: 10.3390/microorganisms10112131

3. Ucciferri C, Caiazzo L, Di Nicola M, Borrelli P, Pontolillo M, Auricchio A, et al. Parameters associated with a diagnosis of COVID-19 in emergency department. *Immun Inflamm Dis*. (2021) 9:851–61. doi: 10.1002/iid3.440

4. Santos TA, Oliveira JE, Fonseca CDD, Barbosa DA, Belasco A, Miura CRM. Sepsis and COVID-19: outcomes in young adults in intensive care. *Rev Bras Enferm*. (2023) 76:e20230037. doi: 10.1590/0034-7167-2023-0037

5. Seymour CW, Liu VX, Iwashyna TJ, Brunkhorst FM, Rea TD, Scherag A, et al. Assessment of clinical criteria for Sepsis: for the third international consensus definitions for Sepsis and septic shock (Sepsis-3). *JAMA*. (2016) 315:762–74. doi: 10.1001/jama.2016.0288

6. Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990-2017: analysis for the global burden of disease study. *Lancet*. (2020) 395:200–11. doi: 10.1016/S0140-6736(19)32989-7

7. Dremsizov T, Clermont G, Kellum JA, Kalassian KG, Fine MJ, Angus DC. Severe sepsis in community-acquired pneumonia: when does it happen, and do systemic inflammatory response syndrome criteria help predict course? *Chest*. (2006) 129:968–78. doi: 10.1378/chest.129.4.968

8. Netea MG, van der Meer JW. Immunodeficiency and genetic defects of pattern-recognition receptors. *N Engl J Med*. (2011) 364:60–70. doi: 10.1056/NEJMra1001976

9. Prescott HC, Dickson RP, Rogers MA, Langa KM, Iwashyna TJ. Hospitalization type and subsequent severe Sepsis. *Am J Respir Crit Care Med*. (2015) 192:581–8. doi: 10.1164/rccm.201503-0483OC

10. Vincent JL, Bihari DJ, Suter PM, Bruining HA, White J, Nicolas-Chanoin MH, et al. The prevalence of nosocomial infection in intensive care units in Europe. Results of the European prevalence of infection in intensive care (EPIC) study. EPIC international advisory committee. *JAMA*. (1995) 274:639–44.

11. Zhou F, Yu T, Du R, Fan G, Liu Y, Liu Z, et al. Clinical course and risk factors for mortality of adult inpatients with COVID-19 in Wuhan, China: a retrospective cohort study. *Lancet*. (2020) 395:1054–62. doi: 10.1016/S0140-6736(20)30566-3

12. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, et al. Bacterial and fungal coinfection in individuals with coronavirus: a rapid review to support COVID-19 antimicrobial prescribing. *Clin Infect Dis*. (2020) 71:2459–68. doi: 10.1093/cid/ciaa530

13. Abelenda-Alonso G, Padulles A, Rombauts A, Gudiol C, Pujol M, Alvarez-Pouso C, et al. Antibiotic prescription during the COVID-19 pandemic: a biphasic pattern. *Infect Control Hosp Epidemiol*. (2020) 41:1371–2. doi: 10.1017/ice.2020.381

14. Vincent JL, Moreno R, Takala J, Willatts S, De Mendonca A, Bruining H, et al. The SOFA (Sepsis-related organ failure assessment) score to describe organ dysfunction/failure. On behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med*. (1996) 22:707–10.

15. Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The third international consensus definitions for Sepsis and septic shock (Sepsis-3). *JAMA*. (2016) 315:801–10. doi: 10.1001/jama.2016.0287

16. Martin GS, Mannino DM, Eaton S, Moss M. The epidemiology of sepsis in the United States from 1979 through 2000. *N Engl J Med*. (2003) 348:1546–54. doi: 10.1056/NEJMoa022139

17. Kaukonen KM, Bailey M, Suzuki S, Pilcher D, Bellomo R. Mortality related to severe sepsis and septic shock among critically ill patients in Australia and New Zealand, 2000-2012. *JAMA*. (2014) 311:1308–16. doi: 10.1001/jama.2014.2637

18. Dombrovskiy Y, Martin AA, Sunderram J, Paz HL. Rapid increase in hospitalization and mortality rates for severe sepsis in the United States: a trend analysis from 1993 to 2003. *Crit Care Med*. (2007) 35:1244–50. doi: 10.1097/01.CCM.0000261890.41311.E9

19. Kaukonen KM, Bailey M, Pilcher D, Cooper DJ, Bellomo R. Systemic inflammatory response syndrome criteria in defining severe sepsis. *N Engl J Med*. (2015) 372:1629–38. doi: 10.1056/NEJMoa1415236

20. Epstein L, Dantes R, Magill S, Fiore A. Varying estimates of Sepsis mortality using death certificates and administrative codes--United States, 1999-2014. *MMWR Morb Mortal Wkly Rep*. (2016) 65:342–5. doi: 10.15585/mmwr.mm6513a2

21. Rangel-Frausto MS, Pittet D, Costigan M, Hwang T, Davis CS, Wenzel RP. The natural history of the systemic inflammatory response syndrome (SIRS). A prospective study. *JAMA*. (1995) 273:117–23.

22. Annane D, Bellissant E, Cavaillon JM. Septic shock. *Lancet*. (2005) 365:63–78. doi: 10.1016/S0140-6736(04)17667-8

23. Lal A, Rayes H, O'Horo JC, Singh TD, Gajic O, Kashyap R. Septic shock definitions and associated outcomes in blood culture positive critically ill patients. *Ann Transl Med*. (2023) 11:192. doi: 10.21037/atm-22-5147

24. Heubner L, Hattenhauer S, Guldner A, Petrick PL, Rossler M, Schmitt J, et al. Characteristics and outcomes of sepsis patients with and without COVID-19. *J Infect Public Health*. (2022) 15:670–6. doi: 10.1016/j.jiph.2022.05.008

25. Buetti N, Tabah A, Loiodice A, Ruckly S, Aslan AT, Montrucchio G, et al. Eurobact 2 study, different epidemiology of bloodstream infections in COVID-19 compared to non-COVID-19 critically ill patients: a descriptive analysis of the Eurobact II study. *Crit Care*. (2022) 26:319. doi: 10.1186/s13054-022-04166-y

26. Di Biagio A, Ricci E, Calza L, Squillace N, Menzaghi B, Rusconi S, et al. Factors associated with hospital admission for COVID-19 in HIV patients. *AIDS*. (2020) 34:1983–5. doi: 10.1097/QAD.0000000000002663

27. Ucciferri C, Vecchiet J, Falasca K. Role of monoclonal antibody drugs in the treatment of COVID-19. *World J Clin Cases*. (2020) 8:4280–5. doi: 10.12998/wjcc.v8.i19.4280

28. Katia F, Myriam DP, Ucciferri C, Auricchio A, Di Nicola M, Marchioni M, et al. Efficacy of canakinumab in mild or severe COVID-19 pneumonia. *Immun Inflamm Dis*. (2021) 9:399–405. doi: 10.1002/iid3.400

29. Ucciferri C, Barone M, Vecchiet J, Falasca K. Pidotimod in Paucisymptomatic SARS-CoV2 infected patients. *Mediterr J Hematol Infect Dis*. (2020) 12:e2020048. doi: 10.4084/mjhid.2020.048

30. Ioannidis JPA, Axfors C, Contopoulos-Ioannidis DG. Population-level COVID-19 mortality risk for non-elderly individuals overall and for non-elderly individuals without underlying diseases in pandemic epicenters. *Environ Res*. (2020) 188:109890. doi: 10.1016/j.envres.2020.109890

31. Harrison DA, Welch CA, Eddleston JM. The epidemiology of severe sepsis in England, Wales and Northern Ireland, 1996 to 2004: secondary analysis of a high quality clinical database, the ICNARC case mix Programme database. *Crit Care*. (2006) 10:R42. doi: 10.1186/cc4854

32. Uslan DZ, Crane SJ, Steckelberg JM, Cockerill FR, St Sauver JL, Wilson WR, et al. Age- and sex-associated trends in bloodstream infection: a population-based study in Olmsted County, Minnesota. *Arch Intern Med*. (2007) 167:834–9. doi: 10.1001/archinte.167.8.834

33. Purdy FR, Tweeddale MG, Merrick PM. Association of mortality with age of blood transfused in septic ICU patients. *Can J Anaesth*. (1997) 44:1256–61. doi: 10.1007/BF03012772

34. Andrew MK, Godin J, LeBlanc J, Boivin G, Valiquette L, McGeer A, et al. Older age and frailty are associated with higher mortality but lower ICU admission with COVID-19. *Can Geriatr J*. (2022) 25:183–96. doi: 10.5770/cgj.25.546

35. Aulakh GK. Neutrophils in the lung: "the first responders". *Cell Tissue Res*. (2018) 371:577–88. doi: 10.1007/s00441-017-2748-z

36. Liu Y, Du X, Chen J, Jin Y, Peng L, Wang HHX, et al. Neutrophil-to-lymphocyte ratio as an independent risk factor for mortality in hospitalized patients with COVID-19. *J Infect*. (2020) 81:e6–e12. doi: 10.1016/j.jinf.2020.04.002

37. Yang SC, Tsai YF, Pan YL, Hwang TL. Understanding the role of neutrophils in acute respiratory distress syndrome. *Biom J*. (2021) 44:439–46. doi: 10.1016/j.bj.2020.09.001

38. Klotz SA, Chasin BS, Powell B, Gaur NK, Lipke PN. Polymicrobial bloodstream infections involving Candida species: analysis of patients and review of the literature. *Diagn Microbiol Infect Dis*. (2007) 59:401–6. doi: 10.1016/j.diagmicrobio.2007.07.001

39. Gupta S, Sakhuja A, Kumar G, McGrath E, Nanchal RS, Kashani KB. Culture-negative severe Sepsis: Nationwide trends and outcomes. *Chest*. (2016) 150:1251–9. doi: 10.1016/j.chest.2016.08.1460

40. Bijkerk P, Monnier AA, Fanoy EB, Kardamanidis K, Friesema IH, Knol MJ. ECDC round Table report and ProMed-mail most useful international information sources for the Netherlands early warning committee. *Euro Surveill*. (2017) 22:502. doi: 10.2807/1560-7917.ES.2017.22.14.30502

41. Sievert DM, Ricks P, Edwards JR, Schneider A, Patel J, Srinivasan A, et al. National Healthcare Safety Network, and N.F. Participating, antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. *Infect Control Hosp Epidemiol*. (2013) 34:1–14. doi: 10.1086/668770

42. Sh R. Study of microbial infections and some immunological parameters among Covid-19 in ICU patients in Najaf governorate. *Iraq Arch Razi Inst*. (2022) 77:1569–74. doi: 10.22092/ARI.2022.358102.2151

43. Ling W, Paterson DL, Harris PNA, Furuya-Kanamori L, Edwards F, Laupland KB. Population-based incidence and characteristics of adult *Escherichia coli* bloodstream infection in Queensland, Australia, from 2000 to 2019. Open forum. *Infect Dis*. (2023) 10:ofad071. doi: 10.1093/ofid/ofad071

44. Giannitsioti E, Louka C, Mamali V, Kousouli E, Velentza L, Papadouli V, et al. Bloodstream infections in a COVID-19 non-ICU department: microbial epidemiology, resistance profiles and comparative analysis of risk factors and Patients' outcome. *Microorganisms*. (2022) 10:314. doi: 10.3390/microorganisms10071314

45. Deana C, Rovida S, Orso D, Bove T, Bassi F, De Monte A, et al. Learning from the Italian experience during COVID-19 pandemic waves: be prepared and mind some crucial aspects. *Acta Biomed*. (2021) 92:e2021097. doi: 10.23750/abm.v92i2.11159

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Molecular network characteristics and drug resistance analysis of 392 newly reported MSM HIV/AIDS cases in Chongqing, China

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To comprehensively investigate the molecular transmission patterns of HIV-1 genotypes among men who have sex with men (MSM) in Chongqing, we employed 392 pol sequences of MSM to construct a phylogenetic tree and gene transmission network. Among the viral subtypes, CRF07_BC accounted for 73.2% (287/392) and CRF01_AE accounted for 20.7% (81/392), emerging as the predominant subtypes in this investigation. Additionally, we observed the presence of CRF55_01B, subtype B, CRF08_BC and other circulating recombinant forms. The HIV-1 molecular network was constructed with a gene distance threshold of 1.5%, resulting in an entry rate of 61.4% (241/392). Within the network, we identified a total of 23 molecular clusters, with the largest cluster being the CRF07_BC molecular cluster comprising 148 node values. Transmitted drug-resistance (TDR) mutations were found in 4.34% of the cases, with 1.79% associated with protease inhibitors (PIs), 0.51% with nucleoside reverse transcriptase inhibitors (NRTIs), and 2.55% with non-nucleoside reverse transcriptase inhibitors (NNRTIs). Statistical analysis indicated a higher enrollment rate in the HIV-1 molecular network among infected individuals with the CRF07_BC subtype, those identifying with same-sex sexual roles as "vers," and individuals with higher education levels. This suggests the need for strengthened investigation and intervention in this population to prevent the formation of larger transmission clusters. Furthermore, continuous monitoring of the HIV-1 molecular dynamics network is necessary to promptly and accurately track changes in molecular epidemic characteristics.

KEYWORDS

molecular network, transmitted drug-resistance, HIV-1, MSM, subtype

Introduction

The increasing burden of HIV/AIDS and comorbidities, along with the emergence of new HIV subtypes, circulating recombinant forms, drug mutations, and changing transmission networks, are likely to exert a significant impact on the HIV epidemic in China. Since the initiation of the National Free Antiretroviral Treatment Program (NFATP) by the Chinese government in 2003, the number of patients participating in this program has consistently increased, leading to broader antiviral medication coverage. However, this rise in treatment coverage has also been accompanied by an escalation in drug resistance. In China, according to the results of the fourth national HIV molecular epidemiology survey, the prevalence of transmitted drug resistance (TDR) was 3.80% in 2015 and increased to 4.40% in 2018 (1).

Resistance mutation analysis revealed that among the newly reported HIV-infected individuals, the highest resistance rates were observed for non-nucleoside reverse transcriptase inhibitors, particularly nevirapine (NVP) and efavirenz (EFV). The specific resistance mutations identified were V179E/D/T, K103N, and V106I/M (2). The evolution of HIV drug resistance (HIVDR) poses a threat to the global expansion of antiretroviral treatment (ART) for HIV infection, thereby elevating the risk of ART failure. Consequently, conducting HIVDR surveys becomes crucial to determine the rate of TDR among ART-naive people living with HIV (PLWH). This survey provides essential baseline data for the successful implementation of ART programs, the mitigation of HIVDR incidence, and the formulation of effective public health policies aimed at curbing HIV prevalence.

The MSM community's high mobility has significantly contributed to the widespread transmission of HIV across various locations and groups throughout China (3, 4). HIV viruses exhibit genetic variability, resulting in a growing number of recombinant subtypes. When coupled with the unique anal sexual behavior, frequent sexual activity, and multiple partners within the MSM population, this exacerbates the transmission of HIV (5–7). Chongqing is one of the areas most severely affected by HIV-1, with over 1,000 new cases of MSM infection reported each year, as documented by the China National Center for AIDS/STD Control and Prevention (NCAIDS). In recent years, Chongqing has exhibited a higher rate of sentinel and HIV Voluntary Counseling and Testing (VCT) infections compared to other Chinese provinces, such as Sichuan and Shaanxi (8, 9). Southwest China reported the highest pooled HIV-1 prevalence among MSM, with Chongqing city exhibiting the highest HIV prevalence (13.8, 95% CI: 12.8–14.9%) due to the region's open attitudes toward homosexuality and sex (10, 11).

Because of the private nature of sexual behavior and the lengthy incubation period of AIDS, conducting an analysis of the social transmission network of HIV based solely on on-site epidemiological information faces numerous challenges and obstacles. To overcome these limitations, we have introduced HIV molecular transmission network analysis techniques, which enable the identification of associations between surveyed subjects at the molecular level. Previous studies have demonstrated that the sequence of the HIV pol coding region varies by less than 1% per year (12). Infected individuals who share transmission relationships exhibit greater genetic similarity in their viral gene sequences and tend to form clusters in phylogenetic analysis (13, 14).

This study enrolled a total of 392 newly reported MSM HIV-1-infected patients between 2019 and 2020. Phylogenetic analyses were conducted to examine the distribution and characteristics of HIV-1 subtypes, TDR, and molecular transmission clusters during this period. The objective of the study was to investigate the local transmission dynamics of prevalent HIV-1 strains within MSM populations and identify risk factors associated with network entry rates. The findings obtained from this study have the potential to inform targeted prevention and control strategies for the MSM population in Chongqing, China, thereby enabling more precise interventions.

Methods

Study participants

A cross-sectional investigation was conducted by the Chongqing Center for Disease Control and Prevention to study the MSM population. On-site surveys and follow-ups were carried out, and blood samples were collected from newly reported MSM individuals with HIV/AIDS between 2019 and 2020. The inclusion criteria were as follows: individuals who reported their cases between January 1, 2019, and December 31, 2020; males aged 18 years or older; individuals who had not initiated antiviral therapy at the time of the survey; individuals who were willing to cooperate and did not have any other serious concurrent illnesses; individuals who understood the purpose of the survey and provided informed consent.

In China, AIDS is a national legal infectious disease, and all newly diagnosed cases of AIDS need to be reported in "China AIDS Comprehensive Prevention and Control Data Information System." And all participants will be screened and matched using personal information in this system to ensure that all subjects included in the study are newly reported HIV cases. A total of 448 participants met the requirements for the survey.

Sample collection and RNA extraction

For eligible study participants, venous blood samples of 5 mL were collected in anticoagulation tubes containing EDTA-K by trained professionals. The blood samples were then processed to separate the plasma, which was subsequently stored in a low-temperature freezer at −80°C. To extract HIV-1 RNA from the plasma samples of infected patients, a Thermo Scientific KingFisher Flex high-throughput automated nucleic acid extractor was employed.

Amplification and sequencing

Nucleic acid amplification was performed using Takara PrimeScript One Step RT-PCR and Tengen 2×Taq PCR (premixed) reagents. The protease (PR) gene region and the first 300 amino acids of the reverse transcriptase (RT) gene region were amplified using the pol gene region of the international standard strain HXB2 (2,253–3,553 nt) as a reference. A nested polymerase chain reaction (PCR) technique was employed to amplify characteristic fragments of the pol region of the HIV-1 virus with commercial primers, following previously published methods (15). The PCR products were then sent to Beijing Nordson Genome Research Center Co., Ltd. for purification and subsequent gene sequencing. The amplification primers and sequencing primers were provided by Beijing Nordson Genome Research Center Co.

Sequence splicing and subtype identification

The resulting pol sequence was spliced using the Sequencher 5.4.6 analytical program. Subsequently, the sequence underwent editing and correction using the BioEdit Sequence Alignment Editor. All sequences used in this study have been uploaded the sequence to the National Microbiological Data Center of China (No:NMDC10018715), which can be accessed through the link.[1] Genotype determination was

1 https://nmdc.cn/resource/genomics/project/detail/NMDC10018715

performed using MEGA X software. To align all assembled sequences with reference sequences from the HIV sequence database of Los Alamos National Laboratory in the United States, the Clustal W algorithm in MEGA X was employed. Manual verification of the alignment was conducted in Bioedit (16).

FastTree software was utilized to estimate an approximate maximum likelihood phylogenetic tree for the pol sequences. The GTR + G + I nucleotide substitution model was applied (17), and the Shimodaira-Hasegawa (SH) test within the software was used to calculate the node (branch point) value of the evolutionary tree. Clades with SH-like support ≥0.90 were defined (18, 19). The preliminary determination of genotype was achieved by clustering the sample sequences with international reference strains. The results were further validated using the online analysis tool HIV Databases BLAST.[2] Recombinant identification was performed using the Recombinant Identification Program (RIP) (20) implemented in the HIV sequence Database.[3] The phylogenetic tree was visualized using FigTree v1.4.3[4] (20). If the genotype of a sequence could not be determined through phylogenetic tree analysis and HIV Databases, it was classified as a Unique Recombinant Form (URF).

Drug resistance mutation analysis

The distribution of HIV drug resistance mutations (DRMs) among the study subjects was analyzed based on 25 commonly used antiretroviral therapeutic drugs listed in the HIVDB database[5] of Stanford University, USA. The analysis aimed to assess drug resistance related to nucleoside reverse transcriptase inhibitors (NRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), and protease inhibitors (PIs) (21, 22).

Molecular network analysis

The hyphy 2.2.4 software was employed to estimate the genetic distances between clusters in the TN93 model. Molecular transmission clusters were generated using Cytoscape 3.9.0 (23). A threshold of 0.5% signifies a nucleotide sequence of 1,000 bp, representing 5 different nucleotides. This threshold suggests the presence of a newly infected molecular cluster, as the maximum time for the virus strains to evolve to 5 different nucleotides is approximately 2–3 years. Conversely, a threshold of 1.5% represents a nucleotide sequence of 1,000 bp, corresponding to 15 different nucleotides. In this case, the threshold indicates long-term infected molecular clusters, as the maximum time for the virus strains to evolve to 15 different nucleotides is around 7–8 years (24).

Statistical analysis

The professional investigators at the STD/AIDS counseling clinic were responsible for recording the questionnaire, which collected basic demographic and epidemiological information from the survey respondents. The epidemiological information encompassed marital status, age, occupation, culture, and sexual roles ("Top"—The individual who assumes the penetrative role during sexual activity. "Bottom"—The individual who assumes the receptive role during sexual activity. "Vers"—Individuals who are flexible and may assume either the top or bottom role, depending on the context or preference) in male-to-male sex. Statistical analysis was conducted using SPSS 26 software (IBM, Chicago, IL, United States). Crude odds ratio (COR) with 95% confidence interval (CI) and adjusted odds ratio (AOR) with 95% CI were determined using univariate and multivariate logistic regression models separately in order to analyze the factors influencing entry into the molecular transmission network. A significance level of $p < 0.05$ was used to determine statistical significance.

Results

Demographic characteristics of the study participants

A total of 392 pol region sequences were successfully obtained from 445 samples after nucleic acid amplification and gene sequencing (88.09% sequencing success rate). The average age of those individuals was 32.2 years (ranging from 18 to 69 years). Of these subjects, 79.8% (313/392) were unmarried, 12.8% (50/392) were married, and 7.4% (29/392) were divorced or widowed. From the education aspect, college and above accounted for 59.70% (234/392). The sex role survey showed that 42.3% (166/392) were the "Top," 23.3% (91/392) were the "Bottom," and 34.2% (135/392) were the "Verse." The majority of the subjects (72.5%, 284/392) had CD4+ T lymphocyte count ≤350 cells/μL before treatment (Table 1).

The prevalence of TDR

Among the 392 individuals included in the sequencing analysis, 17 individuals (4.34%) were found to be infected with HIV strains that harbored at least one drug resistance mutation (DRM). The prevalence of TDR to NNRTIs was 2.55%, followed by TDR to NRTIs at 0.51%, and TDR to PIs at 1.79%. The prevalence of dual-class TDR was 0.06% for both PI and NNRTI, as well as for NRTI and NNRTI. The most common DRMs associated with NNRTIs, NRTIs, and PIs were K103N/ E138A, L74LI/M184I and Q58E, respectively (Table 2).

Identification and characterization of HIV-1 subtype distribution and molecular network

Among the study subjects, a total of 392 samples were successfully sequenced and genotyped, accounting for 88.09% (392/445) of the total sample size. Analysis using the approximately maximum-likelihood (ML) tree in Fast Tree 2.3 revealed that the dominant subtype was CRF07_BC, accounting for 73.20% (287/392) of the samples. This was followed by CRF01_AE at 20.70% (81/392), CRF55_01B at 3.06% (12/392), and subtype CRF08_BC at 1.26% (5/392) (Table 1). Additionally, other subtypes including B, and URFs were also detected

2 https://www.hiv.lanl.gov/content/sequence/BASICBLAST/basicblast.html
3 http://www.hiv.lanl.gov/content/sequence/RIP/RIP.html
4 http://tree.bio.ed.ac.uk/software/figtree/
5 https://hivdb.stanford.edu/

TABLE 1 Characteristics of 392 newly reported cases of MSM HIV/AIDS in Chongqing, China.

Variables	Number	Percent
Total	392	100.00
Age (years)		
18–30	215	54.80
31–40	98	25.00
>40	79	20.20
Occupation		
workers and farmers	36	9.20
Unemployment	73	18.60
Business services	255	65.10
Students	28	7.10
Education level		
Primary and junior high school	58	14.80
High school	100	25.50
College degree and above	234	59.70
CD4+ T lymphocyte count before treatment (cells/μL)		
<200	150	38.30
200–350	134	34.20
≥350	71	18.10
Miss[#]	37	9.40
Marital status		
Unmarried	313	79.80
Married	50	12.80
Divorced/widowed	29	7.40
Sex Roles		
"Top"	166	42.30
"Bottom"	91	23.30
"Vers"	135	34.40
Virus subtypes		
CRF07_BC	287	73.20
CRF01_AE	81	20.70
CRF55_01B	12	3.06
CRF08_BC	5	1.26
B	4	1.02
URF	3	0.76

[#]CD4+ T lymphocyte testing not conducted or results not available.

in sequential order. In the phylogenetic tree, the differing lengths of tree scales among the various viruses resulted in separate clustering of viral sequences with different genotypes (Figure 1).

The HIV-1 molecular network was constructed according to the gene distance threshold of 1.5%. A total of 241 nodes entered the network, with an entry rate of 61.4%. And a total of 23 molecular clusters (≥2 nodes, each node representing a sequence) were found. Visualization by Cytoscape 3.9.0 software revealed several large MSM transmission clusters in the molecular network, with the largest MSM molecular cluster containing 148 nodes, where the viral genotype

TABLE 2 Distribution of DRMs in 392 newly reported cases of MSM HIV/AIDS in Chongqing, China.

DRMs	Number	TDR%	Drug[a]
Total	17	4.34	
NNRTIs			
K103N	3	0.77	EFV/NVP
Y181YC	1	0.26	EFV/ETR/NVP/RPV
E138A	3	0.77	RPV
E138EG, V179E	1	0.26	EFV/ETR/NVP/RPV
V106VI, V179D	1	0.26	EFV/ETR/NVP/RPV
V179IL	1	0.26	RPV
NRTIs			
L74LI	1	0.26	ABC/DDI
M184I	1	0.26	ABC/FTC/3TC
PIs			
G73GRS	1	0.26	IDV/r, SQV/r, NFV
M46MI	1	0.26	NFV
Q58E	3	0.77	TPV/r
M46L	2	0.51	NFV

[a]Drug indicates the 25 drug available on the HIVDB database at Stanford University. EFV, efavirenz; NVP, nevirapine; DDI, desoxynivalenol; D4T, stavudine; FTC, emtricitabine; DRV, darunavir; 3TC, lamivudine; ABC, abacavir; NFV, nelfinavir; IDV, indinavir; SQV/r, saquinavir + ritonavir; TPV/r, tipranavir + ritonavir.

represented by each node was CRF07_BC. Next, a gene distance threshold of 0.5% was chosen to construct the molecular network, and a total of 72 nodes entered the network, with an entry rate of 18.4%. A total of 27 molecular clusters were formed, with the largest one containing 12 nodes. We can also find that 39 nodes in the largest MSM molecular cluster are new additions, and the number of nodes directly or indirectly associated with the propagation network of long-term infections reaches 41, accounting for 56.94% (41/72). By drug resistance transmission cluster analysis, a total of 8 transmission resistant nodes were found in this network, with the main virus subtypes CRF07_BC and CRF01_AE, of which 6 nodes were in the long-term infection transmission network and 2 nodes were in the newly infected network (Figure 2).

Analysis of risk factors of transmitted molecular network

In the multivariable logistic regression model, education level (high School, AOR = 3.535, 95% CI 1.597–7.828; college degree and above, AOR = 2.767, 95% CI 1.389–5.512.), sex role ("Vers," AOR = 2.409, 95% CI 1.366–4.246.), and virus subtypes (CRF01_AE, AOR = 0.106, 95% CI 0.059–0.192; CRF55_01B, AOR = 0.051, 95%CI 0.010–0.248.) were significantly associated with being in clusters compared to those who were not in clusters (Table 3).

