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Emerging and re-emerging infectious diseases in emergency settings

Edited by
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Emerging and re-emerging infectious diseases in emergency settings

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Loop-mediated isothermal amplification combined with lateral flow biosensor for rapid and sensitive detection of monkeypox virus

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The ongoing outbreak of the monkeypox, caused by monkeypox virus (MPXV), has been a public health emergency of international concern, indicating an urgent need for rapid and sensitive MPXV detection. Here, we designed a diagnostic test based on loop-mediated isothermal amplification (LAMP) and nanoparticle-based lateral flow biosensor(LFB)for diagnosis of MPXV infection, termed MPX-LAMP-LFB. A set of six LAMP primers was designed based the ATI gene of MPXV, and LAMP amplification of MPXV templates was performed at 63°C for only 40min. The results were rapidly and visually decided using the LFB test within 2min. The MPX-LAMP-LFB assay can specifically detect MPXV strains without cross-reaction with non-MPXV pathogens. The sensitivity of the MPX-LAMP-LFB assay is as low as 5 copies/μl of plasmid template and 12.5 copies/μl of pseudovirus in human blood samples. The whole process of the MPX-LAMP-LFB assay could be completed ~1h, including rapid template preparation (15min), LAMP reaction (40min)and result reporting (<2min). Collectively, MPX-LAMP-LFB assay developed here is a useful tool for rapid and reliable diagnosis of MPXV infection.

KEYWORDS

monkeypox virus, loop-mediated isothermal amplification, lateral flow biosensor, nanoparticles, diagnosis

Introduction

The monkeypox virus (MPXV), which belongs to the family *Poxviridae*, subfamily *Chordopoxvirinae*, and genus *Orthopoxvirus*, causes monkeypox (MPOX) (1). MPXV was first isolated from cynomolgus monkeys in 1958 and the first human infection case was reported in 1970 (2). MPOX presented symptoms similar to those of smallpox in humans (3). People infected usually started with fever, myalgia, fatigue and headache, followed by macular papules. MPXV infection was typically endemic to the rainforests of Central and West Africa, where a number of ground squirrel species believed to be the hosts are prevalent (4). More recently, MPOX outbreak has occurred in 110 Member States across all 6 WHO (World Health Organization) regions, including 103 non-endemic countries/regions, as of December 23, a total

of 83,497 laboratory confirmed cases (including 72 deaths) has been reported[1]. Particularly, MPXV has been declared a global health emergency by the WHO, in the context of the COVID-19 pandemic (5, 6). Therefore, it is important to develop a simple and efficient laboratory detection method for MPXV detection.

As a re-emerging virus, a number of technologies have been developed for MPXV detection, including viral culture and isolation, electron microscopy, immunohistochemistry and enzyme linked immunosorbent assay (ELISA) and molecular tests. However, these assays are all time-consuming and needed to be performed at a central laboratory with skilled technicians and sophisticated instruments (7). Thus, further establishment of easy-to-use, more rapid and simpler technologies to diagnose MPXV infection are still required for facilitating infection control, clinical care and epidemiologic investigation.

Loop mediated isothermal amplification (LAMP), as one of the most commonly used isothermal detection methods for various pathogens, allows amplification of templates, including DNA and RNA, with high sensitivity and specificity at a fixed temperature of 60–69°C. LAMP results can be reported by the use of spectrophotometric equipment for measuring turbidity, by agarose gel electrophoresis, or by visual inspection of color on turbidity changes (8). However, gel electrophoresis is time consuming, laborious and equipment demanding. Visual inspection with colorimetric indicators were direct and convenient, but visual inspection is subjective and sometimes it is difficult to decide the results through an inconspicuous color change. The spectrophotometric turbidimeters can real-time monitor the turbidity changes and yield quantitative results, but the apparatuses are expensive which limited its popularity.

To help overcome these problems caused by traditional LAMP monitoring methods, nanoparticle-based lateral flow biosensor (LFB) was successfully designed and applied for reporting LAMP results. LFB is a paper-based biosensor, which has the advantages of good robustness, fast speed, cost-effectiveness, high specificity and sensitivity (9, 10). Moreover, reporting LAMP results using LFB permits the visual readout, eliminating the use of sophisticated apparatus (11). Therefore, in this study, a LAMP amplification coupled with a LFB assay was established for the rapid and simple detection of MPXV, termed MPX-LAMP-LFB. The features of simplicity (without the requirement for expensive or complex apparatus), rapidity (results can be reported within 60 min), and excellent specificity and sensitivity make this MPX-LAMP-LFB assay more suitable for application in low-equipment setting laboratory.

Materials and methods

Reagents and instruments

A standard plasmid (ATI-plasmid), containing partial sequence of ATI gene(GenBank accession: MT903346.1) shared by both the Clade one (I) (the former Congo Basin (Central African) clade) and the Clade two (II) (the former West African clade) of the causative agent of MPOX, was constructed by Tianyi-Huiyuan Biotech Co., Ltd. (Beijing, China). A pseudotyped virus, which also contained the ATI gene, was constructed by Sangon Biotech. Co., Ltd. (Shanghai, China). Both common and labeled primers used in this study were synthesized by AOKE Biotech Co., Ltd. (Beijing, China). Visual indicator (VI) [used as visual detection regent (VDR)], DNA Isothermal Amplification Kit and LFB were all provided by HUIDEXIN Biotech Co., Ltd. (Tianjin, China). Genomic DNA kit for nucleic acid extraction and purification was purchased from Beijing TransGen Biotech Co., Ltd. (Beijing, China). Real-time turbidimeter LA-320C was purchased from Eiken Chemical Co., Ltd., Japan.

Primer design

A set of 6 primers, including two outer primers (F3 and B3), two inner primers (FIP/FIP* and BIP) and two loop primers (LF/LF* and LB), were designed based on the ATI gene of MPXV using Primer Premier 5.0 (12) for LAMP reaction, and a set of primers for conventional PCR was also designed using the Primer-Blast tool of NCBI for comparison. All the designed primer sets were subjected to blast against the NCBI database, the primer sets that nonspecifically matched with other microorganisms were excluded and the optimal ones were achieved. The sequences, locations and modifications of the primers used in this report are shown in Figure 1 and Table 1.

Nucleic acid extraction

According to the manufacturers' instructions, the nucleic acid templates were extracted from blood samples using the EasyPure®Viral DNA/ RNA Kit purchased from Beijing TransGen Biotech Co., Ltd. (Beijing, China) and from swab samples using nucleic acid extraction reagent purchased from Capital Bio Technology Co., Ltd. (Sichuan, China). The extracted templates were stored under at −20°C before use.

Preparation of lateral flow biosensor

The nanoparticle-based lateral flow biosensor (LFB) was prepared as previously described (13–15). Briefly, the four components of LFB, including a sample pad, a conjugate pad, a nitrocellulose membrane and an absorbent pad (Jie-Yi Biotechnology), were sequentially assembled on plastic back card. The conjugated region contained dye streptavidin coated polymer nanoparticles (SA-DNPs, 129 nm, 10 mg mL^{-1}, 100 mM borate, pH 8.5 with 0.1% BSA, 0.05% Tween 20 and 10 mM EDTA; Bangs, Laboratories, Inc. Indiana, United States), which was employed as detector regents for visualization of targets. The nitrocellulose membrane (NC), which functioned as the detection region, contained rabbit anti-carboxyfluorescein antibody (anti-FAM, 0.2 mg/ml, Abcam. Co. Ltd.) at the test line (TL) and biotinylated bovine serum albumin (biotin-BSA, 4 mg/ml, Abcam. Co. Ltd.) at the control line (CL), with a distance of 5 mm between the two lines. The assembled LFB were cut into 4-mm dipsticks, packaged in a plastic cassette and stored at room temperature until use.