Discussion

In this study, a cross-sectional survey was conducted to assess the molecular epidemiological status of HIV-1. The focus was on tracking

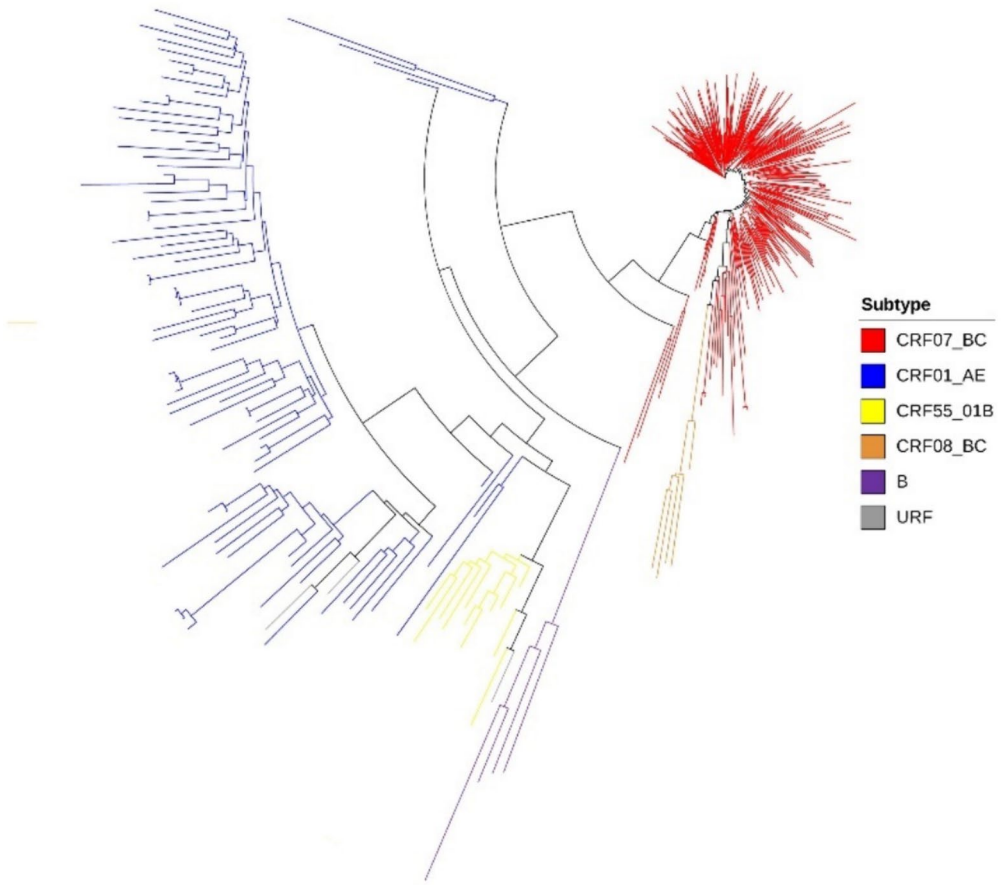

FIGURE 1
Phylogenetic tree of lineages, the phylogenetic tree was constructed using approximately-maximum-likelihood (ML) method based on *pol* region (HXB2: 2, 253 to 3, 306 nt) in Fast Tree 1.4.3. The nucleotide substitution mode was GTR + G + I.

the characteristics and distribution of HIV-1 genotypes, TDR, DRMs, and molecular transmission clusters among newly diagnosed HIV-infected patients in Chongqing. Additionally, the study aimed to explore the factors influencing the rate of entry into the molecular network.

In China, the overall prevalence of TDR in the ART-naïve population was reported to be 3.8% (30), which is slightly lower than the TDR prevalence of 4.34% observed in our study. The distribution of DRMs in our study was found to be similar to that of Yunnan, Heilongjiang, and Sichuan provinces in China (9, 25, 26). Specifically, the prevalence of TDR to NNRTIs (2.55%) was higher compared to NRTIs (0.51%) and PIs (1.79%). This observation can be attributed to the low genetic barrier to resistance of NNRTIs, where a single major mutation often leads to multiple and high-level resistance to NNRTI drugs (27–29). The common DRMs for NNRTIs in our study were K103N (30%) and E138A (30%), which aligns with previous analyses of HIV-1 transmission resistance in newly diagnosed HIV-infected patients in Sichuan Province (30). This finding is related to the widespread use of NNRTI-based (EFV/NVP) first-line treatment in China for over a decade, consistent with data from low- and middle-income countries (31–33). Moving forward, it is essential to strengthen drug resistance monitoring in the MSM population during antiviral treatment. Close attention should be paid to the spread of drug-resistant strains, with concerted efforts to reduce the further proliferation of drug-resistant variants.

Molecular network analysis techniques have become increasingly important in AIDS research in various countries (34–36). They provide valuable insights into the transmission dynamics of HIV-1, aiding in the identification of key transmission clusters, and drug resistance transmission clusters and associated risk factors (37, 38). By comparing the newly infected molecular network with the long-standing infected molecular network, certain key transmission clusters and network segments are identified, which have experienced rapid expansion within a short timeframe. Given the low prevalence of TDR within the MSM population, larger transmission drug-resistant clusters were not observed. However, the transmission drug-resistant clusters mainly consisted of cases with long-term infections. This highlights the importance of enhancing surveillance for drug-resistant transmission clusters and DRMs within the molecular network.

The analysis of subtypes revealed that CRF07_BC and CRF01_AE were the predominant subtypes among the MSM population in Chongqing. This finding is consistent with previous studies conducted in Anhui and Guangzhou (39, 40), and is also in line with the national molecular epidemiological subtype distribution in 2015 (41). CRF07_BC formed 8 molecular clusters, with the largest cluster containing 148 nodes, indicating significant aggregation within the MSM population. Figure 2 demonstrates that the molecular cluster formed

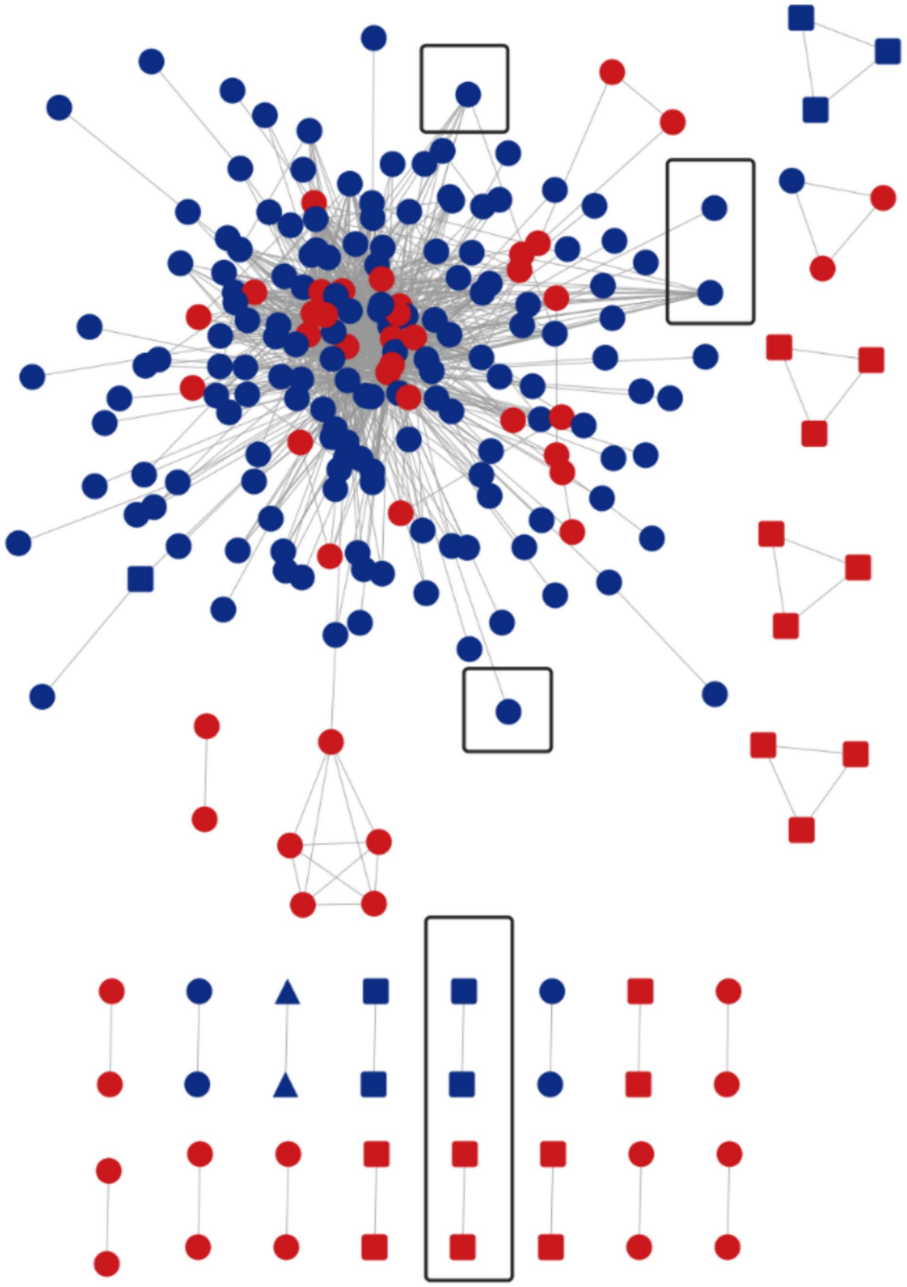

FIGURE 2
Molecular network at a gene distance threshold of 1.5%. Nodes and molecular clusters representing sequences at a gene distance threshold of 0.5% were highlighted in red. The presence of a circle indicated the genotype of HIV at that particular node was CRF07_BC. A rounded rectangle represented the genotype CRF01_AE, while a triangle denoted CRF55_01B. Additionally, black boxes were used to indicate transmitted drug-resistance clusters or nodes.

at a 0.50% threshold is associated with the large cluster of MSM CRF07_BC formed at a 1.50% threshold. This suggests that the CRF07_BC molecular cluster has been gradually expanding over time. To achieve the goal of reducing new infections, it is recommended to implement timely and focused interventions, conduct testing, and promote condom use within this molecular cluster. By identifying the key transmitters in this cluster, targeted strategies can be developed to effectively control and prevent the further spread of CRF07_BC and reduce the incidence of new infections.

In addition to CRF07_BC, it is important to highlight the significance of CRF01_AE and CRF55_01B. Initially, the transmission of CRF01_AE was primarily observed in the eastern coastal regions and southwestern border provinces of China, predominantly affecting heterosexual populations. However, recent findings from the Chinese Center for Disease Control and Prevention indicate that CRF01_AE has rapidly become the most prevalent HIV-1 viral subtype, spreading widely across geographic regions and high-risk populations, particularly among Chinese MSM (42–44).

Similarly, CRF55_01B initially emerged and spread rapidly within the MSM population in Guangdong, China. It has now disseminated to multiple provinces throughout the country (40, 45). The rapid expansion of CRF55_01B highlights the need for ongoing monitoring

TABLE 3 Analysis of factors influencing transmission within clusters of new reported 392 cases of HIV/AIDS in MSM in Chongqing, China.

Variables	Total (*n* = 392)	In cluster (n = 241; 61.4%)	COR (95%CI)	*p*-value	AOR (95%CI)	*P*-value
Age						
18–30	215	135 (62.8%)	1.000	0.066		
31–40	98	66 (67.3%)	1.222 (0.738–2.025)	0.436		
>40	79	40 (50.6%)	0.608 (0.361–1.023)	0.061		
Occupation						
workers and farmers	36	16 (44.4%)	1.000		1.000	
Unemployment	73	44 (60.3%)	1.897 (0.846–4.252)	0.120	1.132 (0.440–2.915)	0.797
Business Services	255	163 (63.9%)	2.215 (1.094–4.484)	0.027	1.765 (0.754–4.129)	0.190
Students	28	18 (64.3%)	2.250 (0.816–6.207)	0.117	2.651 (0.746–9.425)	0.132
Education level						
Primary and junior high school	58	25 (43.1%)	1.000		1.000	
High school	100	64 (64.0%)	2.347 (1.212–4.544)	0.011	3.535 (1.597–7.828)	0.002
College degree and above	234	152 (65.0%)	2.447 (1.363–4.392)	0.003	2.767 (1.389–5.512)	0.004
$CD4^+$ T cells (cells/μL)						
<200	150	94 (62.7%)	1.000			
200–350	134	84 (62.7%)	1.001 (0.618–1.620)	0.997		
>350	71	35 (49.3%)	0.579 (0.327–1.025)	0.061		
Miss	37	28 (75.7%)	1.853 (0.816–4.211)	0.141		
Marital status						
Unmarried	313	197 (62.9%)	1.000			
Married	50	26 (52.0%)	0.638 (0.350–1.163)	0.142		
Divorced/widowed	29	18 (62.1%)	0.964 (0.440–2.111)	0.926		
Sex roles						
"Top"	166	93 (56.0%)	1.000		1.000	
"Bottom"	91	55 (60.4%)	1.199 (0.713–2.017)	0.494	1.669 (0.881–3.160)	0.116
"Vers"	135	93 (68.9%)	1.738 (1.080–2.798)	0.023	2.409 (1.366–4.246)	0.002
Virus subtypes						
CRF07_BC	287	215 (74.9%)	1.000		1.000	
CRF01_AE	81	24 (29.6%)	0.14 (0.082–0.244)	<0.001	0.106 (0.059–0.192)	<0.001
CRF55_01B	12	2 (16.7%)	0.06 (0.014–0.313)	0.001	0.051 (0.010–0.248)	<0.001
Others	12	0 (0.0%)	–	0.998	–	0.998

of this molecular cluster and the implementation of effective interventions to control its transmission within the population.

The results of statistical analysis showed that the variables were CRF07_BC subtype and sex role as "vers" were statistically significant. Further analysis of the "vers" population revealed that this population was generally young and unmarried, and that the viral subtype CRF07_BC was present in both the new-onset and long-term infection networks. A total of 7 individuals with DRMs in the "vers" population, including 4 individuals in the molecular network, suggest that we should strengthen the monitoring of genotypic drug resistance in the "vers" population in the molecular network. The multi-factor analysis of education level shows that the enrollment rate of both high school and above education is high, the possible reason for this result may be because the source of this survey is concentrated in the young group in the main city of Chongqing, and the education level of the young group is generally high. The study showed that new MSM infections in this trial were characterized by young age, high proportion of unmarried people, and good education level. This is similar to the distribution of demographic information in the previous MSM HIV/AIDS transmission cluster analysis in Shanghai and Chongqing (42, 46). The risk factors for entry into the network suggest that we need to strengthen MSM investigation and intervention. Identifying additional risk factors and intervening in a timely manner can stop further expansion of the transmission cluster.

Presently, one of the formidable challenges in the HIV epidemic in China is the substantial proportion of diagnoses made at advanced stages of infection. As highlighted by previous research (47), the incidence of late-stage HIV diagnoses in Chongqing persistently

exceeds the national average, underscoring a critical public health issue that necessitates immediate intervention. In the context of the present study, a significant majority of the participants (72.5%, 284/392) exhibited a CD4+ T lymphocyte count of ≤350 cells/μL prior to initiating treatment. Given the complexities associated with accurately determining the timing of infection, this variable was excluded from the current analysis. Looking forward, our research will integrate HIV new infection testing methodologies and epidemiological assessments to more precisely estimate the time of infection, thereby enhancing our understanding of the relationship between CD4+ T lymphocyte count and the timing of HIV acquisition.

Conclusion

We show the distribution and features of HIV-1 subtypes, TDR, DRMs, and molecular transmission clusters in the MSM population of Chongqing, China, and investigate the risk factors that influence cluster formation. Although the overall incidence of TDRs in this study was low, it is nevertheless vital to underline the need of genotypic and molecular drug resistance surveillance, with a focus on critical drug resistance mutations. Following that, we will aim to support the creation and implementation of an HIV-1 molecular dynamic surveillance network in Chongqing.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: https://nmdc.cn/resource/genomics/project/detail/NMDC10018715.

Ethics statement

The studies involving humans were approved by the institutional review board of Chongqing Center for disease control and prevention. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study.

Author contributions

CB: Writing – original draft, Writing – review & editing. TT: Writing – original draft, Writing – review & editing. LL: Writing – original draft, Writing – review & editing. RL: Writing – original draft, Writing – review & editing. WZ: Writing – original draft, Writing – review & editing. LO: Writing – original draft, Writing – review & editing. GW: Writing – original draft, Writing – review & editing. CZ: Writing – original draft, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1308784/full#supplementary-material

References

1. Hao J, Zheng S, Gan M, Dong A, Kang R, Li M, et al. Changing proportions of HIV-1 subtypes and transmitted drug resistance among newly diagnosed HIV/AIDS individuals - China, 2015 and 2018. *China CDC Weekly*. (2021) 3:1133–8. doi: 10.46234/ccdcw2021.251

2. Zheng S, Wu J, Hao J, Wang D, Hu Z, Liu L, et al. Epidemic characteristics of HIV drug resistance in Hefei, Anhui Province. *Pathogens*. (2022) 11:866. doi: 10.3390/pathogens11080866

3. Wu J, Shen Y, Zhong P, Feng Y, Xing H, Jin L, et al. The predominant cluster of CRF01_AE circulating among newly diagnosed HIV-1-positive people in Anhui Province, China. *AIDS Res Hum Retroviruses*. (2015) 31:926–31. doi: 10.1089/aid.2015.0107

4. Feng Y, Wu Z, Detels R, Qin G, Liu L, Wang X, et al. HIV/STD prevalence among MSM in Chengdu, China and associated risk factors for HIV infection. *J Acquir Immune Defic Syndr*. (2010) 53:S74–80. doi: 10.1097/QAI.0b013e3181c7dd16

5. Fanales-Belasio E, Raimondo M, Suligoi B, Buttò S. HIV virology and pathogenetic mechanisms of infection: a brief overview. *Ann Ist Super Sanita*. (2010) 46:5–14. doi: 10.4415/ANN_10_01_02

6. Li X, Xue Y, Lin Y, Gai J, Zhang L, Cheng H, et al. Evolutionary dynamics and complicated genetic transmission network patterns of HIV-1 CRF01_AE among MSM in Shanghai, China. *Sci Rep*. (2016) 6:34729. doi: 10.1038/srep34729

7. Pang X, Wei H, Huang J, He Q, Tang K, Fang N, et al. Patterns and risk of HIV-1 transmission network among men who have sex with men in Guangxi. *China Sci Rep*. (2021) 11:513. doi: 10.1038/s41598-020-79951-2

8. Chang W, Xing A, Wang B, Li X, Jia H, Zhang L, et al. Analysis of HIV sentinel surveillance among high-risk populations in Shaanxi Province in 2010. *Occup Health*. (2012) 28:399–402. doi: 10.13329/j.cnki.zyyjk.2012.04.050

9. Feng L, Yang Y, Yuan D, Huang J, Pei X. Monitoring results of HIV infection and risky behaviors among MSM population in Sichuan Province in 2011. *Chin J AIDS STD*. (2012) 18:830–2. doi: 10.13419/j.cnki.aids.2012.12.014

10. Dong M-J, Peng B, Liu ZF, Ye QN, Liu H, Lu XL, et al. The prevalence of HIV among MSM in China: a large-scale systematic analysis. *BMC Infect Dis*. (2019) 19:1000. doi: 10.1186/s12879-019-4559-1

11. Wu G, Zhou Y, Zheng J, Zhou Q. Sex behaviors and HIV infection status among men who have sex with men in Chongqing, 2010-2013. *Dis Surveill*. (2014) 29:124–9. doi: 10.3784/j.issn.1003-9961.2014.02.011

12. Hightower GK, May SJ, Pérez-Santiago J, Pacold ME, Wagner GA, Little SJ, et al. HIV-1 clade B pol evolution following primary infection. *PLoS One*. (2013) 8:e68188. doi: 10.1371/journal.pone.0068188

13. Liu L. *Investigation on the sources of HIV transmission, prevalence of drug-resistant strains, and molecular transmission networks in Liangshan, Sichuan*. (2020). Beijing: China CDC Press, 15–6.

14. Zhao S, Feng Y, Xin R, Li Z, Hao M, Liao L, et al. Application of molecular transmission network in studying the transmission characteristics of HIV-1 strains among MSM in Beijing. *Chin J AIDS STD*. (2018) 24:241–245+306. doi: 10.13419/j.cnki.aids.2018.03.08

15. Liao L, Xing H, Shang H, Li J, Zhong P, Kang L, et al. The prevalence of transmitted antiretroviral drug resistance in treatment-naive HIV-infected individuals in China. *J Acquir Immune Defic Syndr*. (2010) 53:S10–4. doi: 10.1097/QAI.0b013e3181c7d363

16. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol Biol Evol*. (2018) 35:1547–9. doi: 10.1093/molbev/msy096

17. Liu K, Linder CR, Warnow T. RAxML and FastTree: comparing two methods for large-scale maximum likelihood phylogeny estimation. *PLoS One*. (2011) 6:e27731. doi: 10.1371/journal.pone.0027731

18. Anisimova M, Gascuel O. Approximate likelihood-ratio test for branches: a fast, accurate, and powerful alternative. *Syst Biol*. (2006) 55:539–52. doi: 10.1080/10635150600755453

19. Patiño-Galindo JÁ, Torres-Puente M, Bracho MA, Alastrué I, Juan A, Navarro D, et al. The molecular epidemiology of HIV-1 in the Comunidad Valenciana (Spain): analysis of transmission clusters. *Sci Rep*. (2017) 7:11584. doi: 10.1038/s41598-017-10286-1

20. Siepel AC, Halpern AL, Macken C, Korber BT. A computer program designed to screen rapidly for HIV type 1 intersubtype recombinant sequences. *AIDS Res Hum Retrovir*. (1995) 11:1413–6. doi: 10.1089/aid.1995.11.1413

21. Shafer RW, Rhee SY, Pillay D, Miller V, Sandstrom P, Schapiro JM, et al. HIV-1 protease and reverse transcriptase mutations for drug resistance surveillance. *AIDS*. (2007) 21:215–23. doi: 10.1097/QAD.0b013e328011e691

22. Liu TF, Shafer RW. Web resources for HIV type 1 genotypic-resistance test interpretation. *Clin Infect Dis*. (2006) 42:1608–18. doi: 10.1086/503914

23. Yuan D, Liu M, Li Y, Jia P, Su L, Ye L, et al. Genetic transmission networks of HIV-1 CRF07_BC strain among HIV-1 infections with virologic failure of ART in a minority area of China: a population-based study. *BMC Infect Dis*. (2020) 20:631. doi: 10.1186/s12879-020-05347-2

24. Shao Y, Han M, Zhong P, Lü F, Li J, Xing H, et al. *Technical Guidelines for Monitoring and Intervening in HIV Transmission Networks in China*. Beijing: China CDC Press, (2021) 15–6.

25. Liu Y. J. *Research on Genetic variation and molecular evolution of HIV-1 epidemic strains and early infection viruses in Yunnan Province*. Beijing: People's Liberation Army Academy of Military Medical Sciences Press, (2011) 15–6.

26. Li Y, Hui S, Zhang J, Liu F, Li T, Zhang X, et al. Molecular clustering and drug resistance of newly identified HIV-1 strains in Heilongjiang Province in 2018. *Chin J AIDS STD*. (2021) 27:680–4. doi: 10.13419/j.cnki.aids.2021.07.02

27. Duani H, Aleixo AW, Tupinambás U. Trends and predictors of HIV-1 acquired drug resistance in Minas Gerais, Brazil: 2002–2012. *Braz J Infect Dis*. (2016) 21:148–54. doi: 10.1016/j.bjid.2016.11.009

28. Paton NI, Kityo C, Hoppe A, Reid A, Kambugu A, Lugemwa A, et al. Assessment of second-line antiretroviral regimens for HIV therapy in Africa. *N Engl J Med*. (2014) 371:234–47. doi: 10.1056/NEJMoa1311274

29. La Rosa AM, Harrison LJ, Taiwo B, Wallis CL, Zheng L, Kim P, et al. Raltegravir in second-line antiretroviral therapy in resource-limited settings (SELECT): a randomised, phase 3, non-inferiority study. *Lancet HIV*. (2016) 3:e247–58. doi: 10.1016/S2352-3018(16)30011-X

30. Zhou C, Liang S, Li Y, Zhang Y, Li L, Ye L, et al. Characterization of HIV-1 molecular epidemiology and transmitted drug-resistance in newly diagnosed HIV-infected patients in Sichuan, China. *BMC Infect Dis*. (2022) 22:602. doi: 10.1186/s12879-022-07576-z

31. TenoRes Study Group. Global epidemiology of drug resistance after failure of WHO recommended first-line regimens for adult HIV-1 infection: a multicentre retrospective cohort study. *Lancet Infect Dis*. (2016) 16:565–75. doi: 10.1016/S1473-3099(15)00536-8E

32. De Clercq E. The role of non-nucleoside reverse transcriptase inhibitors (NNRTIs) in the therapy of HIV-1 infection1Presented at the eleventh international conference on antiviral research, San Diego, CA, 5–10 April 1998.1. *Antivir Res*. (1998) 38:153–79. doi: 10.1016/S0166-3542(98)00025-4

33. Liu J, Wu Y, Yang W, Xue X, Sun G, Liu C, et al. Population-based human immunodeficiency virus 1 drug resistance profiles among individuals who experienced virological failure to first-line antiretroviral therapy in Henan, China during 2010–2011. *AIDS Res Ther*. (2015) 12:22. doi: 10.1186/s12981-015-0062-y

34. Long JE, Tordoff DM, Reisner SL, Dasgupta S, Mayer KH, Mullins JI, et al. HIV transmission patterns among transgender women, their cisgender male partners, and cisgender MSM in Lima, Peru: a molecular epidemiologic and phylodynamic analysis. *Lancet Reg Health Am*. (2022) 6:100121. doi: 10.1016/j.lana.2021.100121

35. Pines HA, Wertheim JO, Liu L, Garfein RS, Little SJ, Karris MY. Concurrency and HIV transmission network characteristics among MSM with recent HIV infection. *AIDS*. (2016) 30:2875–83. doi: 10.1097/QAD.0000000000001256

36. Nduva GM, Otieno F, Kimani J, Wahome E, McKinnon LR, Cholette F, et al. Quantifying rates of HIV-1 flow between risk groups and geographic locations in Kenya: a country-wide phylogenetic study. *Virus Evol*. (2022) 8:veac016. doi: 10.1093/ve/veac016

37. Zhang Y, Dai J, Li Z, Ma Y, Chen H, Dong L, et al. Using molecular network analysis to explore the characteristics of HIV-1 transmission in a China-Myanmar border area. *PLoS One*. (2022) 17:e0268143. doi: 10.1371/journal.pone.0268143

38. Gan M, Zheng S, Hao J, Ruan Y, Liao L, Shao Y, et al. Spatiotemporal patterns of CRF07_BC in China: a population-based study of the HIV strain with the highest infection rates. *Front Immunol*. (2022) 13:824178. doi: 10.3389/fimmu.2022.824178

39. Li K, Ou W, Feng Y, Sun J, Ge Z, Xing H, et al. Near full-length genomic characterization of a novel HIV type 1 recombinant form (CRF01_AE/B) identified from Anhui, China. *AIDS Res Hum Retroviruses*. 34:1100–5. doi: 10.1089/AID.2018.0144

40. Wu Y, Ren X, Yin D, Wang H, Wan Z, Li X, et al. Characterization of a novel HIV-1 unique recombinant form between CRF07_BC and CRF55_01B in men who have sex with men in Guangzhou, China. *PLoS One*. (2017) 12:e0175770. doi: 10.1371/journal.pone.0175770

41. Zhao S, Feng Y, Hu J, Li Y, Zuo Z, Yan J, et al. Prevalence of transmitted HIV drug resistance in antiretroviral treatment naïve newly diagnosed individuals in China. *Sci Rep*. (2018) 8:12273. doi: 10.1038/s41598-018-29202-2

42. Lin Y, Xue Y, Wang X, Zhou L, Wang Y, Shen F, et al. Cluster analysis of CRF01_AE strain transmission among MSM in Shanghai. *Chin J AIDS STD*. (2016) 22:165–9. doi: 10.13419/j.cnki.aids.2016.03.07

43. Feng Y, He X, Hsi JH, Li F, Li X, Wang Q, et al. The rapidly expanding CRF01_AE epidemic in China is driven by multiple lineages of HIV-1 viruses introduced in the 1990s. *AIDS*. (2013) 27:1793–802. doi: 10.1097/QAD.0b013e328360db2d

44. Li Z, Liao L, Feng Y, Zhang J, Yan J, He C, et al. Trends of HIV subtypes and phylogenetic dynamics among young men who have sex with men in China, 2009–2014. *Sci Rep*. (2015) 5:16708. doi: 10.1038/srep16708

45. Han X, Takebe Y, Zhang W, An M, Zhao B, Hu Q, et al. A large-scale survey of CRF55_01B from men-who-have-sex-with-men in China: implying the evolutionary history and public health impact. *Sci Rep*. (2015) 5:18147. doi: 10.1038/srep18147

46. Ouyang L, Wu G, Zhou Y, Zhou C, Chen Z. Qualitative interviews on factors of HIV transmission among MSM in Chongqing. *Pract Prevent Med*. (2016) 23:1348–51. doi: 10.3969/j.issn.1006-3110.2016.11.019

47. Xu CH. Analysis of sample sources and late-stage diagnosis of newly reported HIV/AIDS patients in Chongqing from 2012 to 2017. *Chin J AIDS STD*. Beijing: China CDC Press, (2019) 35–38.

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Recurrent Nipah outbreaks in Kerala: implications for health policy and preparedness

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KEYWORDS

Nipah infection, NiV, outbreak, Kerala, recurrence, policy implications

Summary

Nipah virus (NiV) infection, a highly pathogenic emerging zoonotic disease associated with significant mortality rates, is prevalent in South East Asian countries. This infection typically manifests in small clusters, predominantly presenting as either encephalitis or acute respiratory distress. In India, NiV has been documented in the states of West Bengal and Kerala, with four of the six reported outbreaks occurring in Kerala. This study focuses on the epidemiology of NiV infection in Kerala, offering insights and implications for future policies.

Epidemiologically, three of the four outbreaks in Kerala occurred in a specific geographic belt, suggesting a consistent factor in the spillover of infection from reservoir bats. The average age of affected individuals was 41 years, with a male predominance. The mean incubation period was determined to be 9 days, and transmission primarily occurred within healthcare settings due to lapses in infection prevention and control practices. Intensive response strategies including case isolation, contact tracing, and surveillance were consistently employed during all outbreaks. Challenges related to diagnosis and treatment were addressed through the development and regular updates of state guidelines for outbreak response.

The article emphasizes the need for fortifying the state's health system to enhance preparedness for future outbreaks. This involves proactive measures such as improving infection control practices, expediting diagnostic processes, and exploring advanced treatment options. Strengthening the surveillance system, particularly for monitoring acute encephalitis syndrome (AES) and acute respiratory distress syndrome (ARDS) is crucial for the early detection and containment of potential outbreaks and thereby mitigate the impact of future outbreaks in the region.

Background

India witnessed its sixth outbreak of Nipah virus infection in September 2023, in the southern state of Kerala when the Nipah virus (NiV) was isolated from two patients with epidemiological link to a probable case of NiV infection. Encephalitis caused by NiV is an emerging infectious disease of public health importance reported from South-East Asian countries. Both animal-to-human and human-to-human transmission have been

documented (1). Between 1998 and 2015, over 630 cases of NiV infections were reported in Malaysia, Singapore, Bangladesh and India (2).

While the Malaysian outbreak reported 276 cases with a 38% fatality rate, subsequent outbreaks in India and Bangladesh witnessed significantly higher case fatality rates ranging from 43% to 100% (3). Fruit bats of the genus Pteropus serve as the natural reservoir for the virus and the virus has been isolated from bat urine and partially eaten fruits in Malaysia (4). Human infection typically begin with fever and brain inflammation leading to disorientation or coma (5). Some patients also present with acute respiratory distress syndrome (ARDS). Laboratory confirmation is done by Serum Neutralization antibody detection, Enzyme linked immune sorbent assay (ELISA), or Real time reverse transcriptase polymerase chain reaction (RT-PCR) tests. NiV needs to be handled in a Bio-safety level 4 containment facility and most countries in South East Asia lack diagnostic facilities.

Out of the six outbreaks in India since 2001, four have occurred in Kerala, with three in the Northern district of Kozhikode. The authors being part of the Nipah outbreak response team have been actively involved in the containment of all the three outbreaks in the region. This recurring phenomenon in the district of Kozhikode over the last 5 years raises questions about transmission dynamics.

The authors conducted an analysis of epidemiology, clinical presentations and health system responses of all four outbreaks in Kerala to identify gaps in our current knowledge of Nipah infection epidemiology and its potential policy implications.

The first outbreak-2018

The initial outbreak caught the health system off guard, with limited experience and knowledge to fight the virus. The outbreak was identified due to clustering of cases within a household and later in three health care institutions. Of the 18 cases confirmed by RT-PCR, 16 succumbed (case fatality rate - 88.8%). In addition there were four probable cases (not lab confirmed) identified retrospectively though audits of deaths due to AES & ARDS with symptoms suggestive of NiV infection who expired prior to confirmation of the outbreak. The mean incubation period was 9 days and mean age of the affected was 41 years with male preponderance.

The Primary case, identified retrospectively in a tertiary hospital served as a point source for 15 other cases including two health care workers. The transmission occurred person to person, mainly in health care settings. More than 2,600 contacts were under surveillance for a period of 21 days (maximum incubation period) including 239 contacts from Malappuram district (6). Department of Community medicine at the Government Medical colleges in both districts took the lead in contact tracing and surveillance in collaboration with the District health system. The basic reproduction number (R_0) from May 20th for the ensuing 4 week period was calculated as 0.4, which indicated the epidemic to be dying out. The outbreak was contained and declared over on 10th June 2018 (7).

The source of infection for the primary case could not be identified and was assumed to be contact with fruit bats from the forest or consumption of fruits contaminated with bat secretions. No other animal reservoir could be identified as an intermediate host for spill over infection to humans. A coordinated rapid outbreak response by the health system led to the containment of the outbreak within 3 weeks and was declared closed by July the same year. The authors evaluated district and state coordinated actions using the Management science for health frame work tool (MSH framework) (7).

The second outbreak—2019

In June 2019 Nipah revisited Kerala, this time in Ernakulam district. A 21 year old male student presented with fever and signs of encephalitis. NiV infection was confirmed by RT-PCR of throat swab, urine and serum samples at National Institute of Virology (NIV) Pune (8). Equipped with the 2018 experience and with the management and control guidelines against NiV infection in place, this time no further spread occurred and the patient recovered. The source of infection in this instance also could not be confirmed conclusively and was presumed to be due to consumption of fruits contaminated with bat secretions. Robust contact tracing involving 330 contacts by the district surveillance team categorization of risk using an algorithm developed in the state into high and low risk contributed to effective containment. High risk individuals included those with direct contact with body fluids of confirmed or probable cases or sharing a closed space for more than 12 h with a confirmed case (9). An onsite field laboratory was also set up for conducting Point of care test, RTPCR and ELISA.

The third outbreak in the midst of the COVID-19 pandemic

Nipah hit Kozhikode again in September 2021 while the state was experiencing a second wave of the COVID-19 pandemic (10). A 12 year old boy presented with fever progressing to encephalitis within a period of 5 days. He had sought treatment from three health care facilities prior to confirmation of diagnosis and succumbing to the disease. Contact tracing identified 240 contacts but no further cases were identified. Keeping in view the ongoing COVID-19 pandemic all contacts were also screened for SARS-CoV-2 (12 were positive).

Source of infection in this case was also presumed to be consumption of contaminated fruits from the orchard of exotic fruits owned by the family. Bat studies conducted in the area showed presence of NiV antibodies but viral RNA could not be detected in the bats. We speculate that stringent COVID-19 infection control measures, universal masking at health care facilities and among the public, played a crucial role in limiting the outbreak to a single case.

The fourth outbreak—2023

The most recent outbreak in Kozhikode district in September 2023 reported six confirmed cases and two fatalities. Index case was a 9 year old child who presented with fever rapidly progressing to encephalitis in 5 days. Similar to the first outbreak, the primary case

(father of the child) was identified retrospectively and succumbed in a private health care facility transmitting infection to five others, including one health care worker. Fever was the predominant symptom and contrary to prior outbreaks, respiratory distress was the common presentation (with the exception of a single case of encephalitis). Male gender and mean incubation period of 9 days were consistent with the initial outbreak.

Epidemiological analysis

All four events were considered as outbreaks even though only one case was involved in 2019 and 2021 as NiV is a rare pathogen and moreover the disease was new to the community (11, 12). From an epidemiologists perspective male preponderance in infections potentially linked to engagement in outdoor activities as revealed through in-depth field based case investigations. All the four outbreaks pooled together, lowest age was 9 years with majority between the age of 30–45 years (6, 8, 10). All three spill overs in Kozhikode district occurred in the same geographic belt, suggesting a common link possibly environmental, behavioral or a combination of both. The initial spill overs occurred during May-June coinciding with the breeding season for the Pteropus bats, contrast with later outbreaks in September, raising the possibility of spill over incidents all year through (13). This is in contrast to outbreaks in South East Asia which have occurred during the classic NiV transmission season (December–May) (3).

The affected geographic areas in Kozhikode share common features, including the presence of plantation areas with arecanut and fruit trees, including exotic fruits, adjacent to a natural forest cover of 300 acres which is home to several bat species. Initial epidemiological investigations conducted during outbreaks in these areas revealed presence of large number of fruit bats, with half eaten fruits abundantly found at the outbreak sites. Recent observations during field visits and environmental surveys conducted by the team in the present outbreak point to the primary case's habit of plucking fruits in the peri-domestic area and from his plantations. Multiple fruit trees surround the area, and the primary cases house was ∼4–5 km from the forest belt of Janakikkadu.

Mapping of the bat population and testing for presence of NiV among the bats in all outbreaks by National Institute of Virology (NIV) showed that 19% of the P medius were positive for NiV and Anti NiV antibody in 2018 (14) while 21% of P medius and 37.7% of Rousettus leschenautica exhibited anti NiV Antibody in 2019 outbreak (10). Anti NiV antibodies were demonstrated in bats from the affected area during 2021, confirming bats as the reservoir (10). However the same could not be demonstrated in 2023 from the samples of bats, animal droppings, and half-eaten fruits collected from the village where the initial cases resided (15). NiV human sequences from Kozhikode outbreak and NiV sequences from bat study have shown that the NiV strain circulating in south India are distinct from the Bangladesh strain and a separate "Indian (I) strain has been hypothesized for South India (15).

Considering transmission dynamics, although respiratory secretions and body fluids have been implicated in transmission, gaps in the high risk behaviors predisposing to infection remain unclear. Person to person transmission was established in the 2018 and 2023 outbreak (6) occurring mainly in health care settings indicating that spread occurred in the late symptomatic stage of the disease (6). Most of the secondary cases reported close contact with the primary case. Risk factors for transmission included feeding, contact with body fluids, close contact with a Nipah patient during caregiving and sharing room/space in the hospital (10).

Fever appears to be the predominant symptom in all cases, while encephalitic symptoms like disorientation and seizures were observed more in the 2018 outbreak, the symptoms of respiratory distress were more predominant in the 2023 outbreak. The multiplicity of symptoms pose challenges in early detection by the surveillance system which has to rely on clustering of cases or unexplained deaths as indicators of possible outbreaks. In the 2023 outbreak the survivors were treated with antivirals Remdesvir and Favipiravir, and one case required intensive ventilation. The early initiation of treatment and aggressive supportive measures may account for the low case fatality in the current outbreak. Ribavarin given to a subset of patients in 2018 showed a decrease in encephalitis caused by NiV though the results were not statistically significant (16).

Challenges and policy implications

Exploring the reasons behind the recurring spill over events of NiV infection leading to outbreaks in Kerala, especially in Kozhikode should be prioritized. Unlike Bangladesh where consumption of raw date palm sap is implicated in transmission (17), the source of infection for the primary case and the mechanism of spill over from the reservoir bats remains unestablished in all the outbreaks in the state. Ingestion of fruits coming in contact with saliva or inhalation of tiny droplets produced from infected urine or saliva of bats roosting among braches of trees can be an important mode of transmission of NiV infection to humans (18). Demonstration of the agent in fruit samples remains elusive hinting at viability of the virus outside the reservoir species. This missing link needs to be explored aggressively to complete the natural history of NiV infection.

Outbreak response: Outbreak response in all the instances have been intensive and prompt. Isolation of cases, triaging, contact tracing, risk stratification and surveillance as the basis of containment strategies. Identifying a suitable isolation area and implementing standard operating protocols for isolation and triaging was challenging in earlier outbreaks. Coordination between various sectors viz animal husbandry, health services, medical college, private sector, law enforcement, agriculture and central agencies (NIV, ICMR, NCDC, NIE) was crucial for successful containment. Tracing of community contacts was always a challenge considering the multiple health care institutions visited by the cases and participation in social events (funerals, prayers etc.). The number of health care workers stratified as high risk contacts was of concern indicating lapses in adherence to infection control practices even in the recent outbreak. Hospitals should enforce policies mandating face masks for Health care workers and care-givers, especially in the Emergency and Intensive care settings. In the recent outbreak a Cluster Containment Strategy was undertaken by the district administration by declaring seven village panchayats as containment zones adapting the containment plan guideline for SARS-CoV-2 (19).

Confirmation of diagnosis: Confirmation of diagnosis posed a challenge in the initial outbreak, requiring sample transportation to NIV Pune. This challenge was addressed to a great extent in 2019 with the introduction of point of care (POC) micro PCR assay at designated tertiary care institutions. POC test proved to be a game changer in the outbreak response activities in the subsequent outbreaks as delay in diagnosis could be mitigated to a great extent but confirmation still necessitated a Biosafety Level 3 (BSL3) facility. Establishment of BSL 3 lab in the region is crucial for future outbreak preparedness.

Treatment protocols: There are no drugs or vaccines specific for Nipah virus infection recommended by the WHO or CDC even though WHO has identified NiV infection as a priority disease for research and development (20, 21). The absence of a protocol for management of the outbreak was addressed as early as 2018 with guidelines for contact tracing, surveillance and treatment guidelines framed by the Government of Kerala with contributions from experts from National agencies, and revised in subsequent outbreaks (22). The guidelines should be reviewed based on newer evidences. The early initiation of Remdesvir in the in the latest outbreak showed promising results (23). Its efficacy as a drug for post exposure prophylaxis needs to be explored further (22). Monoclonal antibody (m 102.4) treatment was considered in the first outbreak and Standard operating procedures for administration were developed by the Indian Council of Medical research (ICMR). Considering the fact that it was still an experimental treatment, medical board of the institution along with central agencies (ICMR) decided to make it available for the patients on compassionate grounds. But m 102.4 was not used as in all instances the outbreak was contained quickly and the opportunity for its use did not arise. Research addressing the development of monoclonal antibodies for future use is the need of the hour and the Government of Kerala has taken the initiative of collaborating with central agencies for development of an indigenous monoclonal antibody which may be of use in future outbreaks.

Contact tracing and surveillance in all four outbreaks was resource intensive (more than 2600 contacts in 2018 and 1260 contacts were traced in the 2023 outbreak). Risk stratification as high risk and low risk contacts were based on treatment and surveillance guidelines of Kerala state (20). Risk stratification needs to be updated on the basis of evidence on exposure risks calculated from all four outbreaks. Aggressive contact tracing with improper risk stratification over burdens the health system causing unnecessary panic in the community. Restrictions imposed such as containment zones can lead to adverse economic impact and the suitability of this approach in curtailing Nipah outbreaks needs to be reviewed.

Conclusion and recommendation

Preparedness is a key to containment of any outbreak of infectious origin. Early detection is crucial in containing the spread of the virus and mitigating fatality rates. An Event based surveillance mechanism to detect signals or clusters from hospitals/community is necessary in light of recurring outbreaks. A public private partnership for surveillance can aid early case detection and response. Strengthening of Surveillance of AES and ARDS in the state and subjecting unclassified AES & ARDS to POC test will augment early detection. Reinforcing infection prevention and control practices among health care workers will have broader implications for curtailing other infectious pathogens as well.

Advocating a one health approach with inter sectoral coordination is crucial to address gaps in the natural history of the disease. Exploring alternate routes of spill over and continuing surveillance of NiV among bat population will aid in predicting the risk of potential outbreaks.

Trials with specific antivirals and monoclonal antibodies, research on innovative vaccines and immunological are imperative for evidence based treatment and reducing mortality rates. The political will exhibited by the government in addressing previous outbreaks is commendable and evidence backed policy changes will go a long way in preparing for future challenges.

Author contributions

AR: Conceptualization, Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing. PC: Conceptualization, Formal analysis, Investigation, Methodology, Resources, Supervision, Writing – original draft, Writing – review & editing. VB: Data curation, Investigation, Project administration, Resources, Writing – review & editing. CR: Conceptualization, Resources, Supervision, Validation, Writing – review & editing. AM: Data curation, Investigation, Project administration, Resources, Supervision, Writing – review & editing. LR: Investigation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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References

1. Chadha MS, Comer JA, Lowe L, Rota PA, Rollin PE, Bellini WJ, et al. Nipah virus-associated encephalitis outbreak, Siliguri, India. *Emerg Infect Dis.* (2006) 12:235–40. doi: 10.3201/eid1202.051247

2. Chattu VK, Kumar R, Kumary S, Kajal F, David JK. Nipah virus epidemic in southern India and emphasizing "One Health" approach to ensure global health security. *J Family Med Primary Care.* (2018) 7:275–83. doi: 10.4103/jfmpc.jfmpc_137_18

3. WHO. *Nipah Virus Outbreaks in the WHO South. East Asia Region. Surveillance and Outbreak Alert*. (2024). Available online at: http://www.searo.who.int/entity/emerging_diseases/links/nipah_virus_outbreaks_sear/en (accessed March 25, 2024).

4. Chua KB, Koh CL, Hooi PS, Wee KF, Khong JH, Chua BH, et al. Isolation of Nipah virus from Malaysian Island flying-foxes. *Microbes and infection.* (2002) 4:145–51. doi: 10.1016/S1286-4579(01)01522-2

5. WHO. *Nipah Virus Infection*. (2024). Available online at: https://www.who.int/health-topics/nipah-virus-infection#tab= (accessed April 15, 2024).

6. Thomas B, Chandran P, Lilabi MP, George B, Sivakumar CP, Jayadev VK, et al. Nipah virus infection in Kozhikode, Kerala, South India, in 2018: epidemiology of an outbreak of an emerging disease. *Indian J Commun Med.* (2019) 44:383–7. doi: 10.4103/ijcm.IJCM_198_19

7. Rahim A, Muthukutty S, Jacob S, Ravindran R, Thayyil J, Thavody J. Evaluation of the Nipah epidemic containment and multisectoral involvement in Kerala using an appropriate management framework. *Int J Commun Med Pub Health.* (2020) 7:2813–9. doi: 10.18203/2394-6040.ijcmph20203019

8. Sudeep AB, Yadav PD, Gokhale MD, Balasubramanian R, Gupta N, Shete A, et al. Detection of Nipah virus in Pteropus medius in 2019 outbreak from Ernakulam district, Kerala, India. *BMC Infect Dis.* (2021) 21:1–7. doi: 10.1186/s12879-021-05865-7

9. Nipah Virus Infection. *Guidelines for Surveillance, Diagnosis, Treatment and Control. Department of Family Welfare, Government of Kerala* (2021). Available online at: https://dhs.kerala.gov.in/wp-content/uploads/2021/09/Nipah-Guidelines-9-04-21-2-1.pdf (accessed April 9, 2024).

10. Yadav PD, Sahay RR, Balakrishnan A, Mohandas S, Radhakrishnan C, Gokhale MD, et al. Nipah virus outbreak in Kerala state, India amidst of COVID-19 pandemic. *Front Public Health.* (2022) 10:818545. doi: 10.3389/fpubh.2022.818545

11. Pandemics: Definition, Prevention and Preparation. (2024). Available online at: https://www.webmed.com,cold-and-flu/what-are-epidemics-pandemics-outbreaks (accessed April 24, 2024).

12. *West Virginia Department of Human health Outbreak Investigation Surveillance and Investigation Protocol*. (2018). Available online at: https://oeps.wv.gov/outbreaks/Documents/lhd/community-outbreak-protocol.pdf (accessed January 2024).

13. Epstein JH, Anthony SJ, Islam A, Kilpatrick AM, Ali Khan S, Balkey MD, et al. Nipah virus dynamics in bats and implications for spillover to humans. *Proc Nat Acad Sci.* (2020) 117:29190–201. doi: 10.1073/pnas.2000429117

14. Mourya D, Yadav P, Sudeep A, Gokhale M, Gangakhedkar P, Bhargava B. *Pteropus Bats Positivity for Nipah Virus from Kozhikode, Kerala, India: Possible Link of Infection to Human*. Atlanta, GA: International Conference on Emerging Infectious Diseases (2018).

15. Nipah Virus Infection in India – Disease Outbreak News. World Health Organization. (2023). Available online at: https://www.who.int/emergencies/disease-outbreak-news/item/2023-DON490 (accessed October 9, 2023).

16. Chandni R, Renjith TP, Fazal A, Yoosef N, Ashhar C, Thulaseedharan NK. Clinical manifestations of Nipah virus-infected patients who presented to the emergency department during an outbreak in Kerala state in India, May 2018. *Clin Infect Dis.* (2020) 71:152–7. doi: 10.1093/cid/ciz789

17. Rahman MA, Hossain MJ, Sultana S, Homaira N, Khan SU, et al. Date palm sap linked to Nipah virus outbreak in Bangladesh, 2008. *Vector-Borne Zoonotic Dis.* (2012) 12:65–72. doi: 10.1089/vbz.2011.0656

18. Yadav PD, Shete AM, Kumar GA, Sarkale P, Sahay RR, Radhakrishnan C. Nipah Virus Sequences from Humans and Bats during Nipah Outbreak, Kerala, India, 2018. *Emerg Infect Dis.* (2019) 25:1003–6. doi: 10.3201/eid2505.181076

19. *Containment Plan for Large Outbreaks Novel Coronavirus Disease 2019 (COVID-19). Ministry of Health and Family Welfare. Government of India*. (2020). Available online at: https://www.mohfw.gov.in/pdf/3ContainmentPlanforLargeOutbreaksofCOVID19Final.pdf (accessed December 2023).

20. "Nipah Virus." (2024). Available online at: www.who.int, http://www.who.int/news-room/fact-sheets/detail/nipah-virus (accessed April 17, 2024).

21. Nipah Virus (NiV). *Centre for Diseases Control and Prevention.* (2024). Available online at: https://www.cdc.gov/vhf/nipah/treatment/index.html (accessed April 24, 2024).

22. Nipah Virus Infection – Treatment Guidelines (2023). *GO-Rt-No-2363-2023-HFWD-dated-16-09-2023-with-annexure-Nipah treatment guidelines 2023 September*. Available online at: https://dhs.kerala.gov.in (accessed June 3, 2024).

23. As AK, Sahay RR, Radhakrishnan CPS, Kandath S, Patil DY, Abraham M. Clinico-epidemiological presentations and management of Nipah virus infection during the outbreak in Kozhikode district, Kerala state, India 2023. *J Med Virol.* (2024) 96:e29559. doi: 10.1002/jmv.29559

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Omicron variant and pulmonary involvements: a chest imaging analysis in asymptomatic and mild COVID-19

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Objectives: To identify clinical characteristics and risk factors for pulmonary involvements in asymptomatic and mildly symptomatic patients infected with SARS-CoV-2 Omicron variant by chest imaging analysis.

Methods: Detailed data and chest computed tomography (CT) imaging features were retrospectively analyzed from asymptomatic and mildly symptomatic patients infected with Omicron between 24 April and 10 May 2022. We scored chest CT imaging features and categorized the patients into obvious pulmonary involvements (OPI) (score > 2) and not obvious pulmonary involvements (NOPI) (score ≤ 2) groups based on the median score. The risk factors for OPI were identified with analysis results visualized by nomogram.

Results: In total, 339 patients were included (145 were male and 194 were female), and the most frequent clinical symptoms were cough (75.5%); chest CT imaging features were mostly linear opacities (42.8%). Pulmonary involvements were more likely to be found in the left lower lung lobe, with a significant difference in the lung total severity score of the individual lung lobes ($p < 0.001$). Logistic regression analysis revealed age stratification [odds ratio (OR) = 1.92, 95% confidence interval (CI) (1.548–2.383); $p < 0.001$], prolonged nucleic acid negative conversion time (NCT) (NCT > 8d) [OR = 1.842, 95% CI (1.104–3.073); $p = 0.019$], and pulmonary diseases [OR = 4.698, 95% CI (1.159–19.048); $p = 0.03$] as independent OPI risk factors.

Conclusion: Asymptomatic and mildly symptomatic patients infected with Omicron had pulmonary involvements which were not uncommon. Potential risk factors for age stratification, prolonged NCT, and pulmonary diseases can help clinicians to identify OPI in asymptomatic and mildly symptomatic patients infected with Omicron.

KEYWORDS

chest imaging, clinical features, lung total severity score, pulmonary involvements, SARS-CoV-2 omicron variant

Introduction

Four years have passed since the outbreak of the coronavirus disease (COVID-19) pandemic began, and as of 17 Mar 2024, there have been 7,040,264 deaths reported to the World Health Organization (WHO) (1). The impact of COVID-19 has been profound, affecting the global economy, environment, and tourism, among other sectors. Between 2019 and 2020, the number of unemployed people worldwide increased from 191.93 million to 235.21 million, highlighting just a fraction of the pandemic's socioeconomic ramifications (2). Consequently, countries worldwide have undertaken extensive efforts to curb the spread of the virus, including vaccine development, reinforced prevention and control measures, and implementation of supportive policies (3, 4). However, as a result of genetic variations in SARS-CoV-2 during viral replication to evade the human immune system and achieve self-protection, several SARS-CoV-2 variants have been reported worldwide (5, 6), with the Omicron variant notably becoming predominant (7, 8). Although the Omicron variant has been proven to spread rapidly with less virulence, causing mainly asymptomatic and mildly symptomatic infections which tends to the upper respiratory tract, not the lungs (7, 9), the research has revealed that even among these asymptomatic and mildly symptomatic patients with Omicron who received limited medical attention, some experienced severe outcomes, particularly among the older adult (10). This prompts the hypothesis that potential pulmonary involvements may occur in asymptomatic and mildly symptomatic patients with Omicron, underscoring the importance of early identification to mitigate disease progression.

Chest computed tomography (CT) has become a standard tool for clinicians to evaluate the extent of pulmonary involvement in patients with COVID-19 (11). The Radiological Society of North America (RSNA) guidelines have categorized chest images of COVID-19 pneumonia into four classifications to discern the correlation between pulmonary involvements and COVID-19 (12). While several studies have devised scoring systems to quantify the severity of pulmonary involvements in COVID-19 pneumonia based on chest CT images (13–19), they have predominantly focused on symptomatic patients, neglecting asymptomatic and mildly symptomatic cases. Consequently, there is a paucity of research regarding the chest imaging characteristics of pulmonary involvements in asymptomatic and mildly symptomatic patients with Omicron. Thus, the objective of our study is to elucidate chest CT imaging features indicative of pulmonary involvements in asymptomatic and mildly symptomatic patients with Omicron and to develop a visual model for identifying individuals with potential pulmonary involvements warranting further evaluation via chest CT scans.

Materials and methods

Patients and clinical data

Our study was retrospective in nature and approved by the Ethics Committee (Approval No. 2022-SR-491). All complete data were retrospectively collected from cases infected with Omicron admitted to Shanghai Lin-gang Shelter Hospital from 24 April to 10 May 2022, and the data were de-identified and anonymously analyzed. Population screening was performed according to the following inclusion criteria: (1) patients with the Omicron variant diagnosed in accordance with the Chinese COVID-19 treatment guidelines with asymptomatic and mildly symptomatic disease (20). All patients tested positive for the SARS-CoV-2 nucleocapsid protein gene (*N* gene)/open reading frame 1ab gene (*ORF1ab* gene) using real-time fluorescent quantitative PCR (RT-qPCR) before admission; (2) age ≥ 18 years; (3) finger pulse oximetry >94%; (4) no severe organ dysfunction; (5) asymptomatic patients with clinical symptoms and mildly symptomatic patients with severe or prolonged respiratory symptoms after admission; and (6) chest CT examination was determined necessary by doctors. The exclusion criteria were (1) age < 18 years; (2) finger pulse oximetry ≤93%; (3) respiratory rate ≥ 30 breaths/min; (4) heart, lung, renal, and other important organ dysfunctions; (5) uncontrolled underlying diseases such as hypertension, diabetes, coronary artery disease, and psychiatric diseases; (6) inability to follow up the nucleic acid test results because of serious illness or the need for special treatment (such as chemotherapy); and (7) serious clinical data deficiencies. The data obtained included age, sex, stage, history of vaccination, comorbidities, and major clinical symptoms (e.g., respiratory, gastrointestinal, and other systemic symptoms).

Nucleic acid detection methods and negative conversion time

Specimens were collected from the nasopharynx and oropharynx, and the first test was performed within 24 h after the patients were admitted to the shelter hospital. Subsequently, swab specimens were collected once a day at an interval of 24 h and tested by Shanghai Dean Medical Laboratory Co. Nucleic acid test negativity was judged by two consecutive SARS-CoV-2 nucleic acid tests with Ct values ≥35 for both the *N* and *ORF1ab* gene (RT-qPCR with a cut-off value of 40 and a sampling interval of at least 24 h). The NCT was the time from the date of the first positive nucleic acid test to the date of specimen collection of the first negative nucleic acid test (two consecutive negative tests).

Interpretation of omicron infection

During our study period, Omicron was the predominant strain of COVID-19, and all newly identified viral genomes in Shanghai belonged to the BA.2.2 sub-lineage of the Omicron variant of SARS-CoV-2 (B.1.1.529) (10, 21). Consequently, patients in our study were considered infected with Omicron, and molecular analyses for Omicron identification were not conducted.

Acquisition and interpretation of chest CT images

The patient's first chest CT images were analyzed during hospitalization. Patients were scanned in the supine position with a continuous spiral scan from the lung apex to the lung base. Two radiologists with more than 10 years of experience in the cardiothoracic field reviewed the images individually, and in case of

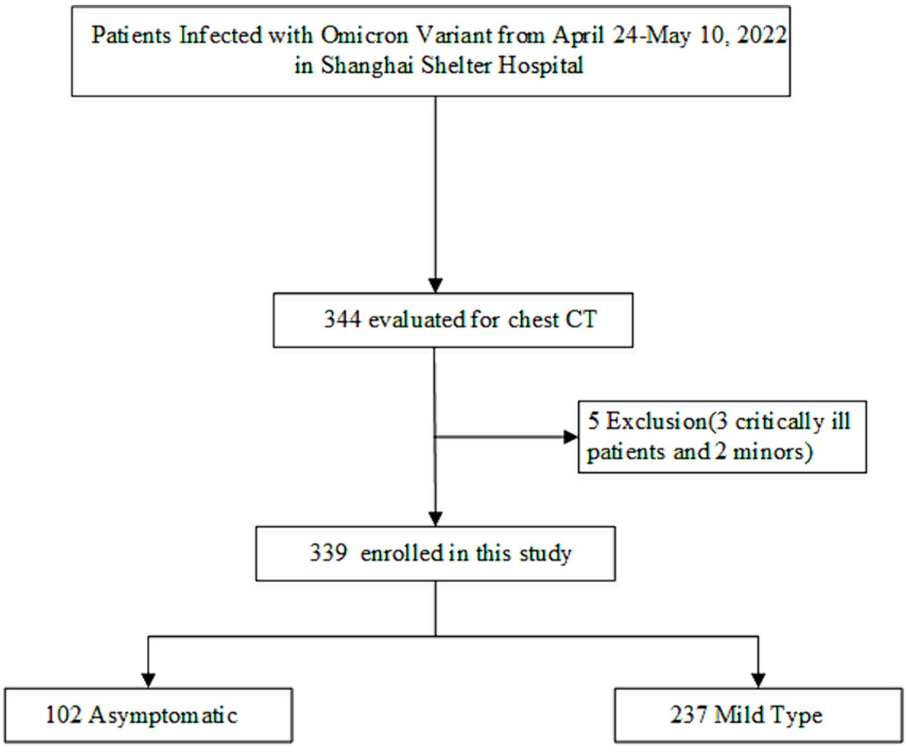

FIGURE 1
Flowchart of study patients.

disagreement, another radiologist with more than 15 years of experience made a comprehensive judgment. The total severity score system was selected for the visual evaluation of pulmonary involvements on chest CT, and TSS was checked and counted by a statistician. The TSS system was based on previously published methods (22).

Statistical analysis

Excel 2016 (Microsoft Corp., Redmond, WA) was used to collect the data. Continuous variables were compared using the Mann–Whitney U test. Categorical variables were compared using the χ^2-test, Fisher's exact test or Yates' correction. The variables of multiple groups were compared using a one-way analysis of variance (ANOVA). The binary logistic regression was used to identify the factors associated with OPI and variables assessed $p < 0.1$ by univariate analysis could be incorporated into multivariate analysis. Statistical analyses were conducted using SPSS version 25.0 (IBM Corp., Armonk, NY) and R software (version 4.2.2[1]; R Foundation for Statistical Computing, Vienna, Austria). The reported statistical significance levels were two-sided, with statistical significance set at $p < 0.05$.