For reporting the amplicons, an aliquot of 5 µl amplification products was added to the sample region of LFB, followed by a 100 µl aliquot of running buffer (10 mM PBS, PH 7.4 with 1% Tween 20) to the same region. Under the capillary force, the target amplicons, which were

1 https://worldhealthorg.shinyapps.io/mpx_global/

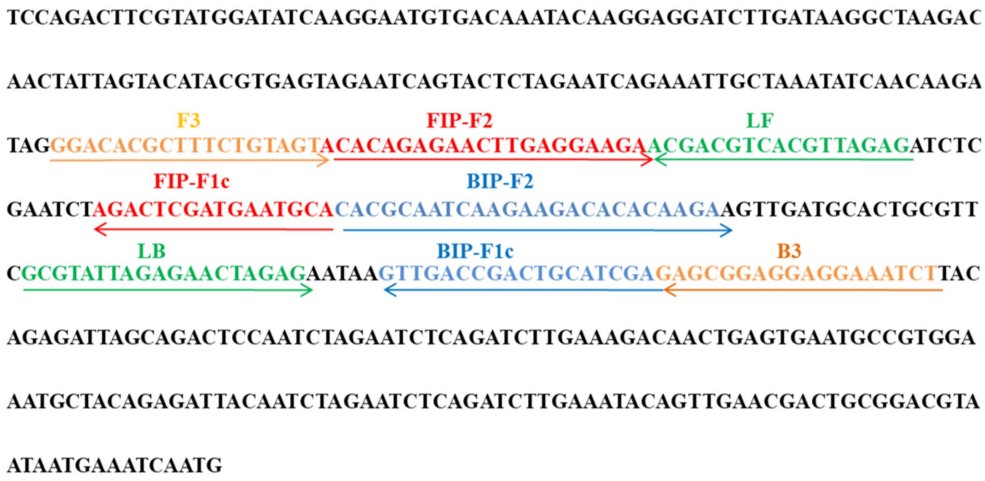

FIGURE 1
Primer sequences and locations used in this study. Right and left arrows show sense and complementary sequences, respectively. The colored text indicates the position of primers, including two outer primers (F3 and B3), two inner primers (FIP and BIP) and two loop primers(LF and LB).

TABLE 1 Sequences and modifications of the MPX-LAMP-LFB primers.

Primers[a]	Sequence (5′-3′) and modifications	Length[b]
F3	GGACACGCTTTCTGTAGT	18 nt
B3	AGATTTCCTCCTCCGCTC	18 nt
FIP	TGCATTCATCGAGTCTAGATTCGACACAGAGAACTTGAGGAAGA	44 mer
FIP*	FAM-TGCATTCATCGAGTCTAGATTCGACACAGAGAACTTGAGGAAGA	46 mer
BIP	CACGCAATCAAGAAGACACACAAGATCGATGCAGTCGGTCAAC	43 mer
LF	CTCTAACGTGACGTCGT	17 nt
LF*	Biotin-CTCTAACGTGACGTCGT	21 nt
LB	GCGTATTAGAGAACTAGAG	19 nt
F	ATCAACAAGATAGGGACACG	20 nt
R	TGCAGTCGGTCAACTTATTC	20 nt

[a]F3, forward outer primer; FIP, forward inner primer; LF, forward loop primer; LB, backward loop primer; BIP, backward inner primer; B3, backward outer primer; FIP*, 5′-labeled with carboxyfluorescein when used in the COM-MPXV-LAMP-LFB assay; LF*, 5′-labeled with biotin when used in COM-MPXV-LAMP-LFB assay. F and R, the forward and reverse primers used for conventional PCR. [b]nt, nucleotide; mer, monomeric unit.

labelled with FAM and biotin at each end, were specially captured by the anti-FAM of the NC region and visualized through biotin and SA-DNPs interaction, resulting to a red band at TL region. The remained SA-DNPs was captured at CL region, indicating the availability of LFB.

The standard MPX-LAMP-LFB reaction

The plasmid DNA was used as positive control to establish the standard MPX-LAMP-LFB assay. The LAMP assay was performed in a 25 µl reaction mixture containing 12.5 µl 2 × reaction buffer, 0.1 µM (each) of F3 and B3 primers, 0.4 µM (each) of FIP* and BIP primers, 0.2 µM (each) of LF* and LB primers, 1.0 µl *Bst* DNA polymerase (8 U), 1.2 µl VDR, 1.0 µl template (5.0 µl in sample detection) and 7.3 µl distilled water (DW). The mixtures were incubated at 63°C for 1 h, and then at 80°C for 5 min to terminate the reaction. Mixture with 1.0 µL genomic DNA of influenza virus A was used as negative control, and with 1 µl DW as the blank control.

The LAMP results were monitored by real-time turbidity, visual reagent (VDR) and LFB. In brief, LFB contains a sample pad, a conjugated pad, a nitrocellulose membrane and an absorbent pad (10, 13). When the reaction products (5 µl) were added in the sample pad, following with about 100 µl running buffers, the results were indicated with two red lines (CL and TL) representing positive reactions, or negative with only one line at CL.

Optimal temperature of MPX-LAMP-LFB assay

Temperatures ranging from 60 to 67°C (with an interval of 1°C) were examined to determine the optimal temperature using the standard LAMP reaction system. LAMP reactions were conducted using the real-time turbidimeter LA-320, with a threshold value of >0.1 as positive reaction. A mixture with DW was regarded as blank control. Each test was repeated three times.

Specificity of the MPX-LAMP-LFB assay

A total of 19 DNA templates, including the ATI-plasmid, pseudotyped virus of MPXV and 17 other virus (Supplementary Table S1) were used to evaluate the analytical specificity of the MPX-LAMP-LFB assay. Genomic DNA of the entire virus was extracted according to the manufactures' instruction. Each test was performed in triplicate.

Sensitivity of the MPX-LAMP-LFB assay

The ATI-plasmid and the pseudotyped virus were serially 10-fold diluted ($5\times10^{5}\sim5\times10^{-1}$copiesper microliter for ATI-plasmid and $1.25\times10^{3}\sim1.25\times10^{-4}$copiesper microliter for the pseudotyped virus) to determine the detection limit of the MPX-LAMP assay. Each serial dilution was tested in duplicate to verify the limit level of the MPX-LAMP-LFB assay. Results of the LAMP reactions were reported by LBF, and further confirmed by real-time turbidity and VDR test. Each test was repeated three times.

Optimal amplification time of MPXV-LAMP–LFB assay

The serially diluted templates of the ATI-plasmid were examined at 63°C to optimize the amplification time of MPX-LAMP-LFB assay. Reaction products were continually analyzed using VDR and LFB test at the 10–40 min with an interval of 10 min. Each reaction was tested in triplicate.

Application of the MPX-LAMP-LFB assay in simulated specimens

In order to verify the feasibility of the MPX-LAMP-LFB assay for clinical diagnosis of MPVX infection, the previously diluted pseudo typed viruses were incubated into human blood samples and lesional swab samples preparing the simulated specimens. Then, the tainted blood and swab samples were applied for nucleic acid extractions, and the resultant supernatants were used as templates for the MPX-LAMP-LFB assay, with the unsimulated samples as blank controls. Moreover, the direct simulated blood and swab samples were further detected by the MPX-LAMP-LFB assay without nucleic acid extraction to test the potential of point-of-care usability of this assay. In addition, 61 nasopharyngeal swab (NPS) samples of non-MPXV infection were enrolled in the process for the evaluation of the MPX-LAMP-LFB assay in the clinical setting. The clinical samples were collected from children in the clinics of the Children's Hospital of Capital Institute of Pediatrics from 1 February to 30 December in 2022, the ethical practice was approved by the Ethical Committee of Capital Institute of Pediatrics, and all the samples were obtained within formed consents signed by the participants' guardians.