1 http://www.Rproject.org

Results

Basic information

In accordance with the inclusion criteria, 344 patients were screened; three seriously ill patients and two minors were excluded according to the exclusion criteria. Finally, 339 patients were included in the study (Figure 1). The median age of all patients was 57 years [interquartile range (IQR), 44–69 years], and 145 patients (42.8%) were male; 257 patients (24.4%) were aged ≥70 years, and 155 patients (45.7%) had underlying diseases. In addition, 112 patients (33.0%) were unvaccinated, and the median NCT was 8 days (IQR, 5–10 days).

The baseline clinical characteristics of the asymptomatic and mildly symptomatic patients are shown in Table 1. The differences were mainly in clinical symptoms, with significant differences in cough (80.6%), sputum (68.4%), stuffy nose (15.2%), muscle aches (22.4%), and malaise (28.3%) in mildly symptomatic patients compared with asymptomatic patients. Notably, no difference was found in vaccination status and NCT between the asymptomatic and mildly symptomatic groups, although the mildly symptomatic group included patients with a longer NCT.

Description of CT imaging features

CT imaging features of 339 patients were analyzed according to the type of parenchymal opacity, opacity (axial) distribution, airway changes, underlying pulmonary changes, and other involvements on CT.

TABLE 1 Baseline clinical characteristics, clinical symptoms, vaccination status, and NCT of the study patients.

Characteristic	Asymptomatic patients (*n* = 102)	Mild type patients (*n* = 237)	*p*-value
Age (years)	58 (46–68)	56 (43–69)	0.726[c]
<70 years	79 (77.5)	178 (75.1)	0.746[a]
Gender			0.835[a]
Female	45 (44.1)	100 (42.2)	
Male	57 (55.9)	137 (57.8)	
Chronic diseases	41 (40.2)	101 (42.6)	0.769[a]
Hypertension	29 (28.4)	79 (33.3)	0.446[a]
Diabetes	19 (18.6)	27 (11.4)	0.107[a]
Coronary heart disease	5 (4.9)	20 (8.4)	0.360[a]
Pulmonary diseases	3 (2.9)	12 (5.1)	0.566[b]
Rheumatic diseases	1 (1.0)	3 (1.3)	1[b]
Other diseases	13 (12.7)	42 (17.7)	0.327[a]
Clinical symptoms			
Fever	15 (14.7)	57 (24.1)	0.074[a]
Sore throat	21 (20.6)	60 (25.3)	0.425[a]
Cough	65 (63.7)	191 (80.6)	0.002[a]
Sputum	56 (54.9)	162 (68.4)	0.025[a]
Nose runny	9 (8.8)	26 (11.0)	0.688[a]
Nose stuffy	6 (5.9)	36 (15.2)	0.027[a]
Chills	5 (4.9)	21 (8.9)	0.301[a]
Muscle soreness	6 (5.9)	53 (22.4)	<0.001[a]
Fatigue	15 (14.7)	67 (28.3)	0.011[a]
Dyspnea	8 (7.8)	27 (11.4)	0.429[a]
Diarrhea	5 (4.9)	23 (9.7)	0.208 [a]
Other symptoms	10 (9.8)	27 (11.4)	0.810[a]
Vaccination status			0.814[a]
Unvaccinated	35 (34.3)	77 (32.5)	
Single or double vaccinated	32 (31.4)	70 (29.5)	
Booster vaccinated	35 (34.3)	90 (38.0)	
Nucleic acid convention time (days)	7 (4–10)	8 (6–10)	0.162[c]
<8 days	46 (45.1)	84 (35.4)	0.120[a]

Data are presented as the median (interquartile range) or number (percentage) of patients. [a]Determined with χ^2-test. [b]Determined with Fisher's exact test. [c]Determined with Mann–Whitney U test.

TABLE 2 The type of parenchymal opacity, opacities (axial) distribution, airways, underlying lung lesions, and other findings of chest CT in two groups.

Findings	Asymptomatic patients (*n* = 102)	Mild type patients (*n* = 237)	*P*-value
Type of parenchymal opacity			
Consolidation (%)	4 (3.9)	12 (5.1)	0.785[b]
Ground glass opacities	23 (22.5)	48 (20.3)	0.740[a]
Linear opacities (%)	52 (51.0)	93 (39.2)	0.060[a]
Rounded morphology (%)	16 (15.7)	40 (16.9)	0.911[a]
Nodules (%)	37 (36.3)	86 (36.3)	1[a]
Reverse halo sign (%)	1 (1.0)	3 (1.3)	1[b]
Crazy-paving pattern	0 (0.0)	1 (0.4)	1[b]
Opacities (axial) distribution			
No axial lung distribution	30 (29.4)	68 (28.7)	0.997[a]
Central distribution (peribronchovascular)	0 (0.0)	4 (1.7)	0.32b[a]
Peripheral distribution	48 (47.1)	104 (43.9)	0.674[a]
Airways			
Bronchial wall thickening	1 (1.0)	4 (1.7)	1[b]
Bronchiectasi	3 (2.9)	14 (5.9)	0.381[a]
Airways secretions	0 (0.0)	0 (0.0)	
Underlying lung lesions			
Pulmonary emphysema	17 (16.7)	38 (16.0)	1[a]
Pulmonary fibrosis	2 (2.0)	15 (6.3)	0.156[a]
Other findings			
Pleural effusion	2 (2.0)	7 (3.0)	0.729[b]
Lymphadenopathy	8 (7.8)	13 (5.5)	0.562[a]
Pericardial effusion	2 (2.0)	2 (0.8)	0.587[b]
Hollow	0 (0.0)	0 (0.0)	
Others	14 (13.7)	45 (19.0)	0.310[a]

Data are presented as the number (percentage) of patients. [a]Determined with χ^2-test. [b]Determined with Fisher's exact test.

Regarding the type of parenchymal opacity, 16 patients (4.7%) had consolidation, with 71 cases (20.9%) of ground glass opacity (GGO); 145 patients (42.8%) had linear opacities, rounded morphology was found in 56 patients (16.5%), and 123 patients (36.3%) had nodules. Interestingly, four patients (1.2%) had reverse halo signs, and only one (0.3%) had a crazy paving pattern. Whereas the opacities were mainly distributed peripherally (44.8%), only four patients (1.2%) showed a central distribution (peribronchovascular), and the remaining 98 patients (28.9%) showed no axial lung distribution. In the study cohort, airway changes were not significantly manifested; in particular, bronchial wall thickening was observed in five cases (1.5%), bronchiectasis in 17 cases (5.0%), and none of the patients had airway secretions. Fifty-five patients (16.2%) showed signs of pulmonary emphysema, whereas pulmonary fibrosis was present in 17 patients (5.0%). The number of patients with pleural effusion and lymphadenopathy was 9 (2.7%) and 21 (6.2%), respectively, while none of the patients had hollow nodules. Finally, other involvements included pulmonary texture thickening and calcification, which were present in 59 cases (17.4%). The CT imaging features of the asymptomatic and mildly symptomatic groups are shown in Table 2.

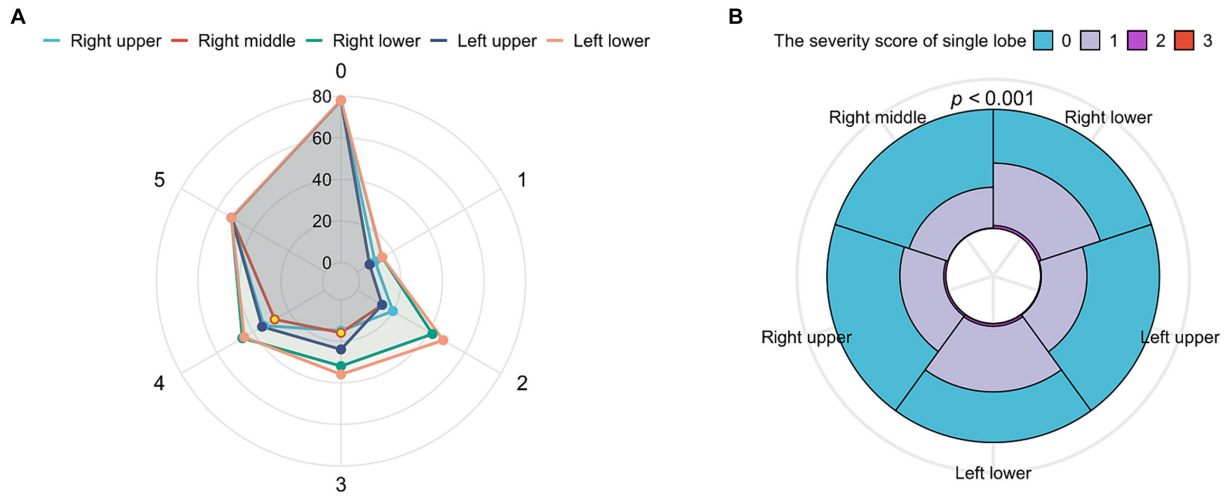

FIGURE 2
The analysis of total severity score (TSS) with lung lobes. **(A)** Radar chart depicting the characteristics of involved lobes. X axis shows the number of involved lobes (0–5), and y axis shows the counterpart number of patients. The wrap-around area of the left lobe was the largest, which represented that the left lobe was seriously involved, and the right lobe conversely. **(B)** Overlay of histograms showing the TSS's characteristics of each lobe. The different color's wrap-around area represented the counterpart number of patients. The cleaned data were counted accordingly to get the percentage of each subgroup in each category and determined with Yates' correction. Radar chart and overlay of histograms were drawn for the data using the ggplot2 package.

TABLE 3 The univariate and multivariate binary logistic regression analysis used for identifying the risk factors for the obvious pulmonary involvements.

Variable	*P*-value	Variable	OR	95% CI	*P*-value
Age stratification	< 0.001	Age stratification	1.92	1.548–2.383	< 0.001
Vaccination status	0.002	NCT > 8d	1.842	1.104–3.073	0.019
NCT > 8d	0.015	Pulmonary diseases	4.698	1.159–19.048	0.03
Hypertension	0.067				
Coronary heart diseases	0.024				
Pulmonary diseases	0.006				
Other diseases	0.004				
Fever	0.099				
Muscle soreness	0.006				

The total severity score

The TSS was counted for the two groups, and the scores of all patients ranged from 0 to 10 with a median of 2 (IQR, 1–4). On the initial CT, all five lobes were involved in 52 patients (15.3%), four lobes in 47 (13.9%), three lobes in 41 (12.1%), two lobes in 69 (20.4%), and one lobe in 52 patients (15.3%), while only 78 patients (23.0%) had no pulmonary involvement. As shown in Figure 2A, the most frequently affected lobe was the left lower lobe (55.2%), with the least involvement in the right middle lobe (34.5%), and pulmonary involvement was most likely to be found in the left lower lobe, followed by the right lower lobe, left upper lobe, right upper lobe, and right middle lobe among patients with involvements in one to four lobes. The analysis of the severity of different lobe involvements revealed that only the left lower lobe had a score of 0–3, with the score ranging from 0 to 2 in all the other lobes. The data are shown in Figure 2B. The difference was statistically significant ($p < 0.001$), and we compared the TSS of the different lung lobes. Supplementary Table S1 shows the CT imaging features of the patients in the asymptomatic and mildly symptomatic groups, and there were no significant differences.

The risk factors for obvious pulmonary involvements and developing the nomogram

We categorized the patients into OPI (TSS >2) and not OPI (NOPI) (TSS ≤2) groups based on the median TSS. The univariate binary logistic regression analysis of patients in both groups was shown in Table 3. Further multivariate analysis revealed age stratification [OR = 1.92, 95% CI (1.548–2.383); $p < 0.001$], prolonged NCT [OR = 1.842, 95% CI (1.104–3.073); $p = 0.019$], and pulmonary diseases [OR = 4.698, 95% CI (1.159–19.048); $p = 0.03$] were independent risk factors for the OPI group patients. To visually depict the effect of the age stratification,

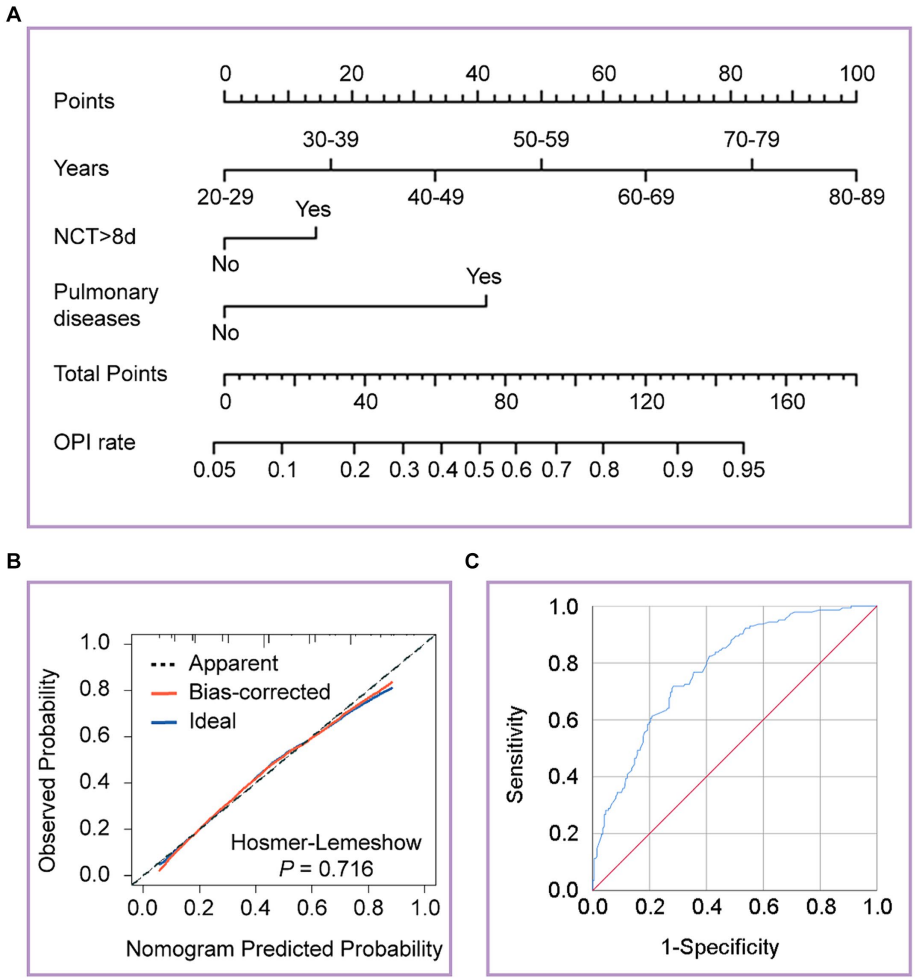

FIGURE 3
Developed the nomogram, calibration curves and receiver operating characteristic (ROC) curves of the nomogram. **(A)** The nomogram was developed on the regression model, with age stratification, prolonged NCT, and pulmonary diseases. **(B)** Calibration curves depict the calibration between the predicted and observed probability of OPI. The y-axis represents the observed OPI rate. The x-axis represents the predicted OPI risk. The diagonal dotted line represents a perfect prediction by an ideal model. The blue solid line represents the performance of the nomogram, of which a closer fit to the diagonal dotted line represents a better prediction. **(C)** ROC curves depict the diagnostic efficacy of the model. The y-axis represents the sensitivity of the model, and the x-axis represents specificity. The blue solid line represents a prediction by the nomogram.

prolonged NCT and pulmonary diseases on the probability of OPI, we visualized the logistic regression model and plotted nomogram. As shown in Figure 3A, the total points obtained by summing the points corresponding to individual factors corresponded to the OPI rate with the vertical line of its score scale. Further, we verified the goodness of fit and detection efficacy of the nomogram using calibration curves and the receiver operating characteristic (ROC) curves (Figures 3B,C). The calibration curve illustrated that our constructed nomogram had a high goodness of fit (Hosmer–Lemeshow test, $p = 0.716$), while the value of the area under the ROC curve (AUC) of the model was 0.779.

Discussion

In this study, we found that almost half of the asymptomatic and mildly symptomatic patients infected with Omicron had pulmonary involvements on CT, which were more likely to be found in the left lower lung lobe. Using the TSS system to assess the severity of pulmonary involvements, we revealed a significant difference in the TSS of the individual lung lobes ($p < 0.001$). Age stratification, prolonged NCT, and pulmonary diseases were identified as the risk factors for OPI by logistic regression analysis.

In our study, the proportion of asymptomatic infections was 30.1%, which is similar to the result of a previous meta-analysis study (32.40%) that included eight studies with 7,640 patients infected with the Omicron variant (23). Compared with the Delta variant (24), the prominent symptoms in patients with the Omicron variant were cough and sputum in our study, while upper respiratory tract symptoms (e.g., runny and stuffy nose) and systemic symptoms (e.g., fever, muscle soreness, and fatigue) occurred in less than 30% of patients. Geng et al. investigated the impact of symptoms on Omicron infection, identifying fever (OR = 6.358, 95% CI 1.748–23.119; $p = 0.005$) and diarrhea (OR = 6.523, 95% CI 1.061–40.110; $p = 0.043$) as risk factors for Omicron infection progression (10). However,

logistic regression analysis in our study did not identify any clinical symptoms as risk factors for OPI, implying challenges in symptom-based identification of pulmonary involvements.

Multiple clinical and experimental studies have found that the virulence of the Omicron variant is significantly weaker than that of the wild-type strain and the Alpha, Beta, and Delta variants (25–28). The study found that Omicron infection has a lower CT severity score (CT-SS) than Delta infection (OR = −7.2, 95% CI (−9.9 to −4.5); $p < 0.001$) and the Delta variant has greater association with severe disease (OR = 4.6, 95% CI(1.2–26); $p = 0.01$) and admission to a critical care unit [OR = 7.0, 95% CI (1.5–66); $p = 0.004$] (28). Moreover, the lower virulence in the lungs, the main target of a viral attack, is reflected in pathological changes that are more symptomatic of acute exudation and less obvious changes in vascular damage and chronic fibrous exudation (29, 30). Compared with the wild-type strain and the Alpha, Beta, and Delta variants, patients with Omicron infection had nontypical peribronchovascular pneumonia and less pulmonary vascular involvement on chest CT images (28, 31). In our study, the types of parenchymal opacity were mainly linear opacities, nodules, GGO, and rounded morphology, whereas the reverse halo sign and crazy paving pattern were almost absent. Conversely, Uysal et al. observed that CT findings in asymptomatic COVID-19 patients primarily consisted of GGO, often localized peripherally (32). This discrepancy may be attributed to the variant types and suggests distinct areas of interest for different variants. Additionally, Tomris et al. (33) explored the distinct spatial distribution of angiotensin-converting enzyme 2 (ACE2) expression in Syrian hamster lung lobes infected with SARS-CoV-2, highlighting ACE2 predominance in the lower regions of the lung lobes, which likely contributes to pulmonary involvements primarily in these areas. Interestingly, the left lower lung lobe was most likely to be involved, while the right lower lung lobe was mostly involved in another study (34). The variations in spatial distribution of pulmonary involvement across different variants offer valuable insights into variant-specific pathogenic mechanisms.

Given the distinctive mutational profile of the virus, numerous radiographers and clinicians have endeavored to devise scoring systems aimed at evaluating the extent of pulmonary involvements on chest CT scans (13, 14). These systems serve the dual purpose of comprehending the impact of various mutant strains and guiding clinical decisions. The more established and widely recognized chest CT severity scoring systems are mainly the chest CT severity score (CT-SS) (15), chest CT score (16), TSS (17), modified TSS (18), and 3-level chest CT severity score (19). In a comparison of these scoring systems, Elmokadem et al. found that the TSS had a higher AUC (0.890) and shorter reporting time, whereas the chest CT score showed the highest specificity (95.2%) in discriminating severe cases (35). In our investigation, the TSS system was selected due to its inherent advantages, coupled with the requirement for detailed characterization of pulmonary involvement; the intricate lobe classification system of CT-SS risked undue dispersion of results, particularly as our study cohort comprised solely asymptomatic and mildly symptomatic cases. For the wild-type strain, one previous study reported a mean TSS of 9.9 (range, 0–19) (22), and another study showed a mean TSS of 9 (range, 4–12) (35). These studies further underscored a strong correlation between higher TSS and disease severity. Viceconte et al. compared TSS values between the Alpha and Delta variants, suggesting a higher TSS association with the Delta variant (36). Similarly, Inui et al. confirmed that patients with the Delta variant had a higher TSS (37) and that the Delta variant was more virulent (38), whereas patients with the Omicron variant exhibited a lower TSS. Our study revealed a median TSS of 2 (range, 0–10), with no significant variance observed between asymptomatic individuals and those presenting with mild symptoms.

We defined patients as having OPI when their TSS exceeded 2. The correlation between OPI and age in asymptomatic and mildly symptomatic patients with Omicron was not previously well known. Our study found age to be an independent risk factor for OPI, which might be due to the positive correlation between age and the number of copies of the virus (39), and another two studies proved that age was independent risk factors for the duration of viral shedding (40, 41). However, several studies (42, 43) did not reach this conclusion. Our study also observed that prolonged NCT indicated a higher level and longer duration of viral replication, with patients exhibiting prolonged NCT being more likely to develop OPI. While our study suggests that pulmonary disease may pose a risk factor for OPI, this conclusion necessitates further investigation, particularly given that patients with underlying pulmonary conditions may be more susceptible to Omicron infection. Other studies had found that the severity of pulmonary involvement was closely related to the variant (28, 31).

This study had several limitations and challenges. Firstly, the clinical symptoms of patients at initial admission to Shanghai Lin-gang Shelter Hospital were mainly collected using a WeChat Application, which may have led to data bias. Secondly, owing to the lack of nucleic acid detection and molecular analyses for Omicron identification in the early period, the time of the first positive nucleic acid test lagged behind the actual infection time in some patients, resulting in a shorter NCT than before, and the diagnosis of Omicron infection based on epidemiological inference might have bias in analysis of baseline data; then, only asymptomatic and mildly symptomatic patients infected with Omicron were analyzed and not compared with previous variants. In addition, Shanghai Lin-gang Shelter Hospital did not carry out laboratory tests in the early period owing to the lack of necessary conditions; hence, we could not provide the laboratory results of these patients. As a result of not reviewing the chest CT images and not following up, we lacked prognostic data, which limited the study to a description of the phenomena, and we could not analyze the clinical outcomes. It is necessary that clinicians should pay greater attention to the pathophysiological mechanisms and assess the follow-up chest CT findings to understand the spatio-temporal variations of the pulmonary involvements in mildly symptomatic and asymptomatic patients infected with Omicron, which will be of benefit in preventing disease progression.

Conclusion

In conclusion, asymptomatic and mildly symptomatic patients infected with Omicron mainly had upper respiratory symptoms, with few and mild systemic symptoms, and Chest CT imaging features were dominated by linear opacities and GGO. However, pulmonary involvements on chest CT were not uncommon. Age stratification, prolonged NCT, and pulmonary diseases were risk factors for OPI, and we developed a nomogram to predict the risk for pulmonary involvements in asymptomatic and mildly symptomatic patients. We hope that our model can be effectively applied in a scenario where

clinicians decide whether to have asymptomatic and mildly symptomatic patients finished CT, neither ignoring patients with underlying pulmonary involvements nor performing redundant CT scans.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

The studies involving humans were approved by the Ethics Committee of the First Affiliated Hospital of Nanjing Medical University. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin because our study was retrospective in nature.

Author contributions

PL: Data curation, Formal analysis, Methodology, Visualization, Writing – original draft, Writing – review & editing. KC: Formal analysis, Investigation, Writing – review & editing. GD: Conceptualization, Data curation, Resources, Writing – review & editing. TC: Validation, Visualization, Writing – review & editing. YifZ: Validation, Visualization, Writing – review & editing. HX: Methodology, Software, Writing – review & editing. XX: Methodology, Software, Writing – review & editing. QC: Project administration, Supervision, Writing – review & editing. YiyZ: Project administration, Supervision, Writing – review & editing. XZ: Data curation, Funding acquisition, Resources, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1325474/full#supplementary-material

References

1. WHO Coronavirus (COVID-19) Dashboard. World Health Organization web site. (2024). Available at: https://covid19.who.int (Accessed April 8, 2024).

2. Statista Web site. (2024). Available at: https://www.statista.com/statistics/266414/unemployed-persons-worldwide (Accessed April 8, 2024).

3. Varenne Z. Pathways to a green and just recovery from COVID-19: promoting systemic change for greater sustainability and resilience. Global Governance Institute Policy Brief Series. London: University College London (2021). 2021 p.

4. Mariita RM, Musila JM. A study on the relationship between Bacillus CalmetteGurin (BCG) vaccination and Covid-19 prevalence: do other confounders warrant investigation? *J Public Health Epidemiol*. (2020) 12:142–50. doi: 10.5897/JPHE2020.1230

5. Tao K, Tzou PL, Nouhin J, Gupta RK, de Oliveira T, Kosakovsky Pond SL, et al. The biological and clinical significance of emerging SARS-CoV-2 variants. *Nat Rev Genet*. (2021) 22:757–73. doi: 10.1038/s41576-021-00408-x

6. Walensky RP, Walke HT, Fauci AS. SARS-CoV-2 variants of concern in the United States-challenges and opportunities. *JAMA*. (2021) 325:1037–8. doi: 10.1001/jama.2021.2294

7. Danielle Iuliano A, Brunkard JM, Boehmer TK, Peterson E, Adjei S, Binder AM, et al. Trends in disease severity and health care utilization during the early omicron variant period compared with previous SARS-CoV-2 high transmission periods — United States, December 2020–January 2022. *MMWR Morb Mortal Wkly Rep*. (2022) 71:146–52. doi: 10.15585/mmwr.mm7104e4

8. Ulloa AC, Buchan SA, Daneman N, Brown KA. Estimates of SARS-CoV-2 omicron variant severity in Ontario, Canada. *JAMA*. (2022) 327:1286–8. doi: 10.1001/jama.2022.2274

9. Chen X, Yan X, Sun K, Zheng N, Sun R, Zhou J, et al. Estimation of disease burden and clinical severity of COVID-19 caused by omicron BA.2 in Shanghai, February-June 2022. *Emerg Microbes Infect*. (2022) 11:2800–7. doi: 10.1080/22221751.2022.2128435

10. Geng Y, Nie Q, Liu F, Pei Y, Chen Q, Zhang H, et al. Understanding clinical characteristics influencing adverse outcomes of omicron infection: a retrospective study with propensity score matching from a Fangcang hospital. *Front Cell Infect Microbiol*. (2023) 13:1115089. doi: 10.3389/fcimb.2023.1115089

11. Som A, Lang M, Yeung T, Carey D, Garrana S, Mendoza DP, et al. Implementation of the Radiological Society of North America expert consensus guidelines on reporting chest CT findings related to COVID-19: a multireader performance study. *Radiol Cardiothorac Imaging*. (2020) 2:e200276. doi: 10.1148/ryct.2020200276

12. Simpson S, Kay FU, Abbara S, Bhalla S, Chung JH, Chung M, et al. Radiological Society of North America expert consensus document on reporting chest CT findings related to COVID-19: endorsed by the Society of Thoracic Radiology, the American College of Radiology, and RSNA. *Radiol Cardiothorac Imaging*. (2020) 2:e200152. doi: 10.1148/ryct.2020200152

13. Verma A, Kumar I, Singh PK, Ansari MS, Singh HA, Sonkar S, et al. Initial comparative analysis of pulmonary involvement on HRCT between vaccinated and non-vaccinated subjects of COVID-19. *Eur Radiol*. (2022) 32:4275–83. doi: 10.1007/s00330-021-08475-8

14. Bai N, Lin R, Wang Z, Cai S, Huang J, Su Z, et al. Exploring new characteristics: using deep learning and 3D reconstruction to compare the original COVID-19 and its Delta variant based on chest CT. *Front Mol Biosci*. (2022) 9:836862. doi: 10.3389/fmolb.2022.836862

15. Yang R, Li X, Liu H, Zhen Y, Zhang X, Xiong Q, et al. Chest CT severity score: an imaging tool for assessing severe COVID-19. *Radiol Cardiothorac Imaging*. (2020) 2:e200047. doi: 10.1148/ryct.2020200047

16. Francone M, Iafrate F, Masci GM, Coco S, Cilia F, Manganaro L, et al. Chest CT score in COVID-19 patients: correlation with disease severity and short-term prognosis. *Eur Radiol*. (2020) 30:6808–17. doi: 10.1007/s00330-020-07033-y

17. Li K, Fang Y, Li W, Pan C, Qin P, Zhong Y, et al. CT image visual quantitative evaluation and clinical classification of coronavirus disease (COVID-19). *Eur Radiol*. (2020) 30:4407–16. doi: 10.1007/s00330-020-06817-6

18. Wasilewski PG, Mruk B, Mazur S, Poltorak-Szymczak G, Sklinda K, Walecki J. COVID-19 severity scoring systems in radiological imaging - a review. *Pol J Radiol*. (2020) 85:361–8. doi: 10.5114/pjr.2020.98009

19. Salaffi F, Carotti M, Tardella M, Borgheresi A, Agostini A, Minorati D, et al. The role of a chest computed tomography severity score in coronavirus disease 2019 pneumonia. *Medicine (Baltimore)*. (2020) 99:e22433. doi: 10.1097/MD.0000000000022433

20. National Health Commission of the People's Republic of China. Diagnosis and treatment plan for COVID-19 (trial version 9). *Int J Epidemiol Infect Dis*. (2022) 2:73–80. doi: 10.3760/cma.j.cn331340-20220325-00065

21. Ao Y, Li J, Wei Z, Wang Z, Tian H, Qiu Y, et al. Clinical and virological characteristics of SARS-CoV-2 omicron BA.2.2 variant outbreaks during April to May, 2022, Shanghai, China. *J Infect*. (2022) 85:573–607. doi: 10.1016/j.jinf.2022.07.027

22. Chung M, Bernheim A, Mei X, Zhang N, Huang M, Zeng X, et al. CT imaging features of 2019 novel coronavirus (2019-nCoV). *Radiology*. (2020) 295:202–7. doi: 10.1148/radiol.2020200230

23. Shang W, Kang L, Cao G, Wang Y, Gao P, Liu J, et al. Percentage of asymptomatic infections among SARS-CoV-2 omicron variant-positive individuals: a systematic review and meta-analysis. *Vaccines*. (2022) 10:1049. doi: 10.3390/vaccines10071049

24. Ong SWX, Chiew CJ, Ang LW, Mak TM, Cui L, Toh M, et al. Clinical and Virological features of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants of concern: a retrospective cohort study comparing B.1.1.7 (alpha), B.1.351 (Beta), and B.1.617.2 (Delta). *Clin Infect Dis*. (2022) 75:e1128–36. doi: 10.1093/cid/ciab721

25. Shuai H, Chan JF, Hu B, Chai Y, Yuen TT, Yin F, et al. Attenuated replication and pathogenicity of SARS-CoV-2 B.1.1.529 omicron. *Nature*. (2022) 603:693–9. doi: 10.1038/s41586-022-04442-5

26. Law N, Chan J, Kelly C, Auffermann WF, Dunn DP. Incidence of pulmonary embolism in COVID-19 infection in the ED: ancestral, Delta, Omicron variants and vaccines. *Emerg Radiol*. (2022) 29:625–9. doi: 10.1007/s10140-022-02039-z

27. Hui KPY, Ho JCW, Cheung MC, Ng KC, Ching RHH, Lai KL, et al. SARS-CoV-2 omicron variant replication in human bronchus and lung *ex vivo*. *Nature*. (2022) 603:715–20. doi: 10.1038/s41586-022-04479-6

28. Tsakok MT, Watson RA, Saujani SJ, Kong M, Xie C, Peschl H, et al. Reduction in chest CT severity and improved hospital outcomes in SARS-CoV-2 omicron compared with Delta variant infection. *Radiology*. (2023) 306:261–9. doi: 10.1148/radiol.220533

29. Kligerman SJ, Franks TJ, Galvin JR. From the radiologic pathology archives: organization and fibrosis as a response to lung injury in diffuse alveolar damage, organizing pneumonia, and acute fibrinous and organizing pneumonia. *Radiographics*. (2013) 33:1951–75. doi: 10.1148/rg.337130057

30. Hariri LP, North CM, Shih AR, Israel RA, Maley JH, Villalba JA, et al. Lung histopathology in coronavirus disease 2019 as compared with severe acute respiratory Sydrome and H1N1 influenza: a systematic review. *Chest*. (2021) 159:73–84. doi: 10.1016/j.chest.2020.09.259

31. Yoon SH, Lee JH, Kim B-N. Chest CT findings in hospitalized patients with SARS-CoV-2: Delta versus omicron variants. *Radiology*. (2023) 306:252–60. doi: 10.1148/radiol.220676

32. Uysal E, Kilincer A, Cebeci H, Ozer H, Demir NA, Ozturk M, et al. Chest CT findings in RT-PCR positive asymptomatic COVID-19 patients. *Clin Imaging*. (2021) 77:37–42. doi: 10.1016/j.clinimag.2021.01.030

33. Tomris I, Bouwman KM, Adolfs Y, Noack D, van der Woude R, Kerster G, et al. Distinct spatial arrangements of ACE2 and TMPRSS2 expression in Syrian hamster lung lobes dictates SARS-CoV-2 infection patterns. *PLoS Pathog*. (2022) 18:e1010340. doi: 10.1371/journal.ppat.1010340

34. Aksu Y, Uslu AU, Tarhan G, Karagulle M, Tiryaki S. The relationship among splenomegaly, lung involvement patterns, and severity score in COVID-19 pneumonia. *Curr Med Imaging*. (2022) 18:1311–7. doi: 10.2174/1573405618666220509212035

35. Elmokadem AH, Mounir AM, Ramadan ZA, Elsedeiq M, Saleh GA. Comparison of chest CT severity scoring systems for COVID-19. *Eur Radiol*. (2022) 32:3501–12. doi: 10.1007/s00330-021-08432-5

36. Viceconte G, Ponsiglione A, Buonomo AR, Camera L, Scotto R, De Giorgi M, et al. COVID-19 chest CT and laboratory features of B.1.617.2 (Delta variant) vs B.1.1.7 (alpha variant) surge: a single center case-control study. *Infez Med*. (2022) 30:555–62. doi: 10.53854/liim-3004-10

37. Inui S, Fujikawa A, Gonoi W, Kawano S, Sakurai K, Uchida Y, et al. Comparison of CT findings of coronavirus disease 2019 (COVID-19) pneumonia caused by different major variants. *Jpn J Radiol*. (2022) 40:1246–56. doi: 10.1007/s11604-022-01301-1

38. Lee JE, Hwang M, Kim YH, Chung MJ, Sim BH, Jeong WG, et al. SARS-CoV-2 variants infection in relationship to imaging-based pneumonia and clinical outcomes. *Radiology*. (2023) 306:e221795. doi: 10.1148/radiol.221795

39. To KK, Tsang OT, Leung WS, Tam AR, Wu TC, Lung DC, et al. Temporal profiles of viral load in posterior oropharyngeal saliva samples and serum antibody responses during infection by SARS-CoV-2: an observational cohort study. *Lancet Infect Dis*. (2020) 20:565–74. doi: 10.1016/S1473-3099(20)30196-1

40. Li R, Jin C, Zhang L, Kong D, Hu K, Xuan M, et al. Clinical characteristics and risk factors analysis of viral shedding time in mildly symptomatic and asymptomatic patients with SARS-CoV-2 omicron variant infection in Shanghai. *Front Public Health*. (2022) 10:1073387. doi: 10.3389/fpubh.2022.1073387

41. Zhong W, Yang X, Jiang X, Duan Z, Wang W, Sun Z, et al. Factors associated with prolonged viral shedding in older patients infected with omicron BA.2.2. Front. *Public Health*. (2022) 10:1087800. doi: 10.3389/fpubh.2022.1087800

42. Lixia S, Jing H, Yongjin Z, Shasha F, Herong W, Shuping H. Influencing factors of negative conversion time of nucleic acid in patients with corona virus disease 2019. *Shanghai Med J*. (2021) 44:576–9. doi: 10.19842/j.cnki.issn.0253-9934.2021.08.009

43. Huili G, Hanping H, Xia Z, Shanshan L, Zhenghua Z, Shaofeng Z, et al. Related factors of the conversion time of virus nucleic acid turning negative in patients with coronavirus disease 2019 and its effect on prognosis. *Herald Med*. (2020) 39:811–4. doi: 10.3870/j.issn.1004-0781.2020.06.015

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Global trends in COVID-19 incidence and case fatality rates (2019–2023): a retrospective analysis

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Objectives: Analyzing and comparing COVID-19 infection and case-fatality rates across different regions can help improve our response to future pandemics.

Methods: We used public data from the WHO to calculate and compare the COVID-19 infection and case-fatality rates in different continents and income levels from 2019 to 2023.

Results: The Global prevalence of COVID-19 increased from 0.011 to 0.098, while case fatality rates declined from 0.024 to 0.009. Europe reported the highest cumulative infection rate (0.326), with Africa showing the lowest (0.011). Conversely, Africa experienced the highest cumulative case fatality rates (0.020), with Oceania the lowest (0.002). Infection rates in Asia showed a steady increase in contrast to other continents which observed initial rises followed by decreases. A correlation between economic status and infection rates was identified; high-income countries had the highest cumulative infection rate (0.353) and lowest case fatality rate (0.006). Low-income countries showed low cumulative infection rates (0.006) but the highest case fatality rate (0.016). Initially, high and upper-middle-income countries experienced elevated initial infection and case fatality rates, which subsequently underwent significant reductions.

Conclusions: COVID-19 rates varied significantly by continent and income level. Europe and the Americas faced surges in infections and low case fatality rates. In contrast, Africa experienced low infection rates and higher case fatality rates, with lower- and middle-income nations exceeding case fatality rates in high-income countries over time.

KEYWORDS

COVID-19, infection rates, case-fatality rates, continents, income levels

1 Introduction

Since its emergence at the end of 2019, the COVID-19 pandemic has rapidly spread globally. As of April 2023, COVID-19 has led to hundreds of millions of infections and millions of fatalities worldwide (1). Reducing the incidence and fatality rate of COVID-19 is a paramount public health concern. Recent advancements in vaccination and therapeutic interventions, along with the decrease in virulence of the COVID-19, have significantly and consistently reduced the mortality rate associated with this disease. However, the degree of the decline in COVID-19 mortality has shown regional and national variations.

The COVID-19 was first identified in China and quickly spread worldwide. During the initial phase of the epidemic, transmission occurred primarily in countries with higher

economic status, particularly in Europe and the Americas. Subsequently, the outbreak spread to the African continent, leading to a second wave of infections that peaked in late 2020, affecting numerous countries across the region (2). Statistical estimates suggest that by 2021, approximately half of the world's countries had undergone all three waves of COVID-19 infections. Preliminary research suggests that during the initial wave of COVID-19 outbreaks, the transmission rate in countries with developing economies was lower than that in developed countries (3). However, the second wave of COVID-19 outbreaks in Africa has shown a concerning trend of increased severity compared to the first wave in the region. Numerous studies have explored the incidence and case fatality rates of COVID-19, revealing significant disparities across different trajectories. However, there remains a gap in longitudinal analyses that consider the entire span of the pandemic from 2019 to 2023 (4, 5). Given the dynamic nature of the COVID-19 pandemic and the evolution of variants, there is a critical need for retrospective analyses that span multiple years. This study aims to fill the existing research gap by providing a comprehensive overview of global COVID-19 trends, including infection and fatality rates, across different continents and income levels from 2019 to 2023.

2 Methods

2.1 Data source

Publicly available data from the World Health Organization (WHO) was utilized to investigate COVID-19 infections and deaths in various countries and regions from 2019 until April 2023. To ensure the reliability and completeness of the data, we included countries and territories that reported valid data on COVID-19 infections and deaths to WHO for each consecutive month from 2019 to 2023. Ultimately, 30 countries that provided incomplete or unpublished data were excluded, resulting in a total of 178 countries being analyzed. The countries included can be found in the Supplementary material.

We computed the COVID-19 infection rate and case fatality rate for each country. The COVID-19 infection rate was determined by calculating the cumulative number of confirmed COVID-19 cases as a percentage of the total population in the respective country or continent. The case fatality rate was calculated as the cumulative number of COVID-19 deaths divided by the cumulative number of confirmed COVID-19 cases. The calculation formulas for the infection rate and case fatality rate per 100,000 population can be expressed as follows: infection rate = (number of confirmed infections/total population) × 100,000. Case fatality rate = (number of COVID-19 deaths/number of confirmed infections) × 100,000. In order to compare differences among countries at various income levels, we followed the World Bank's classification into four categories: high-income, upper-middle-income, lower-middle-income, and low-income (6).

2.2 Statistical analysis

Data about the number of infections and deaths were collected for each time interval (2019–2020/6/1, 2020/7/1–2020/12/31, 2021/1/1–2021/6/30, 2021/7/1–2021/12/31, 2022/1/1–2022/6/30, 2022/7/1–2022/12/31, 2023/1/1–2023/4/1). Descriptive statistics were applied to quantify COVID-19 infection and mortality rates across different geographical areas and time periods. Rates per 100,000 population were calculated using the direct counts from the WHO for confirmed cases, deaths, and population figures. These computations facilitated the straightforward comparison of COVID-19's impact without necessitating inferential statistics. Python 3.12 and GraphPad Prism software were utilized for graphical representations of the calculated rates to enable clear and precise visualization of the trends across regions.

3 Results

3.1 General trends in the global prevalence and case-fatality rate of COVID-19

From the emergence of COVID-19 in late 2019 to April 2023, the number of reported COVID-19 infections totaled 762,133,867, with 6,811,298 associated deaths. Europe had the highest cumulative infection rate (0.326), while Africa had the lowest (0.011). Africa experienced the highest cumulative case-fatality rate (0.020), whereas Oceania saw the lowest (0.002). When categorized by income, high-income countries exhibited the highest cumulative infection rate (0.353) and the lowest case-fatality rate (0.006). In contrast, low-income countries had a lower cumulative infection rate (0.006) and the highest cumulative case-fatality rate (0.016; Figures 1, 2, Supplementary Tables 1, 2).

3.2 Temporal trends in infection rates across continents

The COVID-19 infection rates in the Asian region showed an overall increasing trend from 2019 to April 2023. Conversely, the infection rates in the African region were comparatively lower, displaying an initial increase followed by a subsequent decrease. Similar patterns were observed in Europe, the Americas, and Oceania regions, mirroring those in Africa. Specifically, there were significant increases in infection rates from late 2021 to 2022, with Oceania experiencing minor increments. Since 2022, infection rates have gradually declined in Europe, the Americas, Oceania, and Africa. However, Asia experienced a persistent rise in infection rates, surpassing the other four continents by the first quarter of 2023 (Figure 3).

3.3 Temporal trends in case-fatality rates across continent

All continents experienced a decline in both incidence and case-fatality rates, although with short-term fluctuations. Europe saw a notable decrease from 2019 to the end of 2020, with a slight increase in the first half of 2021 followed by a slow decline to lower lethality levels. Similarly, the Americas exhibited comparable temporal trends to Europe, but with higher case-fatality rates during the initial 6 months of the pandemic. Africa and Asia

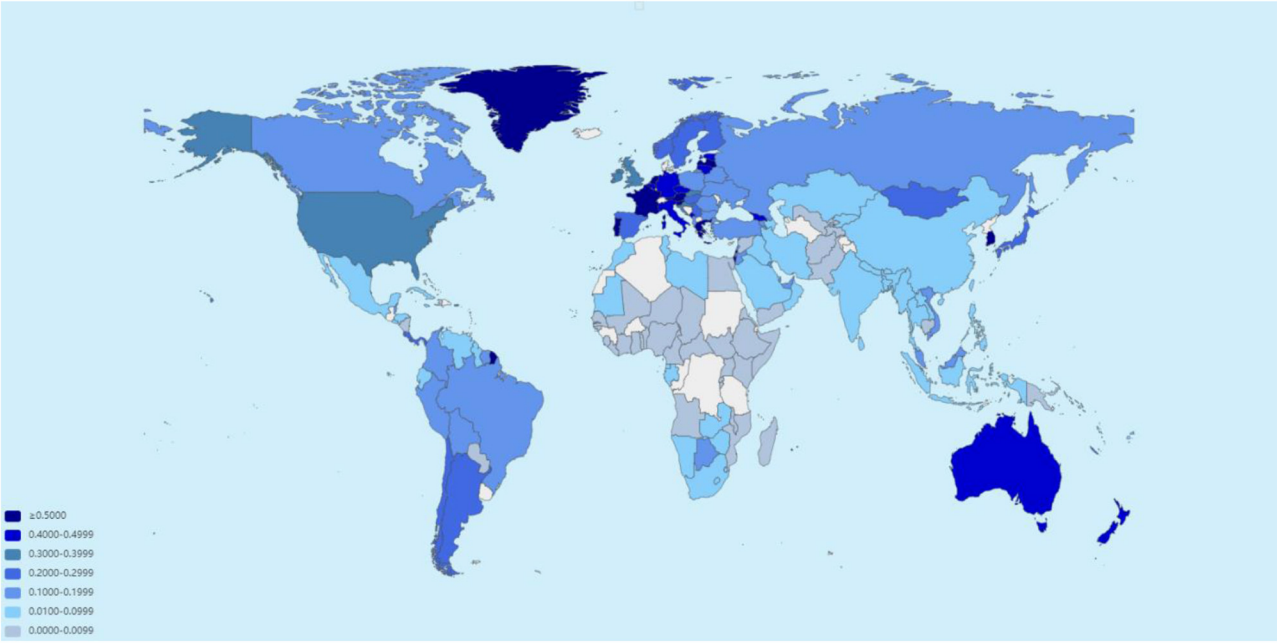

FIGURE 1
Estimated global incidence of COVID-19 as of April 2023 (data sourced from the World Health Organization).

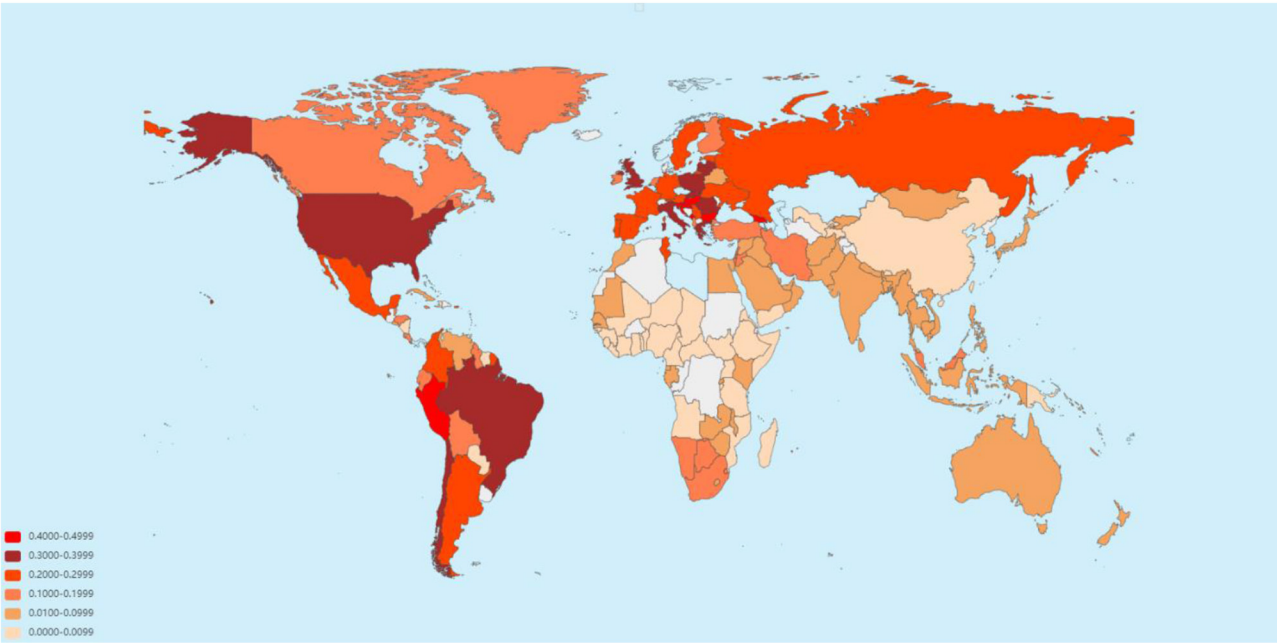

FIGURE 2
Estimated global case fatality rate of COVID-19 as of April 2023 (data sourced from the World Health Organization).

also showed declining case-fatality rates over time, with Africa experiencing a peak in mortality in the first half of 2021 and Asia seeing an upward trend in case-fatality rates compared to other continents from late 2022 to 2023. Oceania had the lowest case-fatality rates among continents, reaching a peaking in late 2020 but maintaining a low level afterward (Figure 4).

3.4 Temporal trends in infection rates in different income countries

There was a clear correlation between COVID-19 infection rates and the economic level of countries across different income categories. Over the past 3

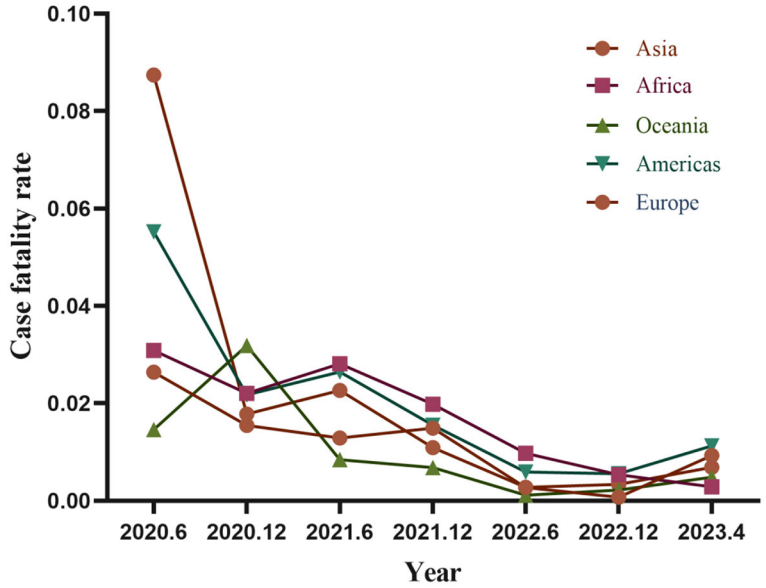

FIGURE 3
Trends in COVID-19 infection rates across continent (data sourced from the World Health Organization).

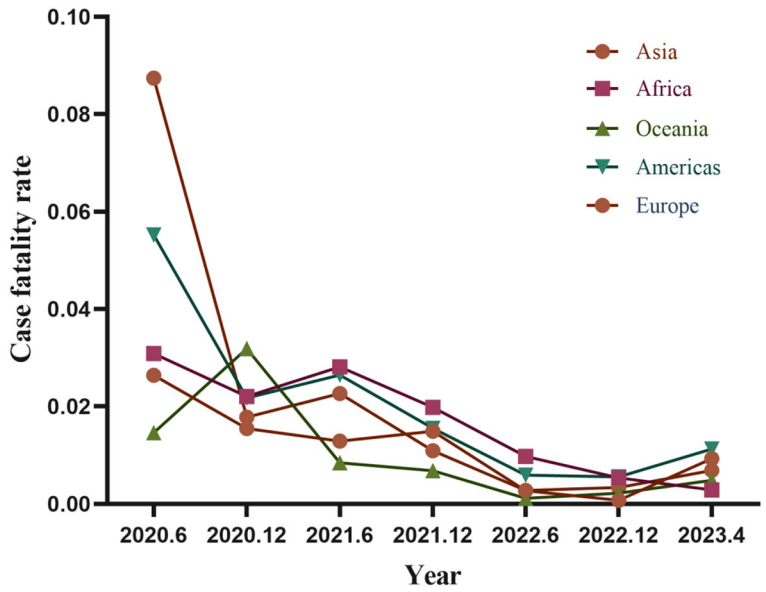

FIGURE 4
Temporal trends in case-fatality rates by continents.

years, high-income countries consistently had the highest infection rates, followed by upper-middle-income, lower-middle-income, and low-income countries with the lowest rates.

The trend in infection rates among high-income and upper-middle-income countries remained consistent, showing a continuous increase followed by a gradual decline in 2022. In contrast, infection rates in low-income and lower-middle-income countries remained low. However, low-income countries experienced a significant increase in infection rates in the first quarter of 2023, converging with the rates observed in upper-middle-income countries (Figure 5).

3.5 Time trends in case-fatality rate in different income countries

Across countries with varying income levels, case-fatality rates generally showed a downward trend. During the first half of 2023, high-income and upper-middle-income countries exhibited

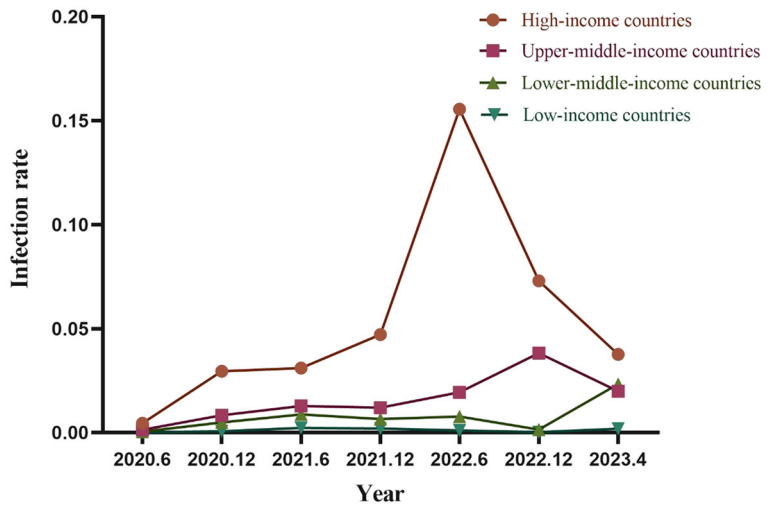

FIGURE 5
Temporal trends in infection rates in different income countries.

higher rates than low-income and lower-middle-income countries, albeit with a more pronounced decline observed among high-income and upper-middle-income countries. Conversely, lower-middle-income and low-income countries experience a slower decline and intermittent fluctuations at different stages. Starting in 2022, lower-middle-income and low-income countries began to outpace high-income and upper-middle-income countries in terms of incidence and case-fatality rates. Nonetheless, except for high-income countries, all others experienced a slight upswing in the early years of 2023, despite still being well below the initial case fatality rates observed during the COVID-19 emergence (Figure 6).

4 Discussion

These findings indicate significant variations in COVID-19 infection and case fatality rates among continents as well as countries with different income levels. Building upon these results, we further analysis to examine the underlying factors contributing to these disparities.

4.1 Prevalence of COVID-19 infections across continents

In this study, we observed that Oceania initially had low infection rates, which then significantly increased in 2022. Several factors were identified as contributing to the initially low infection rates in Oceania. Firstly, Oceania's unique geography limited the movement of people between its islands and with other continents, thereby reducing the transmission channels of the virus (7). Secondly, Oceania is comprised of only 14 countries with low population densities. During the initial stages of COVID-19, Australia and New Zealand implemented strict lockdown measures, which were highly effective in controlling and preventing the spread of COVID-19. However, due to the unsustainable nature of prolonged lockdowns, both countries eventually lifted their isolation and containment policies in 2022 (8, 9). As a result, the number of infections in Oceania showed a significant upward trend in 2022 (10). We suspect that demographics and aging play significant roles in this trend. Countries in Europe exhibit higher population densities and a more significant proportion of an aging population than other regions, with Europe accounting for nine out of the 10 countries with the highest proportion of older adult individuals worldwide. Overall, policies and responses to COVID-19 outbreaks have varied across countries, with countries in Europe and the Americas adopting comparatively more lenient measures to prevent epidemic spread compared to other continents (11). Consequently, these regions have experience consistently high infection rates. The notable increase in COVID-19 infection rates in Asia during the first quarter of 2023 can be attributed to the gradual easing of strict control measures and the relaxation of movement restrictions in China since late 2022.

The persistence of low infection rates in Africa, on the other hand, may be attributed to the age structure of the continent. The report indicates that all the top 10 countries with the highest proportion of population in the 0–14 age group are located in Africa (12). Additionally, it is worth noting that testing for COVID-19 is relatively limited in African countries with lower economic incomes, which may result in an underestimation of mortality from COVID-19 in Africa (13).

Several studies have demonstrated a strong correlation between the prevalence of COVID-19 and environmental conditions. Factors such as the duration, temperatures, humidity, PM2.5, and sunlight exposure values (14). Specifically, some studies have indicated a positive relationship between PM2.5 and COVID-19 cases. Other studies suggest that higher ultraviolet (UV) radiation may inhibit airborne transmission of the virus. However, in outdoor environments, this effect may be influenced by factors such as humidity, temperature, and ventilation (15). Certain studies have found that vitamin D may reduce susceptibility to COVID-19 (16). For instance, one study correlated COVID-19 mortality

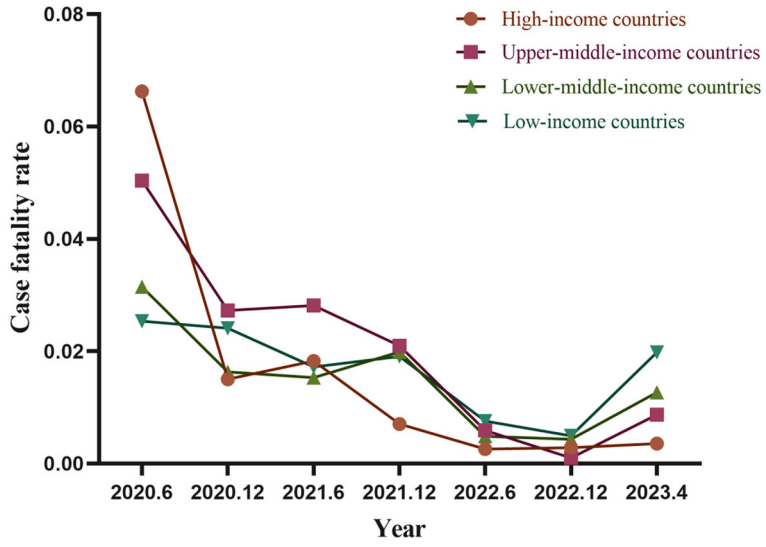

FIGURE 6
Temporal trends in case-fatality rate in different income countries.

data with mean 25(OH)D levels from multiple countries and reported similar observations. Another study noted that severe vitamin D deficiency is associated with comorbidities like chronic kidney disease (CKD), chronic obstructive pulmonary disease (COPD), and cerebrovascular disease (17), which increase the risk of COVID-19 infections. Sunlight is a major source of vitamin D for the body, especially in regions with extended daylight hours such as Oceania and Africa (18). Conversely, countries in Europe and America with shorter periods of sunlight have demonstrated greater susceptibility to COVID-19 infections. However, additional research is necessary to validate these hypotheses and unravel their underlying mechanisms.