Conventional PCR

For confirmation and comparison, a novel conventional PCR method targeting the same gene region of the MPX-LAMP-LFB assay was also developed and used to test the standard ATI-plasmid and the pseudotyped viruses. The conventional PCR reaction was performed in a reaction mixture of 25 µl containing 12.5 µl Premix Ex Taq™ II (Takara Bio, Inc., Otsu, Japan), 0.4 µM F primer, 0.4 µM R primer and 1 µl of DNA template. The conventional PCR reaction procedure contained pre-denaturation at 95°C for 30 s, 30 cycles of denaturation at 95°C for 30 s, annealing at 58°C for 30 s and extension at 72°C for 30 s, and a final extension at 72°C for 5 min. Then, 5 µl of the reaction products were separated by 2% agarose gel electrophoresis (120 V, 40 min). Images were taken using a gel imaging system (GelDoc TM XR1 imager, Bio-Rad Laboratories, Co., Ltd.). The positive result should show a visible band of 177 bp. For precise confirmation of the products, the reaction products were sequenced by Sanger sequencing method provided by Tianyi-Huiyuan Biotech Co., Ltd. (Beijing, China) and the returned sequences were subjected to sequence similarity searching using the Blast tool of NCBI website. In addition to for analytical sensitivity comparison, the conventional PCR was applied to confirm the clinical NPS samples non-MPXV infected.

Results

Confirmation of effectiveness of the MPX-LAMP-LFB assay

The LAMP reaction was performed at 65°C for 60 min to validate the feasibility of the designed primers. Using real-time turbidimeter, a significant increase of turbidity was observed in the mixture with templates of ATI-plasmid, while an almost blunt curve was seen in the negative and blank control (Figure 2A). Using VDR, the color shift of positive results in LAMP tubes from colorless to light green was observed with naked eyes after incubation, and negative results in LAMP tubes remained colorless (Figure 2B). Using LFB, two red bands (CL and TL) were visible in positive results, but only CL was seen in negative and blank controls (Figure 2C). These data above suggested that the selected primers were effective-sufficient for detection of MPXV using LAMP-based assay. In addition, by using the primers of conventional PCR, a visible band was only present in the mixture with templates of ATI-plasmid (Supplementary Figure S1). After sequencing and alignment, it was confirmed that the products was particle region of the ATI-plasmid, implying the availability of the conventional PCR for MPXV test.

Optimal temperature of the MPX-LAMP-LFB assay

We performed the MPX-LAMP-LFB assay at eight different temperatures ranging from 60 to 67°C with a 1°C interval for the optimization of the reaction temperature. As shown in Supplementary Figure S2, 63°C was concluded to be the optimal temperature for the MPX-LAMP-LFB assay, since the threshold value of 0.1 of absorbance was achieved fastest at this condition. Therefore,

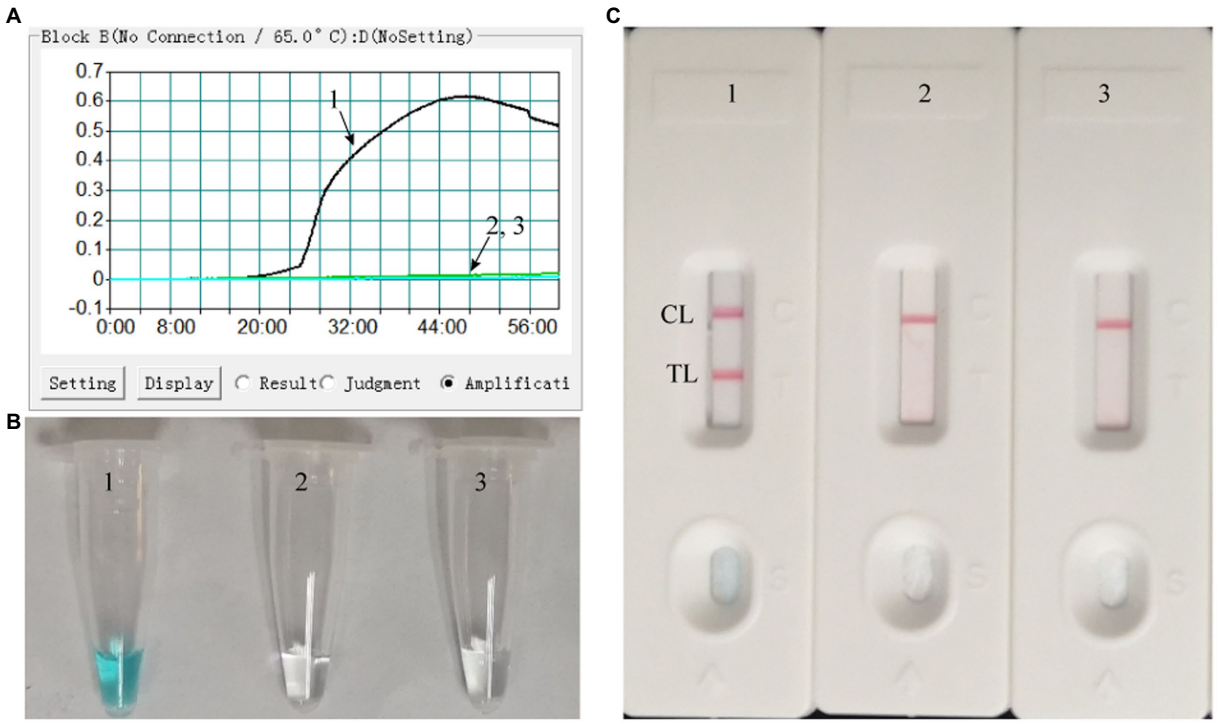

FIGURE 2
Effectiveness of the primer set for the MPX-LAMP-LFB assay. The effectiveness of the primer set for the MPX-LAMP-LFB assay was verified by testing the LAMP products with real-time turbidity **(A)**, visual detection reagent **(B),** and LFB **(C)** methods. Curve/Tube/Biosensor 1, the ATI-plasmid that used as positive control, which was effectively amplified with LAMP reaction at 65°C; Curve/Tube/Biosensor2, the genomic DNA of influenza virus A that used as negative control; Curve/Tube/Biosensor3, the blank control (DW).

63°C was used for the subsequent MPX-LAMP-LFB reaction conducted in this report.

Specificity of MPX-LAMP-LFB assay

DNA templates of MPXV (ATI-plasmid and the pseudotyped virus)and non-MPXV viruses were used to estimate the specificity of the MPX-LAMP-LFB assay under the optimal conditions confirmed above. The results were confirmed using LFB test. Only reactions using templates of ATI-plasmid and pseudotyped virus demonstrated positive results (Figure 3). No cross-reaction was observed within the non-MPXV viruses (Supplementary Table S1).

Sensitivity of the MPX-LAMP-LFB assay

Both the ATI-plasmid and the pseudotyped virus were serially diluted for the examination of the detection limit of the assay. As shown in Figure 4, the results detected by the LFB showed that the detection limit of the MPX-LAMP-LFB assay was 5×10^0copies/μl and that of the pseudotyped virus was 1.25×10^1 copies/μl per reaction, in accordance with those indicated by turbidity and VDR and lower than that of conventional PCR (5×10^1 copies/μl of plasmid template and that of the was 1.25×10^2 copies/μl pseudotyped virus template, Supplementary Figure S3).

Optimal time of the MPX-LAMP-LFB assay

As shown in Supplementary Figure S4, at the time of 40 min, the products of the mixtures with the detection limit level of template were successfully detected by the VDR and LFB test. Thus, 40 min was selected as the optimal incubation time for the MPX-LAMP reaction. Consequently, the whole procedure of MPX-LAMP-LFB analysis only needs no more than 60 min, including rapid DNA extraction (15 min), isothermal reaction (40 min), and result indication (2 min).

Application of MPX-LAMP-LFB assay in simulated specimens

In order to confirm the availability of clinical application, the optimized MPX-LAMP-LFB assay were applied to test the artificial tainted blood and lesional swab samples and 61 clinical NPS samples. The results showed that the MPX-LAMP-LFB assay can effectively detect the pseudotyped virus in the simulated blood and lesional swab samples before or after nucleic acid extraction with the detection limit of 1.25×10^1 copies, which was identical to that of the pure pseudotyped MPXV (Figure 5). Of note, although results from detection of the direct blood samples by real-time turbidity and VDR methods was somewhat weak positive and difficult to recognize, the ones by LFB method were unambiguous

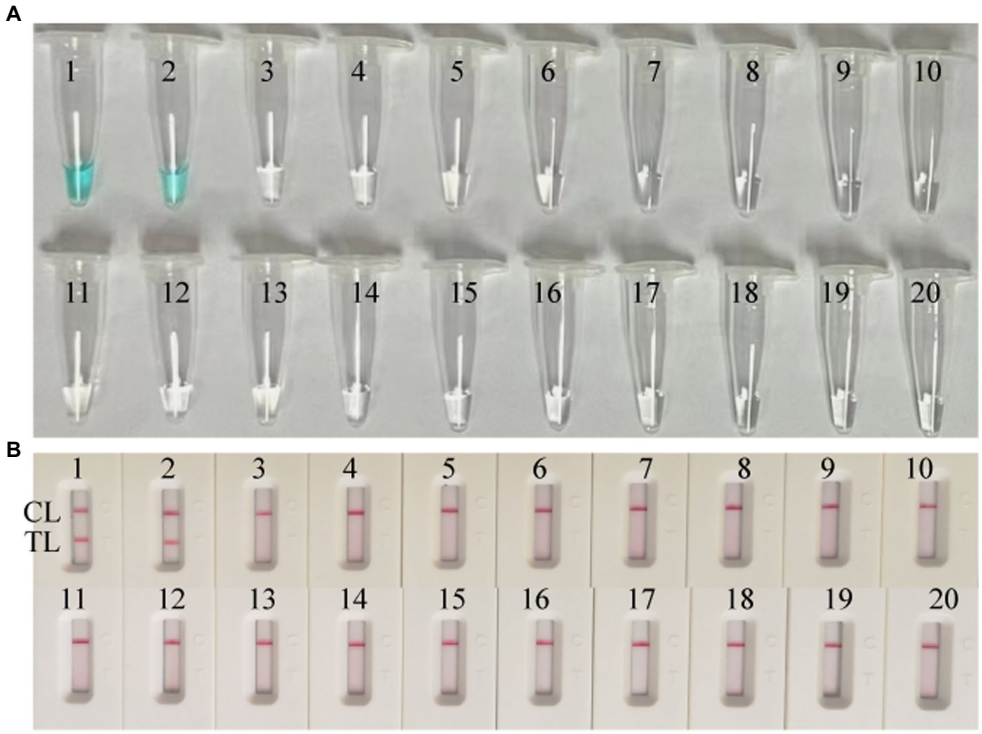

FIGURE 3
Specificity conformation for MPX–LAMP-LFB assay. Specificity of the MPX-LAMP-LFB assay was confirmed by testing the LAMP products with visual detection reagent **(A)** and LFB **(B)** methods. Biosensors/Tubes 1–2 showed the LAMP reaction results of ATI-plasmid and the pseudotyped virus; Biosensors/Tubes 3–19 showed the LAMP reaction results of the 17 non-MPXV viral pathogens (Supplementary Table S1); Biosensor/Tube 20 showed the LAMP reaction results of blank control. TL, test line; CL, control line.

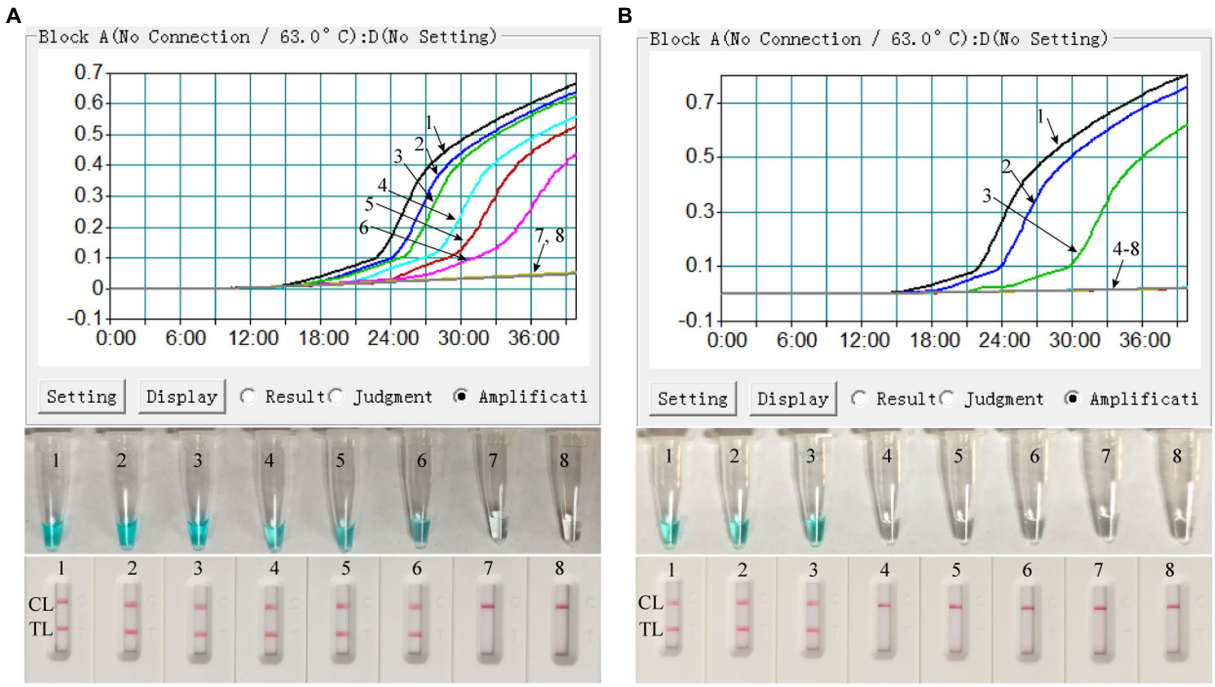

FIGURE 4
Sensitivity confirmation of MPX–LAMP-LFB assay. The sensitivity of the MPX–LAMP-LFB assay was assessed by using the serially diluted ATI-plasmid **(A)** and the constructed pseudotyped virus **(B)**. Three monitoring formats, including turbidity (up row), visual detection reagent (middle row), and LFB (bottom row), were applied to detect LAMP products. Templates 1–8 in A were the ATI-plasmid with concentrations of 5×10^{5}, 5×10^{4}, 5×10^{3}, 5×10^{2}, 5×10^{1}, 5×10^{0}, and 5×10^{-1}copies permicroliter and the uncontaminated blood sample; Templates 1–8 in B were the constructed pseudotyped virus with concentrations of 1.25×10^{3}, 1.25×10^{2}, 1.25×10^{1}, 1.25×10^{0}, 1.25×10^{-1}, 1.25×10^{-2}, and 1.25×10^{-3}copies per microliter and the uncontaminated lesional swab sample. TL, test line; CL, control line.