4.2 Case-fatality rate trends across continents

At the onset of the epidemic, Africa had a higher COVID-19 mortality rate compared to Oceania. However, from 2021, a declining trend in COVID-19 mortality was observed in most continents except Africa, where the mortality rate remained high. As mentioned in the previous section, Oceania exhibited a low rate of COVID-19 infection during the initial year of the outbreak due to its favorable geographic location. The resilient healthcare system in Oceania also played a significant role in maintaining a low rate of COVID-19 mortality (19). Europe and North America are recognized for having the most advanced healthcare systems for COVID-19 with North American countries like the United States featuring advanced healthcare technologies and facilities. Additionally, Canada's universal healthcare system contributes significantly to the prevention and treatment of COVID-19. Asian countries exhibit substantial disparities in healthcare provision, with Japan and Singapore showcasing high rates of healthcare penetration (20). China has made notable progress in healthcare reform and development, although challenges persist regarding inconsistent service quality and resource allocation. African countries face healthcare system challenges, including inadequate resources and limited coverage (21). Undeniably, COVID-19 mortality is closely linked to a country's healthcare system. Accurately evaluating the influence of healthcare resources on COVID-19 mortality requires careful consideration of multiple factors.

In addition, effective vaccination is strongly associated with mortality reduction from COVID-19. Notably, there are significant variations in vaccination rates against COVID-19 across continents. The European and North American regions have demonstrated higher vaccination rates, resulting in a substantial number of deaths averted per vaccination administered within these geographic areas. In contrast, vaccination coverage varies significantly by country in Asian countries, and progress in Africa has been slow (22). Numerous studies have highlighted the potential benefits of vaccination, including the prevention of further mutations through decreased transmission of COVID-19, as well as the potential reduction in case fatality and overall mortality rates associated with COVID-19 (23).

The influence of climate, temperature, and ecology in different regions on COVID-19 mortality cannot be disregarded. A study revealed that cities with over 100,000 inhabitants experienced a higher COVID-19 mortality rate when there was a reduction in forest cover (24). It was also noted that countries in the Americas and Europe tend to have higher forest cover. Temperature has also been found to impact COVID-19 mortality rates. Higher mean monthly temperatures in European countries and temperate climates in the United States were associated with lower daily mortality rates. However, when considering the interaction between monthly temperature and vaccination rates, the effect of vaccination campaigns on mortality was more significant at lower temperatures than at higher temperatures (25). Multicenter research is required to elucidate further the relationship between the natural environment and COVID-19 mortality.

4.3 Prevalence of COVID-19 infections across economically diverse countries

Following the COVID-19 pandemic, nations across various income brackets have exhibited an overall escalation in their infection rates. High-income countries have consistently maintained a high infection rate, while low-income countries exhibit a significantly lower infection rate without an evident upward or downward trend. Within a few months following the spread of COVID-19, the epicenter of the pandemic quickly shifted from Asia to Europe and the Americas. The Asian countries, having experienced the SARS in 2003, responded more promptly to the current situation. In contrast, Western countries were slow in recognizing and responding to COVID-19, resulting in a steep increase in infections due to its high infectivity and delayed government interventions (26). Some low-income countries implemented strict lockdowns and restrictions during the early phases, resulting in limited international travel and population movement. These countries also have lower populations and a higher prevalence of outdoor living and working conditions than high-income countries (27). In addition, as previously mentioned high-income countries generally possess superior healthcare systems and information technology infrastructures, which allow them to collect, compile, and report infection data more accurately and promptly than low-income countries. Therefore, low-income countries may face difficulties in collecting complete data and experience time-consuming processes, leading to a potential underestimation of infection rates.

High-income countries also showed a clear upward trend in infection rates in mid-2022, possibly related to the emergence of omicron following the mutation of COVID-19 in late 2021. With the gradual liberalization of global vaccination measures, the strong transmissibility of omicron did not significantly reduce infection rates, despite the broader availability of vaccines in high-income and upper-middle-income countries.

In addition, aging demographics may play an essential role in the higher prevalence of COVID-19 because older people in low- and middle-income countries tend to be healthier on average than older people in wealthier countries. This difference may be because individuals with diseases in low- and middle-income countries face a higher risk of premature death at a young age (28). Aging demographics are often associated with weakened immune systems and the coexistence of multiple chronic diseases. In addition, high-income countries tend to experience more pronounced population aging due to their superior social security systems, higher life expectancy, and better overall health status (29). According to World Bank data, by 2021, the top 10 countries in terms of aging are predominantly high-income or upper-middle-income countries. This trend may contribute to the increased infection rates observed in high-income countries (30). A review suggests that countries with poorer sanitation and lower socioeconomic status may develop more robust innate immune systems during childhood due to frequent exposure to environmental microbes. This exposure may later reduce susceptibility to COVID-19 (31). In addition, another study found an association between higher rates of obesity and increased prevalence of COVID-19 in these 168 countries with available data. For every 1% increase in the prevalence of obesity, the incidence of COVID-19 cases increased by 6.6% (32). High-income countries, characterized by differences in diet and lifestyle, tend to have higher rates of obesity, which may contribute to a higher incidence of COVID-19 infection. Another study, which collected data from 61 countries worldwide, found a positive correlation between the number of COVID-19 cases and a country's GDP. The study highlighted countries with a higher ecological footprint and greater consumption had higher infection rates (33). Furthermore, high-income countries characterized by increased population mobility, higher levels of urbanization and greater population concentration also had correspondingly higher rates of COVID-19 infection.

4.4 Case-fatality rates in different income countries

A study published in 2022 reported a positive correlation between GDP and COVID-19 mortality, especially in wealthy countries (34). However, the results of our study indicate that as of April 2023, COVID-19 incidence was higher in high-income countries, while mortality rates were higher in low-income countries. This may be because high-income countries were among the first to be affected by virus transmission due to greater population mobility. As a result, during the early stages of the COVID-19 epidemic, high-income countries continued to experience elevated infection and mortality rates. However, they also had sufficient economic and medical resources to support significant reductions in infection rates and deaths.

According to a recent study, countries with higher hospital beds per 100,000 population showed lower COVID-19 incidence and mortality rates. Conversely, inadequate bed capacity and overcrowding in medical centers may contribute to increased mortality (35). Another study found that higher median population age, lower population density, and lower hospital bed availability were significantly associated with higher COVID-19 mortality rates (36). Notably, the median age of a country is highly correlated with its GDP per capita, with high-income countries typically having both a higher median age and a higher GDP (37). While these countries may have higher rates of COVID-19 infection and death, the overall mortality of patients with COVID-19 tends to be lower due to better medical and economic conditions. Globally, there is a significant disparity between the proportion of fully vaccinated population and COVID-19 mortality rates. As countries' income levels rise, vaccination rates increase and deaths per 1,000 cases decrease (38). However, following the emergence of the Omicron variant in December 2021, a significant proportion of the US population is fully vaccinated, highlighting the benefits of high vaccination rates in higher-income countries by showing lower case fatality rates for Omicron. However, by the end of 2022, only 76% of people aged 60 years and older had been fully vaccinated, well below the World Health Organization's target, particularly in low- and middle-income countries (39).

As noted above, several studies have suggested a possible association between obesity and increased susceptibility and mortality from COVID-19 (40). However, it is noteworthy that countries with a higher prevalence of obesity are often middle-

and high-income countries with better healthcare resources. For example, countries such as Qatar, Germany and New Zealand, despite having obesity rates of over 25% in their adult populations, have significantly lower mortality rates despite high infection rates (41). Conversely, some South American countries with moderate levels of obesity still have higher mortality rates, mainly due to limited healthcare facilities. One study found that low BMI is associated with malnutrition, weakness and muscle wasting, all associated with an increased risk of COVID-19 mortality (42). In addition, low-income countries may face challenges such as wasting, weakened immune function and a higher prevalence of burdensome diseases such as diabetes and hypertension due to inadequate health systems and poor socioeconomic conditions. These factors may contribute to an increased risk of severe illness and death following COVID-19 infection.

Another study analyzing data from 81 countries found that countries with a higher proportion of urban population and higher rates of urbanization had, on average, the same or fewer COVID-19 deaths than less urbanized countries (43). Despite the faster spread of COVID-19 in urban areas, the number of deaths may be lower in highly urbanized countries due to economic development, trust in government and well-functioning health systems.

It is essential to acknowledge that in our discussion of case-fatality rates among different continents and income countries, Europe and the Americas consist predominantly of high-income countries. Conversely, out of the 50 countries in Africa, only Seychelles is classified as a high-income country. Therefore, we must recognize the interplay between region and income level.

In addressing the global disparity in COVID-19 infection and fatality rates, it is imperative to consider the impact of public health and social measures. Studies have shown that interventions like mask wearing, hand washing, and social distancing have varied efficacy across different regions, largely dependent on the rigor and consistency of their implementation (44). Furthermore, the economic responses to the pandemic have been significant, with policies ranging from health system investments to fiscal measures aimed at mitigating socio-economic impacts. For instance, OECD reports highlight the necessity of substantial investments in health systems to bolster resilience against public health emergencies, suggesting these investments are crucial components of broader economic stability strategies during crises (45). Additionally, geopolitical responses, including ongoing conflicts, travel restrictions, and changes to international trade policies, have influenced virus transmission rates and the economic interdependencies that shape global health outcomes (46, 47). These measures, while essential in controlling the spread of the virus, have also led to significant social and economic repercussions, which have been unevenly distributed and have exacerbated existing inequalities. Thus, understanding the full impact of these dynamics requires a multidimensional analysis that accounts for the complex interplay between public health initiatives and socio-economic factors.

5 Limitations

This study has several limitations that need to be acknowledged. Firstly, while the data utilized in this analysis comes from the World Health Organization (WHO), which employs rigorous methods to ensure accuracy and reliability, differences in COVID-19 reporting standards and statistics between countries and regions may still affect data comparability. These include variations in testing methods, standards, and reporting mechanisms. Despite these potential inconsistencies, the WHO continuously updates and verifies the data to reflect true trends as accurately as possible, thus maintaining the integrity of a globally recognized health data management system. Secondly, the analytical approach of this study was predominantly descriptive. We acknowledge that descriptive statistics do not permit causal inferences. However, they are invaluable for illustrating global and regional trends in COVID-19 infection and fatality rates, providing a foundational understanding that is essential for hypothesis generation and future analytical explorations. This approach was chosen due to the exploratory nature of the study and the limitations imposed by the available data. Moreover, the potential underreporting of COVID-19 cases and deaths, particularly in regions with limited testing capabilities and varying case definitions, may skew our understanding of the pandemic's true burden.

Despite these limitations, our study provides valuable insights into the global dynamics of the COVID-19 pandemic and underscores significant disparities in infection and mortality rates across different continents and income levels. Future research should not only aim to employ more comprehensive and precise data collection methods but also advance deeper and more analytical studies that consider demographic and other relevant factors. By extending beyond the mitigation of reporting biases, we can explore nuanced interpretations and foster more robust conclusions that will enhance our understanding of the pandemic's impact and support the development of more effective public health strategies and policies.

6 Conclusions

Overall, a decreasing trend in COVID-19 case-fatality rates was observed in different countries and regions, while infection rates showed an increasing trend. However, there were significant differences among different regions and income levels. Although mortality rates have notably decreased, especially in high-income countries, they remain high in many low-income countries. COVID-19 is likely to persist for a long time, requiring continued efforts to reduce infection rates and to conduct active research into prevention and treatment measures according to the geographical and economic conditions of different countries.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary material.

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation

and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and the institutional requirements.

Author contributions

JD: Writing – original draft. H-mL: Writing – review & editing. YM: Writing – review & editing. A-wC: Writing – review & editing. Y-yQ: Writing – review & editing. X-pZ: Writing – review & editing. C-qH: Writing – review & editing, Writing – original draft, Data curation.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1355097/full#supplementary-material

References

1. *WHO Coronavirus disease (COVID-19) dashboard*. Available online at: https://covid19.who.int (accessed April, 2023).

2. Dryden M, Mudara C, Vika C, Blumberg L, Mayet N, Cohen C, et al. Post-COVID-19 condition 3 months after hospitalisation with SARS-CoV-2 in South Africa: a prospective cohort study. *Lancet Glob Health*. (2022) 10:e1247–e56. doi: 10.1016/S2214-109X(22)00286-8

3. Huang C, Wang Y, Li X, Ren L, Zhao J, Hu Y, et al. Clinical features of patients infected with 2019 novel coronavirus in Wuhan, China. *Lancet*. (2020) 395:497–506. doi: 10.1016/S0140-6736(20)30183-5

4. How epidemiology has shaped the COVID pandemic. *Nature*. (2021) 589:491–2. doi: 10.1038/d41586-021-00183-z

5. Gohari K, Kazemnejad A, Sheidaei A, Hajari S. Clustering of countries according to the COVID-19 incidence and mortality rates. *BMC Public Health*. (2022) 22:632. doi: 10.1186/s12889-022-13086-z

6. Bank TW. (2023). Available online at: https://data.worldbank.org/indicator/SP.POP.TOTL (accessed April, 2023).

7. Kopsidas I, Karagiannidou S, Kostaki EG, Kousi D, Douka E, Sfikakis PP, et al. Global distribution, dispersal patterns, and trend of several omicron subvariants of SARS-CoV-2 across the globe. *Trop Med Infect Dis*. (2022) 7:373. doi: 10.3390/tropicalmed7110373

8. Fouda A, Mahmoudi N, Moy N, Paolucci F. The COVID-19 pandemic in Greece, Iceland, New Zealand, and Singapore: health policies and lessons learned. *Health Policy Technol*. (2020) 9:510–24. doi: 10.1016/j.hlpt.2020.08.015

9. Stobart A, Duckett S. Australia's response to COVID-19. *Health Econ Policy Law*. (2022) 17:95–106. doi: 10.1017/S1744133121000244

10. Cao X, Li Y, Zi Y, Zhu Y. The shift of percent excess mortality from zero-COVID policy to living-with-COVID policy in Singapore, South Korea, Australia, New Zealand and Hong Kong SAR. *Front Public Health*. (2023) 11:1085451. doi: 10.3389/fpubh.2023.1085451

11. Kwon S, Joshi AD, Lo CH, Drew DA, Nguyen LH, Guo CG, et al. Association of social distancing and face mask use with risk of COVID-19. *Nat Commun*. (2021) 12:3737. doi: 10.1038/s41467-021-24115-7

12. Chen Y, Klein SL, Garibaldi BT Li H, Wu C, Osevala NM, et al. Aging in COVID-19: vulnerability, immunity and intervention. *Ageing Res Rev*. (2021) 65:101205. doi: 10.1016/j.arr.2020.101205

13. Owoyemi A, Balogun T, Okoro J, Ndoro T, Fasominu O, Atanda A, et al. An assessment of systemic factors and COVID-19 mortality in Africa. *Int J Public Health*. (2022) 67:1604915. doi: 10.3389/ijph.2022.1604915

14. Benedetti F, Pachetti M, Marini B, Ippodrino R, Gallo RC, Ciccozzi M, et al. Inverse correlation between average monthly high temperatures and COVID-19-related death rates in different geographical areas. *J Transl Med*. (2020) 18:251. doi: 10.1186/s12967-020-02418-5

15. Kalluçi E, Noka E, Bani K, Noka E, Bardhi S, Macchia A, et al. Correlation between COVID-19 and air pollution: the effects of PM25 and PM10 on COVID-19 outcomes. *Eur Rev Med Pharmacol Sci*. (2023) 27:39–47. doi: 10.26355/eurrev_202312_34688

16. Padhi S, Suvankar S, Panda VK, Pati A, Panda AK. Lower levels of vitamin D are associated with SARS-CoV-2 infection and mortality in the Indian population: an observational study. *Int Immunopharmacol*. (2020) 88:107001. doi: 10.1016/j.intimp.2020.107001

17. Villasis-Keever MA, López-Alarcón MG, Miranda-Novales G, Zurita-Cruz JN, Barrada-Vázquez AS, González-Ibarra J, et al. Efficacy and safety of vitamin D supplementation to prevent COVID-19 in Frontline healthcare workers. A randomized clinical trial. *Arch Med Res*. (2022) 53:423–30. doi: 10.1016/j.arcmed.2022.04.003

18. Contreras-Bolívar V, García-Fontana B, García-Fontana C, Muñoz-Torres M, Vitamin D and COVID-19: where are we now? *Postgrad Med*. (2023) 135:195–207. doi: 10.1080/00325481.2021.2017647

19. Verguet S, Hailu A, Eregata GT, Memirie ST, Johansson KA, Norheim OF. Toward universal health coverage in the post-COVID-19 era. *Nat Med*. (2021) 27:380–7. doi: 10.1038/s41591-021-01268-y

20. Kayano T, Nishiura H. A comparison of case fatality risk of COVID-19 between Singapore and Japan. *J Clin Med*. (2020) 9:3326. doi: 10.3390/jcm9103326

21. Lal A, Erondu NA, Heymann DL, Gitahi G, Yates R. Fragmented health systems in COVID-19: rectifying the misalignment between global health security and universal health coverage. *Lancet*. (2021) 397:61–7. doi: 10.1016/S0140-6736(20)32228-5

22. Spinardi J, Dantas AC, Carballo C, Thakkar K, Akoury NA, Kyaw MH, et al. Narrative review of the evolution of COVID-19 vaccination recommendations in countries in Latin America, Africa and the Middle East, and Asia. *Infect Dis Ther*. (2023) 12:1237–64. doi: 10.1007/s40121-023-00804-2

23. Zhou F, Hu TJ, Zhang XY, Lai K, Chen JH, Zhou XH. The association of intensity and duration of non-pharmacological interventions and implementation of vaccination with COVID-19 infection, death, and excess mortality: natural experiment in 22 European countries. *J Infect Public Health*. (2022) 15:499–507. doi: 10.1016/j.jiph.2022.03.011

24. Viezzer J, Biondi D. The influence of urban, socio-economic, and eco-environmental aspects on COVID-19 cases, deaths and mortality: a multi-city case in the Atlantic Forest, Brazil. *Sustain Cities Soc*. (2021) 69:102859. doi: 10.1016/j.scs.2021.102859

25. Kumar A, Misra S, Verma V, Vishwakarma RK, Kamal VK, Nath M, et al. Global impact of environmental temperature and BCG vaccination coverage on the transmissibility and fatality rate of COVID-19. *PLoS ONE.* (2020) 15:e0240710. doi: 10.1371/journal.pone.0240710

26. Anderson RM, Heesterbeek H, Klinkenberg D, Hollingsworth TD. How will country-based mitigation measures influence the course of the COVID-19 epidemic? *Lancet.* (2020) 395:931–4. doi: 10.1016/S0140-6736(20)30567-5

27. Wong CKH, Wong JYH, Tang EHM, Au CH, Lau KTK, Wai AKC. Impact of National containment measures on decelerating the increase in daily new cases of COVID-19 in 54 countries and 4 epicenters of the pandemic: comparative observational study. *J Med Internet Res.* (2020) 22:e19904. doi: 10.2196/19904

28. Das M. From crisis to insight: navigating public health challenges in an aging society-lessons from Hong Kong's COVID-19 experience. *J Urban Health.* (2023) 100:852–9. doi: 10.1007/s11524-023-00760-9

29. Yang G, Zhang L. Relationship between aging population, birth rate and disposable income per capita in the context of COVID-19. *PLoS ONE.* (2023) 18:e0289781. doi: 10.1371/journal.pone.0289781

30. WBRF. *World Development Indicators*. Galax, VA: WBRF (2019).

31. Sehrawat S, Rouse BT. Does the hygiene hypothesis apply to COVID-19 susceptibility? *Microbes Infect.* (2020) 22:400–2. doi: 10.1016/j.micinf.2020.07.002

32. Foo O, Hiu S, Teare D, Syed AA, Razvi S. A global country-level analysis of the relationship between obesity and COVID-19 cases and mortality. *Diabetes Obes Metab.* (2021) 23:2697–706. doi: 10.1111/dom.14523

33. Ma L, Yu Z, Jiao Y, Lin L, Zhong W, Day SW, et al. Capacity of transportation and spread of COVID-19-an ironical fact for developed countries. *Environ Sci Pollut Res Int.* (2021) 28:37498–505. doi: 10.1007/s11356-021-12765-5

34. Yao L, Aleya L, Howard SC, Cao Y, Wang C-Y, Day SW, et al. Variations of COVID-19 mortality are affected by economic disparities across countries. *Sci Total Environ.* (2022) 832:154770. doi: 10.1016/j.scitotenv.2022.154770

35. McGowan VJ, Bambra C. COVID-19 mortality and deprivation: pandemic, syndemic, and endemic health inequalities. *Lancet Public health.* (2022) 7:e966–75. doi: 10.1016/S2468-2667(22)00223-7

36. Arbel Y, Arbel Y, Kerner A, Kerner M. What is the optimal country for minimum COVID-19 morbidity and mortality rates? *Environ Sci Pollut Res Int.* (2023) 30:59212–32. doi: 10.1007/s11356-023-26632-y

37. Munford L, Khavandi S, Bambra C. COVID-19 and deprivation amplification: an ecological study of geographical inequalities in mortality in England. *Health Place.* (2022) 78:102933. doi: 10.1016/j.healthplace.2022.102933

38. Rzymski P, Szuster-Ciesielska A. The COVID-19 vaccination still matters: omicron variant is a final wake-up call for the rich to help the poor. *Vaccines.* (2022) 10:1070. doi: 10.3390/vaccines10071070

39. Wong MK, Brooks DJ, Ikejezie J, M Gacic-Dobo, L Dumolard, Y Nedelec, et al. COVID-19 mortality and progress toward vaccinating older adults - World Health Organization, Worldwide, 2020-2022. *MMWR Morb Mortal Wkly Rep.* (2023) 72:113–8. doi: 10.15585/mmwr.mm7205a1

40. Srivastava S, Rathor R, Singh S, Kumar B, Suryakumar G. Obesity: a risk factor for COVID-19. *Adv Exp Med Biol.* (2021) 1352:195–210. doi: 10.1007/978-3-030-85109-5_12

41. de Leeuw AJM, Oude Luttikhuis MAM, Wellen AC, Müller C, Calkhoven CF. Obesity and its impact on COVID-19. *J Mol Med.* (2021) 99:899–915. doi: 10.1007/s00109-021-02072-4

42. Bouziotis J, Arvanitakis M, Preiser JC. Association of body mass index with COVID-19 related in-hospital death. *Clin Nutr.* (2022) 41:2924–6. doi: 10.1016/j.clnu.2022.01.017

43. Naudé W, Nagler P. COVID-19 and the city: did urbanized countries suffer more fatalities? *Cities.* (2022) 131:103909. doi: 10.1016/j.cities.2022.103909

44. Talic S, Shah S, Wild H, Gasevic D, Maharaj A, Ademi Z, et al. Effectiveness of public health measures in reducing the incidence of COVID-19, SARS-CoV-2 transmission, and covid-19 mortality: systematic review and meta-analysis. *BMJ.* (2021) 375:e068302. doi: 10.1136/bmj-2021-068302

45. *How COVID-19 has Changed Public Policy*. (2020). Available online at: https://www.hks.harvard.edu/faculty-research/policy-topics/public-leadership-management/how-covid-19-has-changed-public-policy (accessed April, 2023).

46. *Coronavirus Pandemic (COVID-19).* (2024). Available online at: https://ourworldindata.org/coronavirus (accessed April, 2023).

47. Chumachenko D, Chumachenko T. Impact of war on the dynamics of COVID-19 in Ukraine. *BMJ Glob Health.* (2022) 7:e009173. doi: 10.1136/bmjgh-2022-009173

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Individual risk factors associated with SARS-CoV-2 infection during Alpha variant in high-income countries: a systematic review and meta-analysis

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Objectives: This study aimed to systematically appraise risk factors associated with SARS-CoV-2 infection in high-income countries during the period of predominance of the Alpha variant (January 2020 to April 2021).

Methods: Four electronic databases were used to search observational studies. Literature search, study screening, data extraction and quality assessment were conducted by two authors independently. Meta-analyses were conducted for each risk factor, when appropriate.

Results: From 12,094 studies, 27 were included. The larger sample size was 17,288,532 participants, more women were included, and the age range was 18–117 years old. Meta-analyses identified men [Odds Ratio (OR): 1.23, 95% Confidence Interval (CI): 1.97–1.42], non-white ethnicity (OR: 1.63, 95% CI: 1.39–1.91), household number (OR: 1.08, 95% CI: 1.06–1.10), diabetes (OR: 1.22, 95% CI: 1.08–1.37), cancer (OR: 0.82, 95% CI: 0.68–0.98), cardiovascular diseases (OR: 0.92, 95% CI: 0.84–1.00), asthma (OR: 0.83, 95% CI: 0.75–0.92) and ischemic heart disease (OR: 0.82, 95% CI: 0.74–0.91) as associated with SARS-CoV-2 infection.

Conclusion: This study indicated several risk factors for SARS-CoV-2 infection. Due to the heterogeneity of the studies included, more studies are needed to understand the factors that increase the risk for SARS-CoV-2 infection.

Systematic review registration: https://www.crd.york.ac.uk/prospero/display_record.php?ID=CRD42021244148, PROSPERO registration number, CRD42021244148.

KEYWORDS

SARS-CoV-2, infectious disease, risk-factors, systematic review, meta-analysis

Introduction

In December 2019, an atypical pneumonia outbreak was registered in the Wuhan province. The Chinese authorities later identified a new virus – severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) – as the pathogen originating the outbreak. The globalized world propelled its dissemination, and in just a few months, COVID-19 reached several countries. On March 11th of 2020, the World Health Organization declared COVID-19 as a pandemic (1), which on August 24th of 2022 had already infected almost 600 million people and caused over 6 million deaths globally (2). Due to its utmost impact on overall human life, the United Nations Organization has declared COVID-19 a social, human, and economic crisis (3).

Recognizing the rapid spread and severe impact of the pandemic, researchers have been working to understand the virus and its effects. Hence, a large volume of literature on SARS-CoV-2 infection has been published, namely epidemiological characteristics of positive cases and outcomes. Thus, leading to the development of systematic review (SR) on risk factors for developing infection, severe disease and mortality. SRs identified several factors associated with severe COVID-19, such as being older (4, 5), male (4, 5), having a high body mass index (4, 5) and multiple previous comorbidities [e.g., hypertension, diabetes, cardiovascular disease, chronic obstructive pulmonary disease (4), and active cancer (5)]. Other SRs identified factors associated with SARS-CoV-2 infection, such as lack of protective personal equipment, being female, poor access to healthcare, high volume of tourism and high population density. However, SRs on risk factors for infection were mainly restricted to specific subgroups (i.e., health workers) (6) or continents (i.e., Africa) (7), in which factors might be different to other contexts due to specific contacts and demographics.

Furthermore, when comparing infection rates, a disparity seems to emerge between different economic contexts. High-income countries reported higher infection rates than low-income countries (8), which might be partially explained by different contextual factors, medical infrastructures, and human and technical resources. There was only one SR in high-income countries, conducted in the UK, regarding risk factors for SARS-CoV-2 infection. This SR found that older adults, being male, black, having previous comorbidities, living in urban areas and more deprived areas were associated with a higher risk of SARS-CoV-2 infection. However, this search ended in early pandemic stages (April 2020) and was restricted to England and Wales (9).

Additionally, the evolving understanding of risk factors revealed some unique characteristics of COVID-19. Although COVID-19 is a respiratory manifestation, evidence shows that some risk factors for SARS-CoV-2 infection are different from other infectious respiratory diseases in high-income countries, i.e., pneumonia was more common in women and COVID-19 was more common in men (10). There is also contradictory evidence regarding the effect of some diseases, such as diabetes (11, 12) and cancer (13, 14). Moreover, SARS-CoV-2 variants show different transmissibility between them depending on both characteristics of the variants and the population where it spreads, translating into different case severity (15, 16). The Delta variant already seemed more transmissible than Alpha, i.e., showing differences in the characteristics of index cases (17). In the majority of high-income countries, the Alpha variant was the most predominant variant responsible for SARS-CoV-2 epidemic surges between the end of 2020 and the first half of 2021 (17, 18). Given these complexities and heterogeneity, there is a need for focused research on specific periods and contexts. Thus, given the contextual differences between high- and low-income countries and possible differences in the risk of infection according to different SARS-CoV-2 variants, we aim to systematically appraise and quantify the risk factors associated with SARS-CoV-2 infection during the period of predominance of the Alpha variant in high-income countries.

Methods

This SR protocol has been developed according to the Preferred Reporting Items for Systematic review and Meta-Analysis Protocols (PRISMA-P) (19) and reported in accordance with MOOSE (Meta-analysis Of Observational Studies in Epidemiology) guidelines (Supplementary File 1) (20). We have registered the protocol in the International Prospective Register of Systematic Reviews (PROSPERO), registration ID number: CRD42021244148.

Data sources and search strategy

The data sources comprised PubMed; Web of Science; EMBASE; MedRxiv, and international conferences (European Scientific Conference on Applied Infectious Disease Epidemiology, ESCAIDE) relevant for this matter, from 2020 and 2021. The World Congress on Public Health (WCPH) and the European Public Health Conference (EPHC) were also considered, but the abstracts presented in these two conferences were published, therefore appearing in the searched databases. The databases were searched from 1/1/2020 to 22/4/2021 when the Alpha variant was predominant in the majority of high-income countries (18) and the last search was conducted on 31/5/2021.

Search terms (text words and Mesh terms) were drawn up for three search concepts: SARS-CoV-2, risk factors, and high-income country. The search in the conference abstract book was done using the words "COVID-19" or "SARS-CoV-2." High-income countries were defined according to the classification from the World Bank (21). The detailed search strategy is provided in Supplementary File 2. The literature search was performed by two authors, an investigator and a librarian (M.M. and H.D., respectively).

Inclusion and exclusion criteria

We included all Portuguese, English, French, Spanish and Italian studies that evaluated the risk factors for SARS-CoV-2 infection in high-income countries with a confirmed Polymerase chain reaction (PCR) SARS-CoV-2 positive test result on people 18 years old or more. After polling the articles and eliminating duplicates, a manual review of titles and abstracts was performed, screening for relevant topics and keywords. Similar studies, in title and authors, found in different databases were screened and confirmed to have different objectives. We excluded articles covering reinfection, specific settings and populations (e.g., health workers, schools, hospitalized patients, pregnant women and people with disabilities). We also excluded articles where diagnosis of SARS-CoV-2 infection was self-reported, or the case definition was a composite of various tests (PCR, antigen,

blood samples), and suspected and/or clinically diagnosed cases. Genetic factors were also excluded due to the complexity of the analysis. Environmental factors were excluded due to their specific time–space patterns, thus challenging pooled estimates. Articles lacking information on SARS-CoV-2 infection measurement or population age were excluded for consistency. See Figure 1 for a detailed summary of the selection process.

All individual-based study designs were potentially eligible; however, we have decided to exclude Letters to Editors, Editorials, Comments, Opinions and Ecological studies to analyze more robust information, considering the growing volume of publications. The eligibility criteria were applied by two authors independently (M.M. and S.C.) to titles/abstracts for full-text assessment. References management and screening was carried out using the Rayyan website (22).

Data extraction and quality assessment

We used a random sample (10%) to pilot the extraction form and two authors (M.M and S.C.) extracted the data. After testing, we adjusted the extraction form including more fields to characterize the studies, namely sample description and controlling factors. Thus, data was extracted using a standardized form which included first author, title, year of publication, study design, study location and duration, study population, data source, sample size, sample description (age and sex), factors identified, estimated measure of effect, and control factors for the statistical analysis. Sample description was deemed important for comparison between studies.

Quality assessment of the studies was conducted by the same two authors using the Joanna Briggs Institute tool for analytical cross-sectional studies (23) and Newcastle-Ottawa Scale (NOS) for case–control and cohort studies (24). Since there is no universal criterion for high-quality studies, we considered those scoring ≥7 as high-quality, a cut-off commonly used in the literature (25). Conflicts between raters in classifying individual items of the abovementioned tools were resolved by discussion with a third author (A.L.).

Data analysis

For each study, we undertook a descriptive characterization. When at least two studies reported an exposure in a consistent way (same reference and categories), these were combined in a

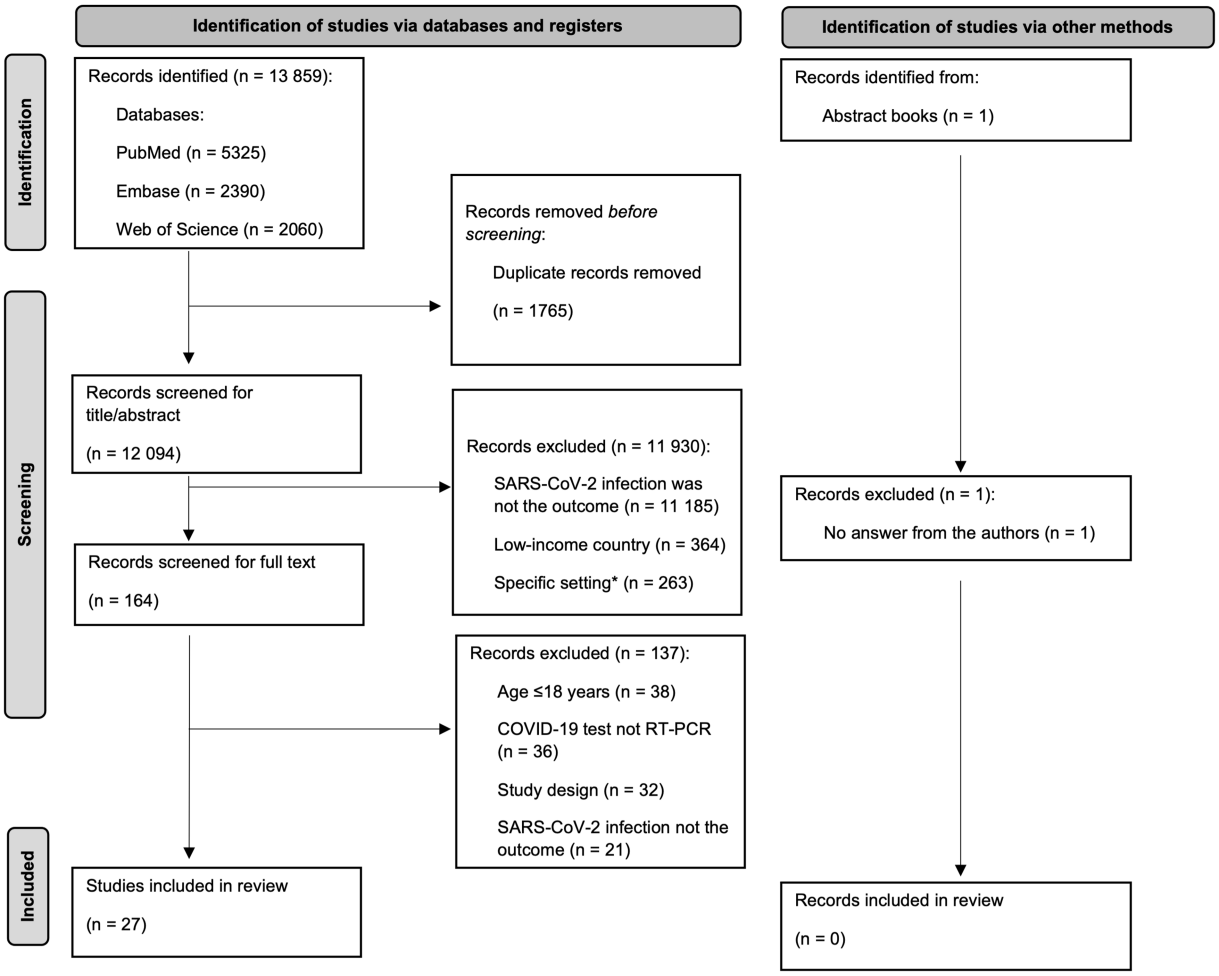

FIGURE 1
Diagram of study selection, adapted from PRISMA group 2020 flow diagram (high-income countries, 2020–2021).

meta-analysis (26, 27). To obtain pooled estimates of SARS-CoV-2 infection risk factors while improving results comparability, we chose to include a single effect measure, decided as the most often reported. In studies that reported several multivariable-adjusted effect estimates, we selected the one that adjusted for more potential confounders (26–28). Each study was weighted in the meta-analysis using the inverse variance of the effect estimate (29).

Heterogeneity between estimates was assessed using the I^2 statistic, with higher values reflecting increased heterogeneity. For higher heterogeneity coefficients (I^2) with statistically significant tests rejecting homogeneity, we used a random-effects model; otherwise, we chose a fixed-effects model (30). We performed sensitivity analyses for ethnicity, diabetes and comorbidities, giving greater depth to the analysis, since they contained various categories within, susceptibly for SARS-CoV-2 infection might be misled. We performed two sensitivity analyses for ethnicity: without the category "other" and dividing the variable into the three ethnicities described (Black, Hispanic and Asian). Comorbidities were arranged into groups of diseases: respiratory diseases (asthma, COPD – chronic obstructive pulmonary disease, respiratory diseases), cardiovascular diseases (arrhythmia, heart failure, cardiomyopathy, ischemic heart disease, cardiovascular diseases), neurological diseases (Alzheimer, degenerative diseases, Parkinson, Parkinsonism and movement disorders, dementia, stroke and transient cerebral ischemia, cerebral hemorrhage) and autoimmune diseases (rheumatoid arthritis, autoimmune diseases). For diabetes we performed an analysis with the studies that analyzed the same type of diabetes. Publication bias was assessed by visually inspecting funnel plots. Statistical analyses were conducted in SPSS version 28.0.1.0 (31).

Results

The search strategy identified 13,859 records. After removing the duplicates, 12,094 records were screened for title and abstract and full-text screening was performed in the 164 remaining records, from which 27 met the inclusion criteria and were included in this systematic review. An abstract from the ESCAIDE 2021 workbook was identified but excluded, as we tried to obtain more information from the authors but did not get any answer (Figure 1).

From the 27 studies, most studies were cohort (48.1%) (10, 32–43), followed by case–control (29.6%) (44–51) and cross-sectional (22.2%) (52–57) studies. Most studies were set in Europe (44.4%) (10, 32–34, 36, 37, 41–43, 46, 47, 50), followed by North America (29.6%) (35, 38, 39, 45, 49, 53–56) and Asia (25.9%) (40, 44, 48, 51, 52, 57). Sample size was heterogenous, ranging from 310 (43) to 17,288,532 (37) individuals. Participants age varied widely (range: 18–117 years old), with the majority of participants being mostly females (70.4%) (10, 32–35, 39–42, 45, 46, 48–57). The odds-ratio (OR) was the effect measure most often used (81.5%) (32, 34, 36, 37, 39–57), followed by risk ratio (7.4%) (10, 34), hazard ratio (3.7%) (33) and median difference (3.7%) (35). One study did not report a measure of effect, and only reported the p-value from the χ^2 test and Fisher's exact test to evaluate the differences between positive and negative cases of SARS-CoV-2 infection (38).

Education was the only factor that demonstrated consistent results in the studies in which it was reported, with lower levels of education indicating a greater risk of SARS-CoV-2 infection (34, 39, 42, 50). Income was measured both with Social Deprivation Index (TSDI) (10, 34, 42, 45) or household income (50, 56) and both approaches found that a lower economic level was associated with a higher risk of infection. Two studies reported alcohol drinking history, one did not show any association with infection risk (45) and the other found an association with negative test (50). Smoking history was reported in seven studies, being one of them related to the influence of early factors in the risk of infection, demonstrating that maternal smoking around birth was associated with a higher risk of SARS-CoV-2 infection (32). Smoking history was associated with a higher risk of infection in two studies (10, 38) and two others reported a negative association (44, 53). The remaining studies did not show any association (46, 50).

Other risk factors were more frequently reported, such as sex (55.5%), ethnicity (44.4%), age (40.7%), economic conditions (25.9%), household conditions (14.8%) and comorbidities (51.9%), with cancer/malignancy and hypertension being the most prevalent.

Despite being reported more than once, for some risk factors [smoking status (10, 33, 34, 38, 44, 46, 53), education (34, 39, 42, 50), alcohol drinking status (34, 40, 45, 50), and economic conditions (10, 34, 42, 45, 50, 56)], it was not possible to conduct a meta-analysis or include all the studies in a single meta-analysis due to different variable categories or different variable types (continuous/categorical), and/or different effect measures.

For insurance (52, 54) and age (39, 42, 46, 49, 50, 53), only a fraction of the studies was combined due to different classifications. Age was reported as continuous or categorical, and for the meta-analysis, we extracted the measure of effect for continuous measurement since it was the type most often reported. From the studies that reported age as a continuous variable, one was not included since the confidence interval was not available, thus we lacked information to perform the meta-analysis (53).

Sex was the only variable reported consistently among all the studies identified, thus, the meta-analysis for sex included all the studies reporting sex. Table 1 summarizes the key characteristics of the studies included, while more detailed information is available in Supplementary File 3.

Quality assessment

Most cohort studies were rated 7 or 8 out of 8 points, mainly lacking representativeness of the exposed cohort or comparability at the baseline; most case–control studies were rated 8 out of 9 points lacking mostly representativeness of cases or with different methods of ascertainment of exposure for cases and controls; and cross-sectional studies were rated 6 out of 8 with the most common gaps being related to the identification and analysis of confounding factors. The full quality assessment of all included studies is in Supplementary File 4.

Meta-analysis

We conducted a meta-analysis for 21 risk factors (Figure 2, detailed results in Supplementary File 5). Two of the factors were continuous variables: age and household number. Most were categorical variables. For sex and ethnicity, the reference was female

TABLE 1 Summary of the included studies considering risk factors for SARS-CoV-2 infection, *N* = 27 (high-income countries, 2020–2021).

First author	Study design	Location	Duration	Data source	Sample size	Factors identified	Quality assessment
Altug Didikoglu, 2021 (32)	Cohort	England, United Kingdom	16 March 2020–21 December 2020	Database – UK Biobank	43,428	Early life factors	5/8
Angel Vila-Córcoles, 2020 (33)	Cohort	Tarragona, Spain	1 March 2020–23 May 2020	Database – CAPAMIS Research	79,083	Underlying comorbidities or using chronic medications	8/8
Ariel Israel, 2020 (44)	Case–control	Israel	Beginning of the disease outbreak – 16 May 2020	Electronic health records – Clalit Health Services	24,906	Smoking habits	8/9
Bing Zhang, 2021 (45)	Case–control	California, United States of America	1 March 2020–10 June 2020	Electronic health records – University of California Health system	861	Use of chronic acid suppressors	7/9
Claire L. Niedzwiedz, 2020 (34)	Cohort	England, United Kingdom	16 March 2020–3 May 2020	Database – UK Biobank	392,116	Ethnicity and socioeconomic position	7/8
Ehab Hamed, 2020 (57)	Cross-sectional	Qatar	10 February 2020–30 April 2020	Electronic health records – publicly funded primary health care settings in the state of Qatar	962	Diagnosis of hematological abnormalities	6/8
Eyrun F. Kjetland, 2020 (46)	Case–control	Norway	1 January 2020–6 April 2020	Electronic records – Oslo University Hospital; Online survey	116,678	Demographic, social, health risk and environmental factors	5/9
Farhaan S. Vahidy, 2020 (56)	Cross-sectional	Houston, United States of America	5 March 2020–31 May 2020	Electronic health records – Houston Methodist	20,228	Ethnicity and race	5/8
Farhaan S. Vahidy, 2021 (55)	Cross-sectional	Houston, United States of America	6 March 2020–22 August 2020	Electronic health records – Houston Methodist	96,473	Sex	6/8
Frederick K Ho, 2020 (10)	Cohort	England, United Kingdom	16 March 2020–3 May 2020	Database – UK Biobank	1,525	Demographic, lifestyle, socioeconomic and clinical risk factors	7/8
Giuseppe Mancia, 2020 (47)	Case–control	Lombardy, Italy	21 February 2020–11 March 2020	Databases – Lombardy Regional Health Service	37,031	Use of angiotensin-receptor blockers (ARBs) and angiotensin-converting–enzyme (ACE) inhibitors	7/9
Harmony R. Reynolds, 2020 (35)	Cohort	New York, United States of America	1 March 2020–15 April 2020	Electronic health records – New York University (NYU) Langone Health	12,594	Use of renin-angiotensin-aldosterone system inhibitors	7/8
Jeongkuk Seo, 2020 (58)	Case–control	South Korea	Beginning of the disease outbreak – 15 May 2020	Database – South Korea Health Insurance Review and Assessment Service	4,932	Use of renin-angiotensin-aldosterone system inhibitors	7/9

(Continued)

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TABLE 1 (Continued)

First author	Study design	Location	Duration	Data source	Sample size	Factors identified	Quality assessment
Jose L. Pablos, 2020 (36)	Cohort	Spain	7 April 2020–17 April 2020	Database – public Research network for the Investigation of Inflammation and Rheumatic Diseases (RIER)	29,931	Diagnosis of chronic inflammatory and autoimmune rheumatic disease	5/8
Kuan-Han H. Wu, 2021 (49)	Case–control	Michigan, United States of America	1 March 2020–29 July 2020	Michigan Medicine biorepository; Online survey	8,041	Demographic, lifestyle, socioeconomic and clinical risk factors	5/9
L. Silvia Muñoz-Price, 2020 (54)	Cross-sectional	Milwaukee, United States of America	12 March 2020–31 March 2020	Electronic health records – Froedtert and the Medical College of Wisconsin	2,595	Race	8/8
Leonard E Egede, 2020 (53)	Cross-sectional	Wisconsin, United States of America	1 March 2020–10 July 2020	Electronic health records – Froedtert and the Medical College of Wisconsin	31,549	Ethnicity and race	8/8
Marc Chadeau-Hyam, 2020 (50)	Case–control	England, United Kingdom	16 March 2020–18 May 2020	UK Biobank	4,509	Demographic, social, health risk, medical and environmental factors	6/9
Rohini Mathur, 2021 (37)	Cohort	England, United Kingdom	1 February 2020–3 August 2020; 1 September 2020–31 December 2020	Database – OpenSAFELY platform	17,288,532	Ethnicity	7/8
Sachin J Shah, 2020 (38)	Cohort	San Francisco, United States of America	3 February 2020–31 March 2020	Electronic health records – University of California, San Francisco	316	Demographic and medical and factors	5/8
Sara J. Cromer, 2020 (39)	Cohort	New England, United States of America	1 February 2020–21 June 2020	Electronic health records – Mass General Brigham	57,865	Demographic risk factors	8/8
Seon Cheol Park, 2021 (52)	Cross-sectional	South Korea	3 January 2020–31 May 2020	Database – South Korea Health Insurance Review and Assessment Service	219,729	Underlying comorbidities	6/8
Seung Won Lee, 2020 (40)	Cohort	South Korea	1 January 2020–15 May 2020	Database – South Korea Health Insurance Review and Assessment Service	216,418	Diagnosis of mental illness	7/8
Wonjun Ji, 2020 (51)	Case–control	South Korea	Beginning of the disease outbreak – 15 May 2020	Database – South Korea Health Insurance Review and Assessment Service	219,961	Underlying comorbidities	7/9
Xiude Fan, 2021 (41)	Cohort	United Kingdom	16 March 2020–29 June 2020	Database – UK Biobank	9,469	Use of acid- suppressants	7/8
Yizhou Yu, 2021 (42)	Cohort	United Kingdom	16 March 2020–26 July 2020	Database – UK Biobank	13,338	Diagnosis of dementia, Alzheimer disease or Parkinson disease	7/8
Zahra Raisi-Estabragh, 2021 (43)	Cohort	England, United Kingdom	16 March 2020–22 August 2020	Database – UK Biobank	310	Baseline cardiovascular magnetic resonance (CMR) phenotypes	5/8

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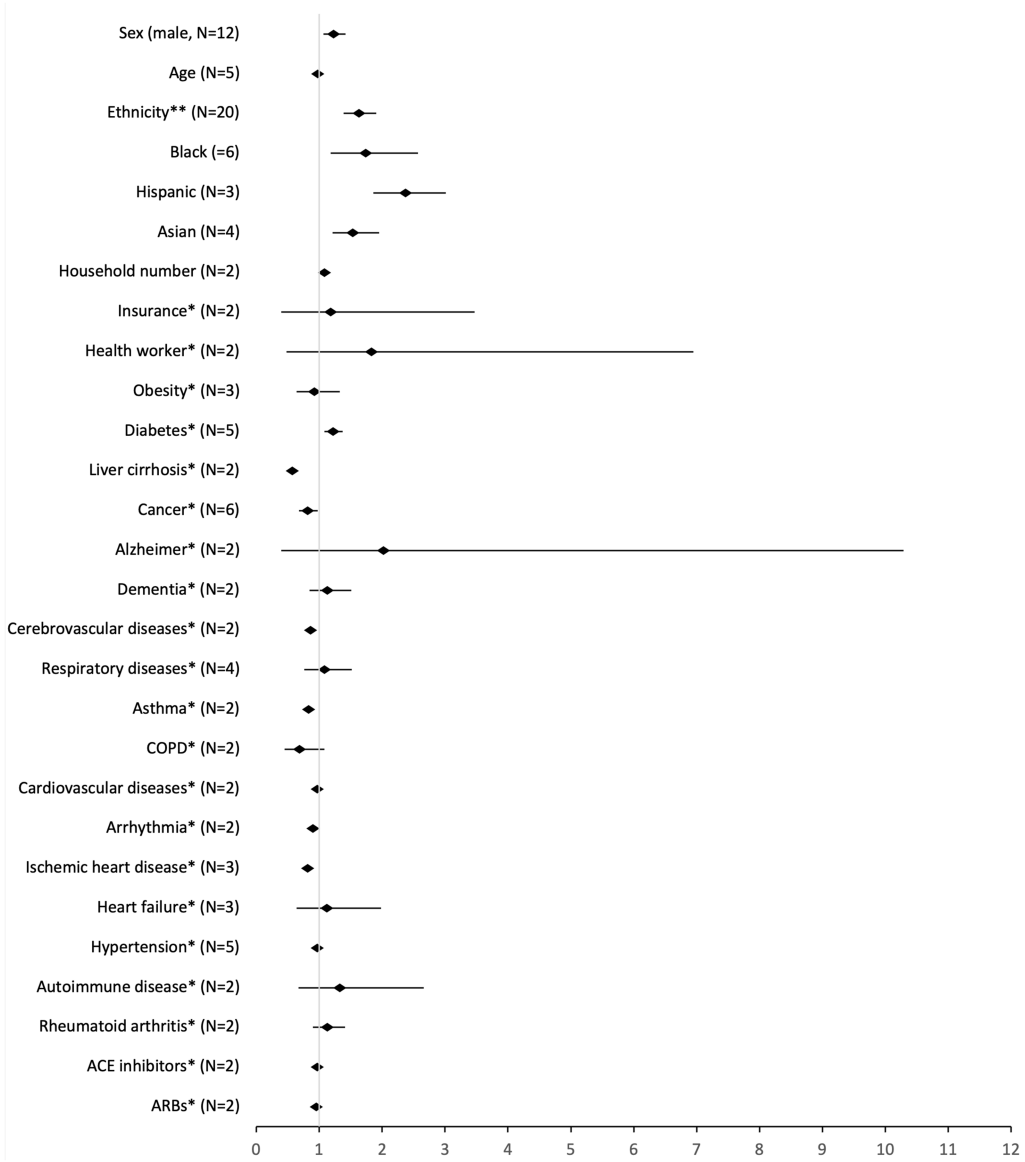

FIGURE 2
Pooled analysis of the risk factors for SARS-CoV-2 infection (high-income countries, 2020–2021). *reference: no; **reference: white.

and white, respectively. The remaining variables were classified as yes or no, presence or absence. In these cases, the reference was no/absence. The variables in this situation were: health worker, insurance, asthma, obesity, diabetes, hypertension, angiotensin-converting enzyme (ACE) inhibitors, angiotensin receptor blockers (ARBs), Alzheimer, dementia, chronic obstructive pulmonary disease (COPD), arrhythmia, ischemic heart disease, heart failure, liver cirrhosis, rheumatoid arthritis, cancer, cerebrovascular, respiratory and cardiovascular diseases. Variable cancer includes any diagnosis of cancer, and variable cerebrovascular diseases include stroke, transient cerebral ischemia and cerebral hemorrhages. The diseases included in each group are in Supplementary File 6.

Being a man (OR: 1.23, 95% CI: 1.97–1.42), of non-white ethnicity (OR: 1.63, 95% CI: 1.39–1.91), increasing household number (OR: 1.08, 95% CI: 1.06–1.10), or having diabetes (OR: 1.22, 95% CI: 1.08–1.37), were associated with an increased odds of getting SARS-CoV-2 infection. In contrast, having asthma (OR: 0.83, 95% CI: 0.75–0.92), ischemic heart disease (OR: 0.82, 95% CI: 0.74–0.91), cancer (OR: 0.82, 95% CI: 0.68–0.98), or cardiovascular diseases (OR: 0.92, 95% CI: 0.84–1.00) were associated with a decreased odds for the infection.

The sensitivity analysis for ethnicity without the category "Other" yielded results almost identical to the main analysis (OR: 1.70, 95% CI: 1.40–2.07). Comparing different ethnicities, Hispanics had a higher OR for SARS-CoV-2 infection (OR: 2.37, 95% CI: 1.86–3.01), followed by Black people (OR: 1.74, 95% CI: 1.18–2.57) and Asians (OR: 1.53, 95% CI: 1.21–1.95). The sensitivity analysis for type II diabetes yielded different results (OR: 1.08, 95% CI: 0.77–1.50) from the main analysis, not being a significant factor for the risk of infection. None of the sensitivity analyses for comorbidities were statistically significant.

All funnel plots suggested eventual publication bias, which is expected in a meta-analysis with observational studies (59).

Discussion

This review aimed to synthesize the available evidence on risk factors associated with SARS-CoV-2 infection in high-income countries and quantify them. The high infection rate in high-income countries and the contradictory evidence found for some underlying diseases motivated this SR. Sex, ethnicity, household number and diabetes were associated with an increased risk of SARS-CoV-2 infection and asthma, ischemic heart disease, cancer and cardiovascular disease were associated with a decreased odds of infection.

Men showed a higher likelihood of becoming infected with SARS-CoV-2. This is consistent with records from other countries and meta-analyses related to SARS-CoV-2 infection (60, 61) and susceptibility trends for other respiratory viruses (62). Other studies suggest that this difference might be caused by biological and immune differences between females and males (63–65), namely neurological manifestations (66). Although there are several explanations for sex variations, the most common reason seems to be gender roles (60). A SR that specifically analyzed sex differences in COVID-19 (pooled prevalence men: 55.0, 51.4–56.6, $I^2 = 99.5\%$) states the differences found were due to the role and behaviors of men and women in the society (60). Detailing, more men are working in essential sectors and occupations that require them to continue being active, to work outside their homes and interact with other people even during lockdowns (e.g., manufacturing and sales, agriculture, food production and distribution, transportation, and security) (60). An independent initiative, to promote gender equality in health, stated that the number of cases between men and women vary with age and stay apparently balanced (67). This can support the behavior's theory, as occupational issues also become less significant as people age. It is important to note that this report includes worldwide data, and the number of cases depends directly on the availability of tests. Data on testing disaggregated by sex is only available from a small number of countries, which makes it difficult to know if the case numbers suffer from ascertainment bias (67). This is also true for other respiratory viruses, like influenza, where it is difficult to ascertain the precise number of cases worldwide, for which a laboratory test is also necessary to confirm the disease, and because there is also insufficient data from less developed countries (62). Further studies are required to address underlying factors explaining such differences.

Non-white individuals also showed higher odds of getting infected, particularly Hispanic. Health determinants could explain this result, as ethnic minorities are commonly at the lower socioeconomic levels. Consequently, these populations tend to be over-represented in essential jobs with more contact with the public, living in worse neighborhoods or overcrowded houses, increasing the infection risk (68). This trend was consistent with literature from other studies, mainly from the United States of America and the United Kingdom (69). However, these results should be interpreted with caution because they are mainly from only two countries whose results might be specific to its social context (70). It is worth mentioning that studies often include a category "other," not described in detail, which makes interpreting the results challenging since we cannot know which ethnicities are included (37, 39, 50, 53, 56). Nevertheless, our results add to the body of literature in this area.

We have identified several studies assessing pre-existing conditions with distinct results in our meta-analyses (36, 42, 44, 45, 47, 49–52, 56). From all the comorbidities analyzed, having a diabetes diagnosis was the only one with a higher chance of SARS-CoV-2 infection. A meta-analysis found that diabetes was the second most prevalent comorbidity in SARS-CoV-2 infected patients (9.7, 95% CI: 7.2–12.2%) (71). This disease could affect the immune system and weaken the immune response against SARS-CoV-2 infection (72) which is also affected by the nutritional uptake that is influenced by diabetes (73). Having asthma was identified as having lower odds of SARS-CoV-2 infection. An earlier literature review focused on the influence of this comorbidity in SARS-CoV-2 infection found no association between asthma and SARS-CoV-2 infection (74). This finding could suggest corticosteroids and bronchodilators, treatments for respiratory diseases, may reduce SARS-CoV-2 infection risk or reduce symptoms development leading to diagnosis (75). However, this has contrasting evidence (76, 77), being at the moment unclear the benefits and harms of respiratory disease treatments to the risk of COVID-19 infection.

Having a cancer diagnosis or a cardiovascular disease also showed lower odds for SARS-CoV-2 infection. However, there is evidence that cardiovascular diseases are important risk factors for respiratory viruses (78). Specifically related to cancer, there is evidence mentioning the weakened immune system of these patients (13, 79), and that regular visits to healthcare facilities for therapy may expose them to the virus (80). The high heterogeneity found between studies could be a reason for the apparent contradictory effect related to comorbidities.

Of the five comorbidities that showed significative ORs, four had protective results. To the best of our knowledge there is no underlying biological mechanism that explains this. Thus, similar to other studies, we hypothesize that these findings might be related to the evidence that people with comorbidities are more cautious toward their health, being more likely to avoid social gatherings, wear masks in situations where distancing is not an option and adhere to lockdown measures, possibly because they perceive their risk of being infected with SARS-CoV-2 as higher (81). That is, individuals with underlying conditions are unlikely to be less prone to SARS-CoV-2 infection, but their risk can be lowered through protective behaviors. Additionally, for asthma, our meta-analysis was performed with only two studies, where asthma was present in 21% (52) and 4% (44) of SARS-CoV-2 cases. However, more evidence is needed to ascertain the effect of the aforementioned comorbidities on SARS-CoV-2 infection risk.

Antihypertensors, namely ACE and ARB, were not significantly associated with SARS-CoV-2 infection, as other meta-analyses also indicate (82, 83). One of these studies analyzed the combined effect of these two medications (OR: 0.95, 95% CI: 0.89–1.02), showing that there is no evidence that this medication significantly increases the risk of infection (83). These results might be associated with SARS-CoV-2 transmission dynamics, which is mainly transmitted through the respiratory tract ACE2 receptors. There is no evidence to date reporting the expression of ACE2 receptors in lung tissue after ACEI/ARB treatment (83). This suggested reduction could not be confirmed in our analysis, which only included two articles for this variable.

Other risk factors, such as income, education, smoking status and drinking status, were reported in the included studies, but meta-analyses could not be performed due to heterogeneity of classification and analysis. These challenges understanding who has a higher risk of getting infected and what behaviors contribute to a higher risk of infection. Furthermore, the aforementioned factors had contradictory results in the individual studies that reported them, confirming the heterogeneity that could result in confusing guidelines to control the spreading and infection rate. In future studies on individual risk factors for SARS-CoV-2 infection, authors should analyze the variables more consistently, considering the published literature on the subject. For example, in one study, education was analyzed according to specific levels from the UK education system (42), challenging comparison with other international results. Additionally, it would be important for authors to provide more detailed information, as previously pointed out, in improving reporting initiatives (84).

Contextual factors could also have an important association with SARS-CoV-2 risk. However, since we excluded ecological studies, we only analyzed sociodemographic and behavioral factors. Although individual factors are important, the effect of contextual factors should also be assessed, i.e., where individuals live and/or work, type of transportation they use, and their access to health services. We urge authors to consider both factors in future analyses since analyzing them jointly strengthens SARS-CoV-2 research, providing a more comprehensive understanding of this disease (85). Although this systematic review is focused on the Alpha variant, new variants were emerging with an increasingly transmissibility and different patterns each time (86). Thus, would be important to replicate this type of studies for new variants to enhance SARS-CoV-2 epidemiologic surveillance.