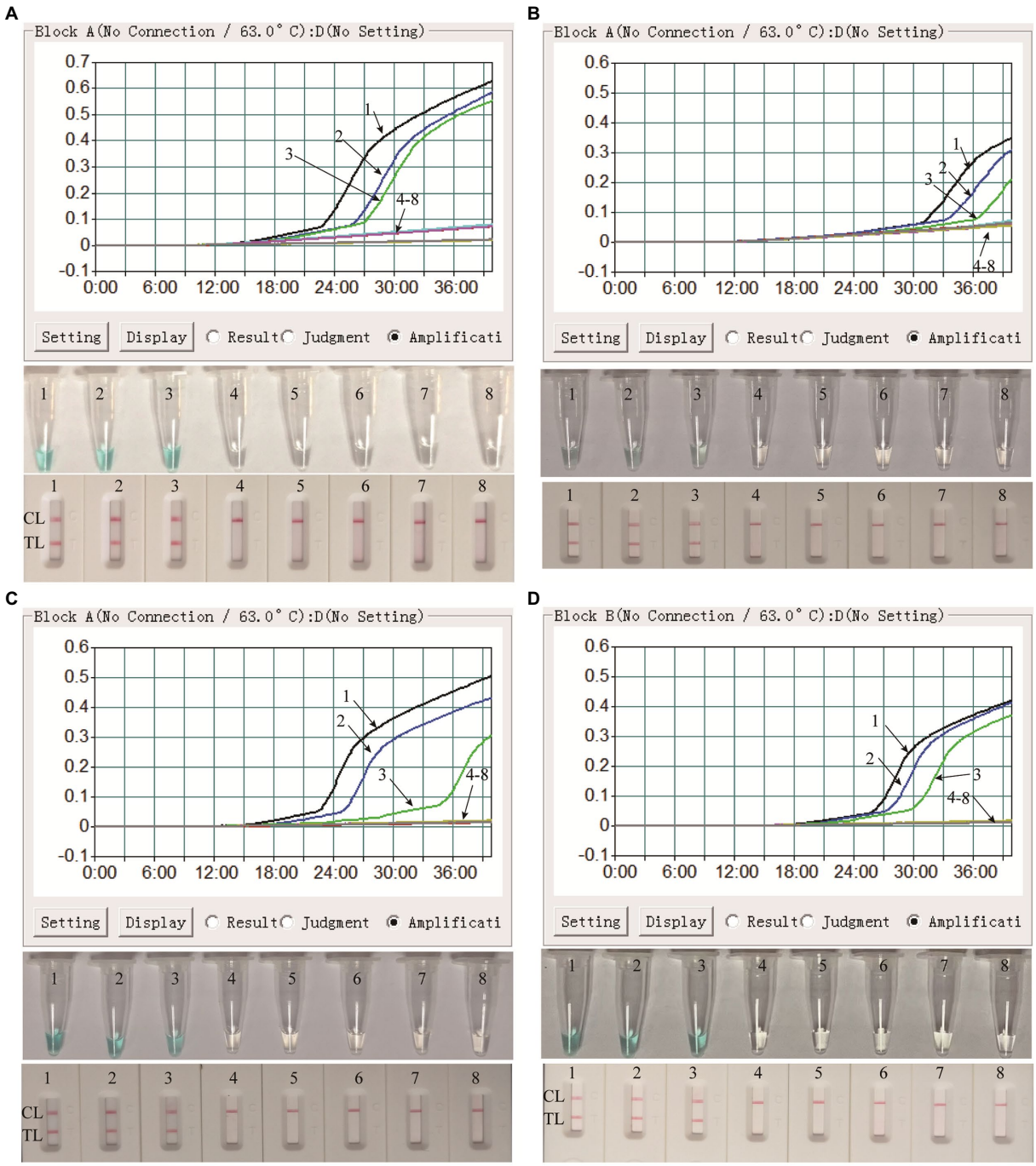

FIGURE 5
Clinical feasibility confirmation of the MPX-LAMP-LFB assay in simulated blood and lesional swab specimens. Clinical feasibility of the MPX-LAMP-LFB assay was confirmed by using the serially diluted simulated blood specimens with nucleic acid extraction process **(A)** and without nucleic acid extraction process **(B)**, and the serially diluted simulated lesional swab specimens with nucleic acid extraction process **(C)** and without nucleic acid extraction process **(D)**. All the specimens were detected using turbidity (top row), visual detection reagent (middle row)and LFB (bottom row). Curves/Tubes/Biosensors 1–8 represented the amplification results of the pseudotyped virus levels in blood samples of 1.25×10^{3}, 1.25×10^{2}, 1.25×10^{1}, 1.25×10^{0}, 1.25×10^{-1}, 1.25×10^{-2}, and 1.25×10^{-3}copies permicroliter and DW.TL, test line; CL, control line.

and easy to read. Moreover, all the clinical samples diagnosed as non-MPXV infection by conventional PCR (Supplementary Figure S5) were also detected negative of MPXV by MPX-LAMP-LFB (Supplementary Figure S6), indicating the high specificity of the MPX-LAMP-LFB assay.

Discussion

Since the beginning of May 2022, human MPOX outbreaks reemerged and have spread to many countries, even as to areas where the disease was not endemic previously (16). The

reemergence of the human MPOX outbreak has made the MPXV become the focus of attention again. Timely and accurate identification of those infected with MPXV is critical to prevent further spread of the disease. Here, we developed a rapid, ultra-sensitive, specific and user-friendly method for the diagnosis of MPXV and named it the MPX-LAMP-LFB assay.

In the MPX-LAMP-LFB assay, isothermal LAMP technique was used to amplify target sequences and LFB was used for indicating the results. In order to perform the MPX-LAMP-LFB test, only a simple instrument (such as a hot block or water bath) is required, maintaining a constant temperature (63°C). Particularly, LAMP assay can achieve exponential amplification of target template within 40 min (8, 17, 18). What's more, the nanoparticle-based LFB platform could visually and objectively decode the pre-amplified targets without expensive and professional laboratory conditions (9). The feature of not limited by site and instrument enables the LFB test to avoid workplace and equipment contamination, which always presents through transfer of nucleic acid amplification test products. In our study, we carried out the LFB test in the clean bench apart from the amplification room, which effectively avoid contamination from the products. The used LFB strips could be easily handled without contamination of any equipment. The whole detection process, including rapid sample processing (15 min), LAMP reaction (40 min), and LFB detection (2 min), could be completed within 60 min. Therefore, the MPX-LAMP-LFB test was a simple, rapid and user-friendly method and may be applied in resource-limited areas or bed-site for MPX infections diagnosis.

Sensitivity analysis showed that the MPX-LAMP-LFB assay had high sensitivity in detecting MPXV. The MPX-LAMP-LFB assay could detect as low as 5 copies of pure plasmid templates and 12.5 copies of pure pseudotyped virus templates. Obviously, the MPX-LAMP-LFB method was highly sensitive for MPXV detection, which was comparable to the real-time PCR based method (~3.5 genomes) (19), and more sensitive than the newly developed conventional PCR method and the previously reported LAMP-based method ($10^2 \sim 10^3$copies per reaction) (20) and RPA-based method (16 molecules per reaction) (21). In addition, the MPX-LAMP-LFB assay correctly reported negative results for the 17 non-MPXV virus, suggesting the highly specificity for MPXV detection. Moreover, although no available non-MPXV *Orthopoxvirus* was obtained, the specificity of the MPX-LAMP-LFB assay between MPXV and other *Orthopoxvirus* members was confirmed by using the blast tool of NCBI database to obtain sequence similarity between the amplified sequence of MPXV strains and that of the other *Orthopoxvirus* members (Supplementary Table S2 and Supplementary Figure S7). It will be preferable if non-MPXV *Orthopoxvirus* strains were obtained and validated in the future study.