There are several limitations to this study. First, we included studies whose outcome was only laboratory-confirmed SARS-CoV-2 infection through RT-PCR tests. This can potentially exclude a considerable volume of studies detected by another method. Thus, underestimating our results since severe cases, often associated with more risk factors, were not always laboratory confirmed. Additionally, in the early stages of the pandemic, RT-PCR tests mainly supported diagnosis. Thus, their scarcity meant that their use was directed toward healthcare professionals, residents and professionals in residential homes and symptomatic people. This is reported in some of the articles included, which may skew the results toward populations with higher risk. Although rapid antigen SARS-CoV-2 tests could reach more people, they were only available in late 2020 (87) or early 2021 (88), influencing the case definition between countries. RT-PCR tests remained the gold standard method of diagnosis during the study period, providing more consistent and reliable results. Additionally, restricting the systematic review only to high-income countries could also left out countries with high incidence and potentially with important information to the study of this disease. Another limitation is the rapid evolution of the SARS-CoV-2 virus, which results in the appearance of variants that differ in transmissibility, meaning that as new variants appear, risk factors may also change. Comorbidities can also pose another limitation because, in some studies, they were self-reported and it was unclear whether it was an acute or chronic illness. For this meta-analysis, we extracted the ORs of the most complete analyses, whose variables adjusted had some variation between studies. Values adjusted for the higher number of confounders tend to be closer to the real effect. Still, they can also increase heterogeneity between studies, which results should be interpreted with caution. The contradictory evidence found, namely for cancer and cardiovascular diseases, possibly due to inconsistent terminology describing the diseases and methodology used to extract data, highlights the challenging task of comprehending the true effect of the underlying risk factors for SARS-CoV-2 infection. Results for diabetes should be interpreted cautiously since articles for meta-analysis were included regarding the type of diabetes (I and II). We performed a sensitivity analysis with the articles analyzing the same type of diabetes (type II), which were only two articles. Another limitation could be related to vaccination against COVID-19 since vaccines started to be administrated in late 2020, during our study period. However, vaccine effectiveness against SARS-CoV-2 infection is lower than against severe COVID-19 (89). One study found that vaccine effectiveness against SARS-CoV-2 infection was lower among individuals with comorbidities than individuals without. Thus, it remains crucial to understand who is at higher risk for infection (90).

This review has several strengths since it is, to our knowledge, the first SR and comprehensive meta-analysis of risk factors for SARS-CoV-2 infection in high-income countries, thus adding important knowledge to the SARS-CoV-2 infection. The meta-analyses were conducted using methods that were most suited for the data extracted, considering the heterogeneity of the studies included. Choosing only one measure of effect for the meta-analysis ensured the homogeneity between studies and thus yielded more robust results. The comprehensive search strategy and the databases included, returning a high number of studies, also strengthens this study. The reliability of the study selection criteria was confirmed by double screening of included articles and by testing a random sample (10%) of the extraction form. The study quality was also verified with quality assessment tools there are robust and widely used in literature.

In conclusion, our study demonstrated that men, people of black ethnicity, increased household number, and having diabetes diagnosis were associated with an increased risk for SARS-CoV-2 infection. However, cardiovascular diseases, asthma and ischemic heart disease were shown to be protective factors for this disease. One of the limitations of this meta-analysis relates to the heterogeneity between studies. Thus, future studies should consider how variables are measured to improve comparison between studies and enable a more robust gathering of information from academics.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary material, further inquiries can be directed to the corresponding author.

Author contributions

MM: Conceptualization, Formal analysis, Investigation, Methodology, Software, Writing – original draft, Writing – review & editing. SP: Data curation, Investigation, Validation, Writing – review

& editing. PS: Conceptualization, Formal analysis, Investigation, Methodology, Supervision, Validation, Writing – review & editing. PA: Formal analysis, Methodology, Validation, Writing – review & editing. HD: Data curation, Software, Writing – review & editing. AL: Conceptualization, Formal analysis, Investigation, Methodology, Software, Supervision, Validation, Writing – review & editing.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1367480/full#supplementary-material

References

1. World Health Organization. WHO director-general's opening remarks at the media briefing on COVID-19: 11 March 2020. Washington, D.C. (2020). Available at: https://www.who.int/dg/speeches/detail/who-director-general-s-opening-remarks-at-the-media-briefing-on-covid-19---11-march-2020

2. World Health Organization. WHO Coronavirus Disease (COVID-19) Dashboard. (2021). Available at: https://covid19.who.int/

3. United Nations. UN response to COVID-19. New York City: United Nations (2021).

4. Xu L, Mao Y, Chen G. Risk factors for 2019 novel coronavirus disease (COVID-19) patients progressing to critical illness: a systematic review and meta-analysis. *Aging*. (2020) 12:12410–21. doi: 10.18632/aging.103383

5. Booth A, Reed AB, Ponzo S, Yassaee A, Aral M, Plans D, et al. Population risk factors for severe disease and mortality in COVID-19: a global systematic review and meta-analysis. *medRxiv*. (2020) 16:e0247461. doi: 10.1371/journal.pone.0247461

6. Dzinamarira T, Nkambule SJ, Hlongwa M, Mhango M, Iradukunda PG, Chitungo I, et al. Risk factors for COVID-19 infection among healthcare workers. A first report from a living systematic review and meta-analysis. *Safety Health Work*. (2022) 13:263–8. doi: 10.1016/j.shaw.2022.04.001

7. Gesesew HA, DiN K, Fetene DM, Woldegiorgis M, Kinfu Y, Geleto AB, et al. Risk factors for COVID-19 infection, disease severity and related deaths in Africa: a systematic review. *BMJ Open*. (2021) 11:e044618-10. doi: 10.1136/bmjopen-2020-044618

8. Bayati M. Why is COVID-19 more concentrated in countries with high economic status? *Iran J Public Health*. (2021) 50:1926–9. doi: 10.18502/ijph.v50i9.7081

9. de Lusignan S, Dorward J, Correa A, Jones N, Akinyemi O, Amirthalingam G, et al. Risk factors for SARS-CoV-2 among patients in the Oxford Royal College of general practitioners research and surveillance Centre primary care network: a cross-sectional study. *Lancet Infect Dis*. (2020) 20:1034. doi: 10.1016/S1473-3099(20)30371-6

10. Ho FK, Celis-Morales CA, Gray SR, Katikireddi SV, Niedzwiedz CL, Hastie C, et al. Modifiable and non-modifiable risk factors for COVID-19, and comparison to risk factors for influenza and pneumonia: results from a UK biobank prospective cohort study. *BMJ Open*. (2020) 10:e040402. doi: 10.1136/bmjopen-2020-040402

11. Chun SY, Kim DW, Lee SA, Lee SJ, Chang JH, Choi YJ, et al. Does diabetes increase the risk of contracting COVID-19? A population-based study in Korea. *Diabetes Metab J*. (2020) 44:897–907. doi: 10.4093/dmj.2020.0199

12. Pugliese G, Vitale M, Resi V, Orsi E. Is diabetes mellitus a risk factor for COronaVIrus disease 19 (COVID-19)? *Acta Diabetol*. (2020) 57:1275–85. doi: 10.1007/s00592-020-01586-6

13. Roel E, Pistillo A, Recalde M, Fernández-Bertolín S, Aragón M, Soerjomataram I, et al. Cancer and the risk of COVID-19 diagnosis, hospitalisation, and death: a population-based multi-state cohort study including 4,618,377 adults in Catalonia, Spain. *Int J Cancer*. (2022) 150:782–94. doi: 10.1002/ijc.33846

14. Mangone L, Gioia F, Mancuso P, Bisceglia I, Ottone M, Vicentini M, et al. Cumulative COVID-19 incidence, mortality, and prognosis in cancer survivors: a population-based study in Reggio Emilia, northern Italy. *medRxiv*. (2021) 149:820–826. doi: 10.1002/ijc.33601

15. Earnest R, Uddin R, Matluk N, Renzette N, Turbett SE, Siddle KJ, et al. Comparative transmissibility of SARS-CoV-2 variants Delta and alpha in New England, USA. *Cell Rep Med*. (2022) 3:100583. doi: 10.1016/j.xcrm.2022.100583

16. Varea-Jiménez E, Aznar Cano E, Vega-Piris L, Martínez Sánchez EV, Mazagatos C, García San Miguel Rodríguez Alarcón L, et al. Comparative severity of COVID-19 cases caused by alpha, delta or omicron SARS-CoV-2 variants and its association with vaccination. Enferm Infecc Microbiol Clin. (2022). Available at: https://linkinghub.elsevier.com/retrieve/pii/S0213005X22002816

17. Trobajo-Sanmartín C, Martínez-Baz I, Miqueleiz A, Fernández-Huerta M, Burgui C, Casado I, et al. Differences in transmission between SARS-CoV-2 alpha (B.1.1.7) and Delta (B.1.617.2) variants. *Microbiol Spectr*. (2022) 10:1–10. doi: 10.1128/spectrum.00008-22

18. Hodcrof EB. Covariants: SARS-CoV-2 mutations and variants of interest. (2021). Available at: https://covariants.org/per-country

19. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *BMJ*. (2021) 372:n71. doi: 10.1136/bmj.n71

20. Stroup DF, Berlin JA, Morton SC, Olkin I, Williamson GD, Rennie D, et al. Meta-analysis of observational studies in epidemiology: a proposal for reporting. Meta-analysis of observational studies in epidemiology (MOOSE) group. *JAMA*. (2000) 283:2008–12. doi: 10.1001/jama.283.15.2008

21. World Bank. High income: data. (2021). Available at: https://data.worldbank.org/income-level/high-income?view=chart

22. Ouzzani M, Hammady H, Fedorowicz Z, Elmagarmid A. Rayyan – intelligent systematic review. (2022). Available at: https://www.rayyan.ai/

23. Moola S, Munn Z, Tufanaru C, Aromataris E, Sears K, Sfetcu R, et al. Joanna Briggs Institute. Checklist for qualitative research: analytical cross sectional studies. Adelaide: Joanna Briggs Institute. (2017). 6 p. Available at: https://jbi.global/critical-appraisal-tools (Accessed January 13, 2020).

24. Wells G, Shea B, O'Connell D, Peterson J, Welch V, Losos M, et al. Ottawa Health Institute. The Newcastle-Ottawa Scale (NOS) for assessing the quality of nonrandomised studies in meta-analyses. Available at: https://www.ohri.ca/programs/clinical_epidemiology/oxford.asp

25. Islam MM, Iqbal U, Walther B, Atique S, Dubey NK, Nguyen PA, et al. Benzodiazepine use and risk of dementia in the elderly population: a systematic review and meta-analysis. *Neuroepidemiology*. (2017) 47:181–91. doi: 10.1159/000454881

26. Ryan R. Cochrane consumers and communication review group reviews: meta-analysis. *Cochrane Cons Commun Rev Group*. (2016) 2016:1–6. doi: 10.1002/14651858.CD010825.pub2

27. Michelen M, Manoharan L, Elkheir N, Cheng V, Dagens A, Hastie C, et al. Characterising long COVID: a living systematic review. *BMJ Glob Health*. (2021) 6:1–12. doi: 10.1136/bmjgh-2021-005427

28. Tang B, Liu X, Liu Y, Xue C, Zhang L. A meta-analysis of risk factors for depression in adults and children after natural disasters. *BMC Public Health*. (2014) 14:623. doi: 10.1186/1471-2458-14-623

29. Deeks JJ, Higgins JPT, Altman DG. Chapter 10: Analysing data and undertaking meta-analyses. In: Higgins JPT, Thomas J, Chandler J, Cumpston M, Li T, Page MJ, et al., editors. Cochrane handbook for systematic reviews of interventions version 6.3 (updated February 2022). Cochrane (2022). Available at: https://www.training.cochrane.org/handbook (Accessed August 26, 2022).

30. Borenstein M, Hedges LV, Higgins JPT, Rothstein HR. Introduction to meta-analysis. Chapter 13: Fixed-Effect Versus Random-Effects Models (Pages: 77–86).

31. IBM Corp. IBM SPSS statistics for Macintosh, version 28.0.1.0. Armonk, NY: IBM Corp. (2021).

32. Didikoglu A, Maharani A, Pendleton N, Canal MM, Payton A. Early life factors and COVID-19 infection in England: a prospective analysis of UK biobank participants. *Early Hum Dev*. (2021) 155:105326. doi: 10.1016/j.earlhumdev.2021.105326

33. Vila-Córcoles A, Ochoa-Gondar O, Satué-Gracia EM, Torrente-Fraga C, Gomez-Bertomeu F, Vila-Rovira A, et al. Influence of prior comorbidities and chronic medications use on the risk of COVID-19 in adults: a population-based cohort study in Tarragona, Spain. *BMJ Open*. (2020) 10:e041577-13. doi: 10.1136/bmjopen-2020-041577

34. Niedzwiedz CL, O'Donnell CA, Jani BD, Demou E, Ho FK, Celis-Morales C, et al. Ethnic and socioeconomic differences in SARS-CoV-2 infection: prospective cohort study using UK biobank. *BMC Med*. (2020) 18:160. doi: 10.1186/s12916-020-01640-8

35. Reynolds HR, Adhikari S, Pulgarin C, Troxel AB, Iturrate E, Johnson SB, et al. Renin–angiotensin–aldosterone system inhibitors and risk of COVID-19. *N Engl J Med*. (2020) 382:2441–8. doi: 10.1056/NEJMoa2008975

36. Pablos JL, Abasolo L, Alvaro-Gracia JM, Blanco FJ, Blanco R, Castrejon I, et al. Prevalence of hospital PCR-confirmed COVID-19 cases in patients with chronic inflammatory and autoimmune rheumatic diseases. *Ann Rheum Dis*. (2020) 79:1170–3. doi: 10.1136/annrheumdis-2020-217763

37. Mathur R, Rentsch CT, Morton CE, Hulme WJ, Schultze A, MacKenna B, et al. Ethnic differences in SARS-CoV-2 infection and COVID-19-related hospitalisation, intensive care unit admission, and death in 17 million adults in England: an observational cohort study using the OpenSAFELY platform. *Lancet*. (2021) 397:1711–24. doi: 10.1016/S0140-6736(21)00634-6

38. Shah SJ, Barish PN, Prasad PA, Kistler A, Neff N, Kamm J, et al. Clinical features, diagnostics, and outcomes of patients presenting with acute respiratory illness: a retrospective cohort study of patients with and without COVID-19. *EClinicalMedicine*. (2020) 27:100518. doi: 10.1016/j.eclinm.2020.100518

39. Cromer SJ, Lakhani CM, Wexler DJ, Burnett-Bowie SAM, Udler M, Patel CJ. Geospatial analysis of individual and community-level socioeconomic factors impacting SARS-CoV-2 prevalence and outcomes. *medRxiv*. (2020). doi: 10.1101/2020.09.30.20201830

40. Lee SW, Yang JM, Moon SY, Yoo IK, Ha EK, Kim SY, et al. Association between mental illness and COVID-19 susceptibility and clinical outcomes in South Korea: a nationwide cohort study. *Lancet Psychiatry*. (2020) 7:1025–31. doi: 10.1016/S2215-0366(20)30421-1

41. Fan X, Liu Z, Miyata T, Dasarathy S, Rotroff DM, Wu X, et al. Effect of acid suppressants on the risk of COVID-19: a propensity score-matched study using UK biobank. *Gastroenterology*. (2021) 160:455–458.e5. doi: 10.1053/j.gastro.2020.09.028

42. Yu YZ, Travaglio M, Popovic R, Leal NS, Martins LM. Alzheimer's and Parkinson's diseases predict different COVID-19 outcomes: a UK biobank study. *Geriatrics*. (2021) 6:10. doi: 10.3390/geriatrics6010010

43. Raisi-Estabragh Z, McCracken C, Cooper J, Fung K, Paiva JM, Khanji MY, et al. Adverse cardiovascular magnetic resonance phenotypes are associated with greater likelihood of incident coronavirus disease 2019: findings from the UK biobank. *Aging Clin Exp Res*. (2021) 33:1133–44. doi: 10.1007/s40520-021-01808-z

44. Israel A, Feldhamer I, Lahad A, Levin-Zamir D, Lavie G. Smoking and the risk of COVID-19 in a large observational population study. *medRxiv*. doi: 10.1101/2020.06.01.20118877

45. Zhang B, Silverman AL, Bangaru S, Arneson D, Dasharathy S, Nguyen N, et al. Case–control study of the association of chronic acid suppression and social determinants of health with COVID-19 infection. *Sci Rep*. (2021) 11:20987. doi: 10.1038/s41598-021-00367-7

46. Kjetland EF, Kalleberg KT, Søraas CL, Hammarström B, Myklebust Å, Jenum S, et al. Risk factors for community transmission of SARS-CoV-2: a cross-sectional study in 116,678 people. *medRxiv*. (2020). doi: 10.1101/2020.12.23.20248514v1

47. Mancia G, Rea F, Ludergnani M, Apolone G, Corrao G. Renin–angiotensin–aldosterone system blockers and the risk of COVID-19. *New England J Med*. (2020) 382:2431–40. doi: 10.1056/NEJMoa2006923

48. Seo J, Son M. Update on association between exposure to renin-angiotensin-aldosterone system inhibitors and coronavirus disease 2019 in South Korea. *Korean J Intern Med*. (2021) 36:S114–22. doi: 10.3904/kjim.2020.380

49. Wu KHH, Hornsby WE, Klunder B, Krause A, Driscoll A, Kulka J, et al. Exposure and risk factors for COVID-19 and the impact of staying home on Michigan residents. *PLoS One*. (2021) 16:e0246447. doi: 10.1371/journal.pone.0246447

50. Chadeau-Hyam M, Bodinier B, Elliott J, Whitaker MD, Tzoulaki I, Vermeulen R, et al. Risk factors for positive and negative COVID-19 tests: a cautious and in-depth analysis of UK biobank data. *Int J Epidemiol*. (2020) 49:1454–67. doi: 10.1093/ije/dyaa134

51. Ji W, Huh K, Kang M, Hong J, Bae GH, Lee R, et al. Effect of underlying comorbidities on the infection and severity of COVID-19 in Korea: a Nationwide case-control study. *J Korean Med Sci*. (2020) 35:e237. doi: 10.3346/jkms.2020.35.e237

52. Park SC, Won SY, Kim NH, Choi H, Youk TM, Lee HJ, et al. Risk factors for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infections: a nationwide population-based study. *Ann Transl Med*. (2021) 9:211. doi: 10.21037/atm-20-5958

53. Egede LE, Walker RJ, Garacci E, Raymond JR. Racial/ethnic differences in COVID-19 screening, hospitalization, and mortality in Southeast Wisconsin. *Health Aff*. (2020) 39:1926–34. doi: 10.1377/hlthaff.2020.01081

54. Muñoz-Price LS, Nattinger AB, Rivera F, Hanson R, Gmehlin CG, Perez A, et al. Racial disparities in incidence and outcomes among patients with COVID-19. *JAMA Netw Open*. (2020) 3:e2021892. doi: 10.1001/jamanetworkopen.2020.21892

55. Vahidy FS, Pan AP, Ahnstedt H, Munshi Y, Choi HA, Tiruneh Y, et al. Sex differences in susceptibility, severity, and outcomes of coronavirus disease 2019: cross-sectional analysis from a diverse US metropolitan area. *PLoS One*. (2021) 16:e0245556. doi: 10.1371/journal.pone.0245556

56. Vahidy FS, Nicolas JC, Meeks JR, Khan O, Pan A, Jones SL, et al. Racial and ethnic disparities in SARS-CoV-2 pandemic: analysis of a COVID-19 observational registry for a diverse US metropolitan population. *BMJ Open*. (2020) 10:e039849. doi: 10.1136/bmjopen-2020-039849

57. Hamed E, Syed MA, Alnuaimi AS, Soliman M, Alemrayat B, Muktar AAM, et al. Haematological abnormalities and risk of COVID-19 infection in adult patients attending primary healthcare settings. *Hematol Rep*. (2020) 12:8829. doi: 10.4081/hr.2020.8829

58. Son M, Seo J, Yang S. Association between renin-angiotensin-aldosterone system inhibitors and COVID-19 infection in South Korea. *Hypertension*. (2020) 76:742–9. doi: 10.1161/HYPERTENSIONAHA.120.15464

59. Metelli S, Chaimani A. Challenges in meta-analyses with observational studies. *Evid Based Ment Health*. (2020) 23:83–7. doi: 10.1136/ebmental-2019-300129

60. Abate BB, Kassie AM, Kassaw MW, Aragie TG, Masresha SA. Sex difference in coronavirus disease (COVID-19): a systematic review and meta-analysis. *BMJ Open*. (2020) 10:e040129. doi: 10.1136/bmjopen-2020-040129

61. Pijls BG, Jolani S, Atherley A, Derckx RT, Dijkstra JIR, Franssen GHL, et al. Demographic risk factors for COVID-19 infection, severity, ICU admission and death: a meta-analysis of 59 studies. *BMJ Open*. (2021) 11:e044640. doi: 10.1136/bmjopen-2020-044640

62. World Health Organization. Sex, gender and influenza. Geneva: World Health Organization.

63. Wambier CG, Goren A. Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection is likely to be androgen mediated. *J Am Acad Dermatol*. (2020) 83:308–9. doi: 10.1016/j.jaad.2020.04.032

64. Public Health England. Disparities in the risk and outcomes of COVID-19. London, GOV UK: PHE Publications (2020). 89 p.

65. Takahashi T, Ellingson MK, Wong P, Israelow B, Lucas C, Klein J, et al. Sex differences in immune responses that underlie COVID-19 disease outcomes. *Nature*. (2020) 588:315–20. doi: 10.1038/s41586-020-2700-3

66. Sompa AW, Harun H, Mimika RA, Riksanto R. Neurological manifestations of COVID-19 in Indonesia: assessment of the role of sex and age. *Narra J*. (2023) 3:e277–7. doi: 10.52225/narra.v3i3.277

67. Global Health 50/50. The sex, gender and COVID-19 project. (2022). Available at: https://globalhealth5050.org/covid19

68. Agyemang C, Richters A, Jolani S, Hendriks S, Zalpuri S, Yu E, et al. Ethnic minority status as social determinant for COVID-19 infection, hospitalisation, severity, ICU admission and deaths in the early phase of the pandemic: a meta-analysis. *BMJ Glob Health*. (2021) 6:7433. doi: 10.1136/bmjgh-2021-007433

69. Raharja A, Tamara A, Kok LT. Association between ethnicity and severe COVID-19 disease: a systematic review and meta-analysis. *J Racial Ethn Health Disparities*. (2021) 8:1563–72. doi: 10.1007/s40615-020-00921-5

70. McAuliffe M. AT. World migration report 2022. Geneva: International Organization of Migration (IOM) (2021).

71. Yang J, Zheng Y, Gou X, Pu K, Chen Z, Guo Q, et al. Prevalence of comorbidities and its effects in coronavirus disease 2019 patients: a systematic review and meta-analysis. *Int J Infect Dis*. (2020) 94:91–5. doi: 10.1016/j.ijid.2020.03.017

72. Sharifi Y, Payab M, Mohammadi-Vajari E, Aghili SMM, Sharifi F, Mehrdad N, et al. Association between cardiometabolic risk factors and COVID-19 susceptibility, severity and mortality: a review (2021) 20:1743–65. doi: 10.1007/s40200-021-00822-2,

73. James PT, Ali Z, Armitage AE, Bonell A, Cerami C, Drakesmith H, et al. The role of nutrition in COVID-19 susceptibility and severity of disease: a systematic review. *J Nutr*. (2021) 151:1854–78. doi: 10.1093/jn/nxab059

74. Morais-Almeida M, Pité H, Aguiar R, Ansotegui I, Bousquet J. Asthma and the coronavirus disease 2019 pandemic: a literature review. *Int Arch Allergy Immunol*. (2020) 181:680–8. doi: 10.1159/000509057

75. Halpin DMG, Faner R, Sibila O, Badia JR, Agusti A. Do chronic respiratory diseases or their treatment affect the risk of SARS-CoV-2 infection? *Lancet Respir Med*. (2020) 8:436–8. doi: 10.1016/S2213-2600(20)30167-3

76. Matsuyama S, Kawase M, Nao N, Shirato K, Ujike M, Kamitani W, et al. The Inhaled Steroid Ciclesonide Blocks SARS-CoV-2 RNA Replication by Targeting the Viral Replication-Transcription Complex in Cultured Cells. *J Virol*. (2020) 95:e01648–20. doi: 10.1128/jvi.01648-20

77. Stockman LJ, Bellamy R, Garner P. SARS: systematic review of treatment effects. *PLoS Med*. (2006) 3:1525–31. doi: 10.1371/journal.pmed.0030343

78. Chen L, Hao G. The role of angiotensin-converting enzyme 2 in coronaviruses/influenza viruses and cardiovascular disease. *Cardiovasc Res*. (2020) 116:1932–1936. doi: 10.1093/cvr/cvaa093

79. Wang QQ, Berger NA, Xu R. Analyses of risk, racial disparity, and outcomes among US patients with cancer and COVID-19 infection. *JAMA Oncol*. (2021) 7:220–7. doi: 10.1001/jamaoncol.2020.6178

80. Liu C, Zhao Y, Okwan-Duodu D, Basho R, Cui X. COVID-19 in cancer patients: risk, clinical features, and management. *Cancer Biol Med*. (2020) 17:519–27. doi: 10.20892/j.issn.2095-3941.2020.0289

81. De NN, Moniz M, Gama A, Laires PA, Goes AR, Pedro AR, et al. Non-adherence to COVID-19 lockdown: who are they? A cross-sectional study in Portugal. *Public Health*. (2020) 211:5–13. doi: 10.1016/j.puhe.2022.07.001

82. Qu G, Shu L, Song EJ, Verghese D, Uy JP, Cheng C, et al. Association between angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers use and the risk of infection and clinical outcome of COVID-19: a comprehensive systematic review and meta-analysis. *medRxiv*. (2020) 20144717. doi: 10.1101/2020.07.02.20144717

83. Liu X, Long C, Xiong Q, Chen C, Ma J, Su Y, et al. Association of angiotensin converting enzyme inhibitors and angiotensin II receptor blockers with risk of COVID-19, inflammation level, severity, and death in patients with COVID-19: A rapid systematic review and meta-analysis. *Clin Cardiol*. (2020):1–10. doi: 10.1002/clc.23421

84. von Elm E, Altman DG, Egger M, Pocock SJ, Gøtzsche PC, Vandenbroucke JP. STROBE Initiative. The strengthening the reporting of observational studies in epidemiology (STROBE) statement: guidelines for reporting observational studies. *Lancet*. (2007) 370:1453–7. doi: 10.1016/S0140-6736(07)61602-X

85. Leite A, Leão T, Soares P, Severo M, Moniz M, Lucas R, et al. A case-control study of contextual factors for SARS-CoV-2 transmission. *Front Public Health*. (2021) 9:772782. doi: 10.3389/fpubh.2021.772782

86. Manjunath R, Gaonkar SL, Saleh EAM, Husain K. A comprehensive review on Covid-19 omicron (B.1.1.529) variant. *Saudi J Biol Sci*. (2022) 29:103372. doi: 10.1016/j.sjbs.2022.103372

87. Food and Drug Administration. Coronavirus (COVID-19) update: FDA Issues New Authorization for the BinaxNOW COVID-19 Ag Card Home Test. (2020). Available at: https://www.fda.gov/news-events/press-announcements/coronavirus-covid-19-update-fda-issues-new-authorization-binaxnow-covid-19-ag-card-home-test

88. European Commission. Coronavirus: commission puts forward rules on rapid antigen tests and secures 20 million tests for member states. (2020). Available at: https://ec.europa.eu/commission/presscorner/detail/en/ip_20_2483

89. Zheng C, Shao W, Chen X, Zhang B, Wang G, Zhang W. Real-world effectiveness of COVID-19 vaccines: a literature review and meta-analysis. *Int J Infect Dis*. (2022) 114:252–60. doi: 10.1016/j.ijid.2021.11.009

90. Petráš M, Máčalík R, Janovská D, Čelko AM, Dáňová J, Selinger E, et al. Risk factors affecting COVID-19 vaccine effectiveness identified from 290 cross-country observational studies until February 2022: a meta-analysis and meta-regression. *BMC Med*. (2022) 20:461. doi: 10.1186/s12916-022-02663-z

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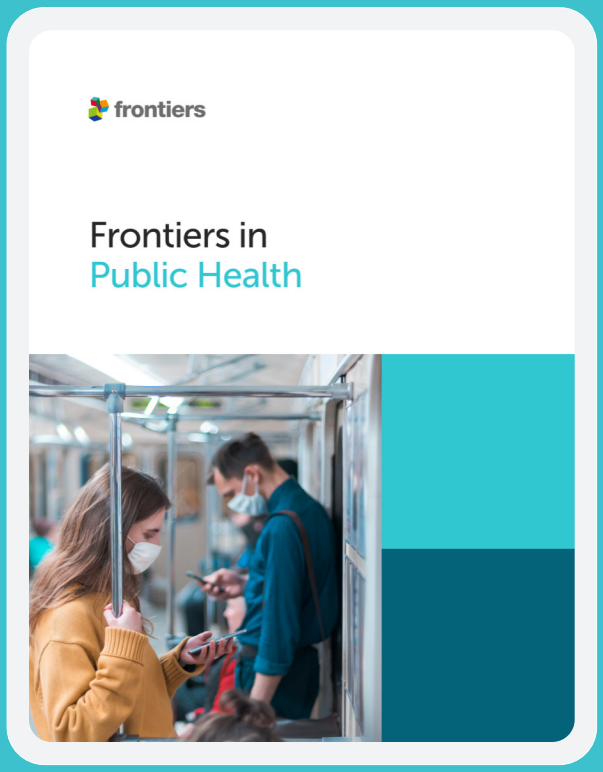

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