Simulated blood and lesional swab specimens were tested to reveal the feasibility of MPX-LAMP-LFB assay in clinical practice. No matter in the directed simulated samples or the ones with nucleic acid extraction process, the MPX-LAMP-LFB assay can successfully characterize and identify the presence of the pseudovirus of MPXV. Moreover, the MPX-LAMP-LFB assay was able to test positive in the simulated blood and lesional swab specimens with down to 12.5 copies of pseudovirus, which further verifying the high sensitivity of this method in clinical settings. In addition, negative results of 61swabs collected from non-MPXV infected patients further demonstrate the analytical specificity of the MPXV-LAMP-LFB assay. The feasibility of MPX-LAMP-LFB assay in clinical settings enables more opportunity to rapid and accurate identify the individuals with MPOX, especially the application in direct simulated blood and lesional swab specimens, implying its great potential in bed-site settings for MPXV infection diagnosis. Thus, the MPXV carriers can be isolated and treated timely, avoiding cross infection and further spread of MPXV, which is of vital importance for the MPOX surveillance in the world.

In summary, we combined LAMP with LFB to develop a diagnostic method for MPXV infection (termed MPX-LAMP-LFB assay). The results showed that the MPX-LAMP-LFB assay was a rapid, efficient, sensitive, specific and simple method for the detection of MPXV infection, and could be used for the detection of MPXV infection in clinic. The MPX-LAMP-LFB assay does not require complex instruments or skilled technicians and can be completed within 60 min. Therefore, the MPX-LAMP-LFB test developed here is an effective tool for the rapid and reliable diagnosis of MPXV infection in both the scientific research field and clinical settings, especially for point-of-care testing or in rough conditions.

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

This study was approved by the Ethical Committee of Capital Institute of Pediatrics, and all the samples were obtained with informed consents signed by the participants' guardians.

Author contributions

XH performed the experiments, analyzed the data, and drafted the manuscript. FX and NJ performed the experiments. CS analyzed the data and drafted the manuscript. JF, ZX, and XC contributed reagents and materials. HH, DQ, and JZ supervised the clinical guidance and study, as well as revised the manuscript. YW conceived the study, designed the experiments, revised the manuscript, supervised and funded this study. 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.2023.1132896/full#supplementary-material

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Involving hard-to-reach populations is pivotal for the tailoring and implementation of an epidemiological study in cross-border communities of French Guiana and Suriname

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Background: Hard-to-reach, vulnerable and cross-border populations are often disproportionately affected by communicable diseases. Epidemiological data on viral hepatitis in French Guiana and Suriname are available for urban areas, but not for remote communities. The Maroni River, which separates FG and Suriname, is home to Tribal and Indigenous communities. Reaching these populations is challenging due to logistical constraints, cultural and language barriers, and mistrust of outsiders.

Objectives: We aimed to conduct an epidemiological study of viral hepatitis [Maroni Hepatites Virales (MaHeVi)] in this remote and complex area. Here, we describe the operational hurdles and solutions required to achieve this.

Methods: We undertook a preliminary assessment of the area with local community leaders and health workers to gain approval of MaHeVi, acceptance of blood sampling, and suggestions for adapting the study to cultural and logistical constraints. Anthropological assessments were conducted through focus groups and interviews with key individuals to assess knowledge, beliefs and risk factors for VH.

Results: MaHeVi was well received by the local communities. The approval of the community leaders was crucial for the implementation and acceptance of the study. The main adaptations were hiring community health mediators to overcome cultural and language differences, using blotting paper instead of venipuncture for logistical and acceptability reasons, and adapting communication materials.

Conclusion: Careful preparation and tailoring of the communication materials and research protocol have enabled the successful implementation of the study. This process could be replicated in this area and transferred to other complex

contexts combining borders, logistical hurdles and populations requiring cultural adaptations.

KEYWORDS

hepatitis B virus, public health, cultural differences, Amazon, research

Background

Vulnerable, migrant and indigenous populations are at an increased risk of chronic hepatitis B virus (HBV) (1, 2) and chronic hepatitis C virus (HCV) infection (3–5). These populations are key in epidemiological context and lowering-and ultimately eliminating – the burden of communicable diseases, and are often disproportionately affected by communicable diseases (6–8). Low-income and lack of health insurance enhance the vulnerability of these populations (9). Rural populations also suffer from health inequities worldwide, having a diminished access to healthcare compared to urban populations (10). Furthermore, vulnerable populations are prone to view researchers as outsiders, feel distrust, which can lead to recruitment barriers and challenges, especially in Indigenous communities (11). Therefore, disease prevalence rates in hard-to-reach or vulnerable populations are often calculated by proxy, using data from blood bank donors or healthier than average participants.

French Guiana (FG) and Suriname have a multi-ethnic and multicultural population reflecting their respective histories, including Tribal and Indigenous communities with a traditional lifestyle. Language, socio-economic level, education, and lifestyle vary widely depending on ethnicity and rural–urban location. The border between FG and Suriname – delineated by the Maroni river – is a remote, poorly accessible area, situated in the rainforest, home mainly to various Indigenous and Tribal communities, who generally have limited access to healthcare (12, 13) (Figure 1).

The Maroni river is mainly inhabited by Maroons (descendants from runaway enslaved individuals) and Amerindians (14), who live, respectively, on the northern and southern part of the river (Figure 1). The settlement of the different ethnic communities along the Maroni river follows historical events and natural boundaries formed by rapids that are difficult to cross, instead of administrative considerations (15). Except for Maripasoula and Saint-Laurent-du-Maroni which are multi-ethnic cities, the different communities live side by side in mainly mono-ethnic villages (14, 16, 17). Moreover, numerous gold-miner settlements deep in the forest contribute substantially to the migrant border population. These settlements concern thousands of mainly Brazilian gold-miners, but also traveling vendors, sex workers (SW) and others (18). Thus, despite the border, many people live alternatively in both countries and cross the border on a daily basis (19).

Each ethnic community has its own language, but most, apart from the Wayana, communicate together for daily life matters through *Mawinatongo*, the "language of the river." Each community has their main leader, and each village their local leaders. People in these

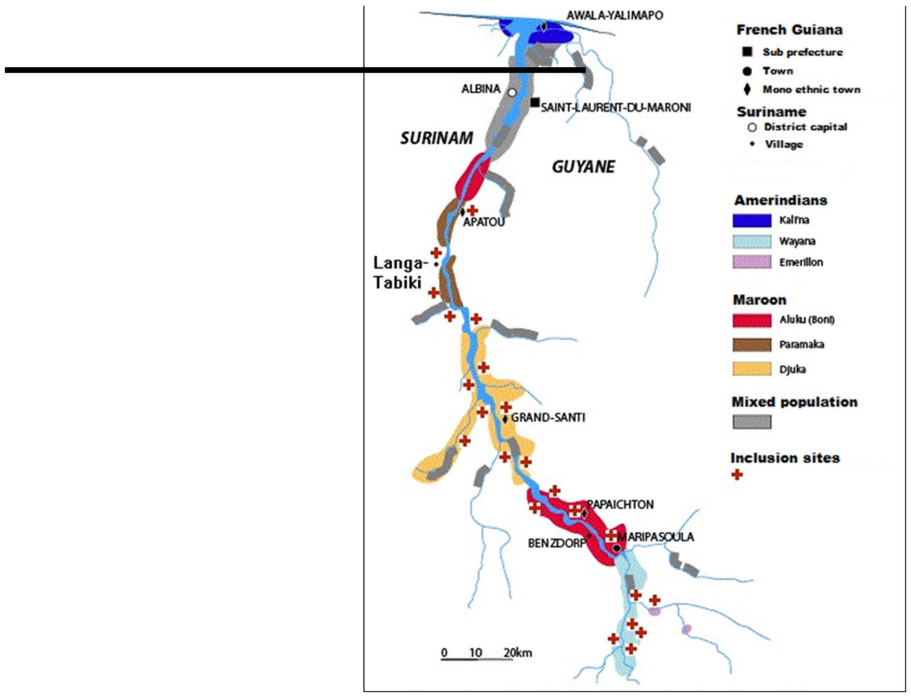

FIGURE 1
Map of the study region and the main population groups.

communities are often semi-nomadic, with frequent moves between villages (20). Men have a dominant role in both communities and most important public positions such as mayor, community leader, and village chief are held by men. On the French side, 5% of children aged 6–11 years and 8% of those aged 12–16 years do not attend school (21). In Suriname, 79.6% of Amerindian women and 45.3% of Maroon women have no or primary education (22). Traditional lifestyle predominates and most households have no electricity, running water or sanitation.

The Maroni basin is remote, with many transportation and communication problems. Apart from the main villages that concentrate the administrative, educational and health infrastructures, several hundred small settlements called *kampus* (about 100 on the Suriname side and 200 on the French side) – mostly remote and unregistered – are scattered along the river and its tributaries. These *kampus* are home to up to 50% of the inhabitants in parts of the Maroni basin. In FG, Maripasoula and Grand-Santi are accessible by plane and only Apatou is linked to the regional paved road network. In Suriname, most villages on the Maroni river are only accessible by air and/or by river (17). The journey may take up several hours depending on the river's water level. In this area, FG and Suriname provide health care only through health clinics and medical mission posts in the main villages (23). Some of these clinics have doctors on duty, while others are staffed by nurses (Figure 1). Yet with health worker shortages, increasing costs, financial instability, political and social unrest, sustainable health care remains a challenge.

Data on viral hepatitis (VH) prevalence and VH health literacy in this border population are scarce. Available data in FG and Suriname are mainly confined to the urban areas: in Cayenne (FG), VH has strong ethnic clustering with HBV and HCV prevalence ranging between 0 and 11%, and 0 to 4.7% respectively, among various ethnic groups (24, 25). Similarly, in Paramaribo (Suriname), prevalence varies greatly, between 0.5 to 6.5% for HBV (26) and 0.6 to 4.1% for HCV (27).

Objectives

We aimed to assess the burden of VH among populations living along this remote border between Suriname and FG. Their knowledge about hepatitis was assumed to be limited and therefore we needed to identify methods to inform, engage and finally test the hard-to-reach communities.

In this context, local teams in FG and Suriname tried to identify feasible and acceptable ways to assess knowledge, attitudes and beliefs (KAP-B) and study the epidemiology of VH in the river border-population between FG and Suriname—the aim of the MaHeVi study. The difficulties of implementing such a project in complex logistical and intercultural conditions are not unique to the context of the border between FG and Suriname. Therefore, here, we describe the operational hurdles and solutions required to implement our VH epidemiological study.

Methods

Study design

The MaHeVi study is a population-based, cross-sectional, non-interventional epidemiological study, whose main objective was to estimate the prevalence of HBV, HCV, Hepatitis D and HIV infection in the general adult population of the Maroni River basin upstream Apatou. The inclusion criteria were being 18 years or older, planning to stay on the Maroni within 2 months of inclusion, and signing informed consent to participate. The MaHeVi study (ClinicalTrials.gov, Identifier: NCT05002907) received ethics approval from the Ethics Committee of the Ministry of Health of Suriname (VG 023–16) and from the Institutional Review Board (IRB00003888) of the French Institute of Medical Research And Health.

In order to improve the feasibility and acceptability of the MaHeVi study, preliminary field missions were carried out to meet with local health actors (health centre staff and associations) and community leaders and authorities, to gather the support of community agents in communicating about VH and the study. In parallel, anthropologists assessed the acceptability of the study in the general population and collected information for the development of a relevant questionnaire, in particular on questions concerning body modifications and sexual and hygiene practices. They also assessed the criteria for involvement of the different communities in order to provide suggestions for improving acceptability and participation in the study.

Preliminary field work for study adaptation

Awareness and communication with community leaders and community health care workers

The initial phase entailed an extensive communication campaign to engage the local community, starting with an audience with community leaders of the different ethnic communities and villages. These key persons were identified through local health centre managers and anthropologists. Topics covered were information on VH and possible risk-factors for transmission. We informed them about the purpose of the research project and requested their approval, acknowledgement and advice on how to proceed. The best ways to communicate about the study to potential participants and appropriate locations for inclusions were discussed with community leaders and local health care workers (HCWs) in each community. We also discussed the feasibility and acceptability of future participation concerning the process of inclusion, participation, reporting of results to participants and follow-up. We asked the community leaders for advice on how to carry out the project in accordance with local habits and customs.

Additionally, training on VH for HCWs was done prior to the study. The training was available for all HCWs from the health care centres, but also to the very rare private practitioners and pharmacists, and to employees and volunteers of local non-government organizations involved in healthcare. The training was conducted by specialists in VH from local hospitals, and covered general knowledge of VH (pathophysiology, epidemiology), management and treatment, and information on the MaHeVi study.

Anthropological studies

Preliminary qualitative anthropological studies were conducted in order to adapt the project to the local communities on different levels. First, to improve the understanding and acceptability of the project by exploring KAP-B on VH before implementation of the study. Second, we wanted to precisely identify and estimate the importance of various potential risk practices that are specific to these

communities as well as the reality of known risk practices in these communities, in order to adapt the risk factors questionnaire in terms of accuracy, completeness, understanding and acceptability. Indeed, in these communities, there were reports of practices like homemade penile implants (28), dry sex (29), vaginal steam baths and multiple sexual partnerships (30, 31), which are suspected or confirmed risk factors for sexually transmitted infections. Polygamous marriage is traditional and widespread in the Maroon community, but not among Amerindians.

Two independent teams of anthropologists worked on each side of the river. The study sites were selected based on the ethnic groups residing in the villages/settlements. Triangulation, by involving multiple researchers and the combination of various data collection methods, was used to reduce bias and ensure the validity of the study.

In Suriname, two anthropologists and two assistants collected data in 3 villages and one Brazilian gold miners' settlement (Antonio do Brinco), each with populations belonging to a different ethnic community (Figure 1). They performed 4 focus group discussions (FGD) per location, with a total of 120 participants, to obtain insights on common general behavioral patterns that people see around them: girls aged 15–20, women aged >20, boys aged 15–20 and men aged >20 years old, as well as 2 FGDs with SWs in Antonio do Brinco. The participants were selected by purposing sampling and the data collection was made with the help of brochures and picture cards and a problem tree exercise. Additionally, qualitative in-depth semi-structured interviews were conducted with 30 key persons and target individuals (health care workers, teachers, school leaders, and persons working in areas with a risk for transmission), to gain insight into personal knowledge and experiences with hepatitis and general insights of the community. The interviews explored knowledge, attitudes, behaviors around viral hepatitis, sexual and other practices that may lead to transmission.

In FG the anthropologist collected data in 12 sites, covering all ethnic communities (Figure 1). He used an ethnographic approach with 20 participatory observations, as well as 52 in-depth face-to-face semi-structured interviews with respondents aged ≥18, about their own experiences, and 27 interviews with informants with expertise in different aspects of the topic (teachers, HCWs, community spokespersons or chiefs, traditional practitioners, and SWs), all recruited through informants or acquaintances or during participatory observations. The anthropologists had an inductive approach to data analysis, meanings emerged from the data through exploration of different data sets, obtained from different sources (interviews, observations, documents) to generate a more comprehensive understanding in the analysis. Supplementary Tables S1–S3 show more details of the studied participants.

Results

Lessons from anthropological studies

KAP-B about VH varied widely among different populations, but overall knowledge was very low. Most people had never heard of hepatitis. Young people had more knowledge and understanding of sexual and health issues, having learned about them in biology classes, or from the school nurse. However, there were misconceptions about the modes of transmission of VH, including sharing of eating utensils and drinking glasses, and transmission through mosquito bites.

In the villages, anal and oral sex were somewhat taboo and uncommon, and men having sex with men occurred but was not talked about. In the gold mining settlements, neither oral nor anal sex was taboo. Commercial sex was not common in the villages, but available in nearby gold mining settlements. Focus group participants acknowledged the need for condoms but condoms were rarely used in steady relationships. In the Amerindian village, the negative impact of alcohol on condom use was explicit. In the gold mining settlements, condom use was reported to be consistent.

Sharing of toothbrushes, razors and other personal toiletries was not reported. Vaginal steam baths were very popular in Maroon communities and were used regularly. However, contrary to popular belief elsewhere, this practice in Maroon communities was not associated with sexual activity, but rather with an old tradition of achieving "cleanliness." The term "dry sex" was considered offensive. Among Amerindian women, vaginal steam baths were only used after pregnancy.

For invasive skin procedures, earrings were always inserted with clean disposable needles. Other body piercings and tattoos were not always done professionally but participants often traveled to Paramaribo or FG to have them done. Piercings were particularly popular with young Amerindians. In the gold mining areas, cosmetic tattoos were popular and were often done in the country of origin. Aesthetic scarification is no longer practiced, but there is medicinal scarification called *koti*, where incisions on the forehead or back of the hand are coated with homemade medicines. Golden teeth, a status symbol, were mainly done in professional settings. Circumcision is not practiced.

Injection drug use was very rare in the area. Drug use was limited to smoking marijuana, crack and heroin, although "rape drugs" have recently been introduced in gold mining settlements.

Antenatal and postnatal care is available at health centres and includes VH and HIV screening for pregnant women and HBV vaccination at birth.

Following the preliminary anthropological studies, the anthropologists made recommendations on general organisation, communication and questionnaire content, which were implemented as far as possible for the MaHeVi study.

Adaptation of MaHeVi communication, study design, and conduct

Following the preliminary steps, we adapted the MaHeVi study design and organisation on several ways, as summarized in Table 1.

Discussions with local key persons, HCWs and non-government organizations, helped us to identify and anticipate communication difficulties linked to poor knowledge and awareness on VH, and low literacy. They suggested favoring oral communication with public meetings, live interviews and radio infomercials broadcasted on local stations, and poster campaigns including drawings rather than writing and allowing the population to identify with the characters. The posters were developed using the well-known logos of local health structures, and involved a not-for-profit organisation with experience of developing visual information materials with local communities. A poster was developed to introduce the MaHeVI project (Figure 2).

TABLE 1 Recruitment barriers and challenges for the MaHeVi project, and resulting study adaptation.

Recruitment barriers and challenges	Study adaptation
Traditional social structure	Consultation of community leaders and local health workers prior to study
Potential distrust in community outsiders	Extended stay in each inclusion location to allow time for potential participants to become familiar with the project and the field team and working hand in hand with local health structures
Cultural differences between researchers and participants and between participants from different ethnic communities, language barrier	Hiring health mediators from the community, ideally not living in the same village as the one surveyed because of sensitive questions
Mostly oral communication and low literacy rate	Adaptation of recruitment material through audio communication on local radio stations and display communication promoting illustration
Acceptability of blood sampling and sample transportation	Choice of dried blood spot instead of tube blood sampling
Individual transportation difficulties for participants, due to transportation costs and remoteness	Travel of field teams instead of participants
Transborder situation involving administrative constraints and highly mobile potential participants	Operation based on 1 field team in each country, moving in a coordinated way in the territories of each community and using the same communication and identification

These posters and flyers were distributed in key locations, e.g., health centres, shops and other community gathering places.

A short repeat communication campaign, including another visit to the local authorities and airing of radio-infomercials, kicked off the inclusions of participants in the MaHeVi study. Local radio stations announced the dates of arrival of MaHeVi teams on both sides of the border shortly before inclusions.

In addition to gaining the consent of community leaders, we improved the acceptability of the study by making a visible commitment to our cross-border partnership and to working with local health centres that people knew and trusted. The study therefore took place primarily in or near the health centres and usual places of regular health outreach, following local health workers on their regular outreach in collaboration with community volunteers, before moving further into the field where possible.

Trained health mediators had to be fluent in the language of the specific communities. In addition, as a result of preliminary discussions, mediators were required to have no relationship or connection with the village where inclusions were taking place, if possible, to ensure maximum participation and honest answers to questions. Participants often preferred non-locals to administer the questionnaires and report the results, as they were seen as less likely to divulge confidential information in the village.

We considered using fingerpricks and filter paper, i.e., DBS, rather than conventional blood sampling, due to limited or no access to centrifugation facilities and sufficient refrigerated storage space, coupled with uncertain logistics of repatriating samples. In addition, the cold chain could not be guaranteed because the electricity supply was intermittent, with only the larger settlements having access to a generator. In addition, people are used to capillary blood samples for rapid HIV and malaria testing.

Conventional geographical cluster sampling, which would have been more accurate in obtaining a more representative sample of the target population, was not feasible due to poor accessibility, especially in the hundreds of unregistered *kampus*. The necessary elements for a relevant random selection of participants were not available, either individually or by residential area. In addition, visiting a *kampu* directly could be perceived as intrusive. Not giving everyone the opportunity to participate could also be misinterpreted. We therefore decided to focus on communication, particularly radio broadcasts, and hypothesized that almost the entire target population would be informed about the study in an area where radio is popular and word of mouth is very effective. In order to compensate for the selection bias, we decided to adjust the sample according to the number of people included per community, since we did not refuse participants even if the target number of participants per community was reached, and opted for a post-stratification weighting method based on the population structure.

Conclusion

Lessons from the field

In the setting of the Maroni River, contrary to the widespread belief that epidemiological research would face insurmountable difficulties, MaHeVi was well accepted by the local community leaders and the people of the various communities on both sides of the border.

Following an initial field communication mission, the local authorities acknowledged the seriousness of the disease and agreed to the study and our presence in the area. In addition, the Granman of the Ndyuka Maroon community requested that the area of inclusion be extended to include the Tapanahony River, a tributary further inland in Suriname, in order to provide screening and treatment options for the entire community. The approval of the local community authorities was crucial for the acceptance and implementation of the study.

Focus group and key-person discussions within the various local populations were important for a tailored development of communication materials on VH, adaptation of the study questionnaire, as well as acceptance of the sample collection. The choice of DBS as the sampling method was mainly motivated by its indication in populational studies and its use in challenging settings (32). Furthermore, preference for DBS instead of venepuncture was confirmed in our study by several key persons, HCWs and community chiefs, but also by random respondents. In general, it was clear that this blood sampling method would increase acceptability to participate in the MaHeVi project.

The involvement of the local community in the adaptation of the study protocol, especially community leaders, as well as in the development and finalization of the posters and radio announcements,

FIGURE 2
Posture used to introduce the MaHeVi project (Ndyuka version).

was essential for the communities' commitment to the project, as has been showed elsewhere (33).

Limitations

There are several limitations to this preliminary study. First, not all *kampus* are registered, possibly creating a selection bias in the people interviewed. However, we believe that working simultaneously on both sides of the border, involving all known ethnic groups in FGDs and interviewing local HCWs, community chiefs and authorities, provides an accurate reflection of the populations' KAP-B.

Second, it is necessary to continue our efforts to go further in involving local communities in health interventions to ultimately improve health outcomes (34). In particular, for the epidemiological studies needed to establish appropriate and evaluable health studies and interventions, we need to improve the level of community participation and progress toward interactive participation (33). This requires the promotion of intercultural dialog between Western and Traditional communities, through study co-design and evaluation and the establishment of a sustainable climate of trust and partnership (35).

In conclusion, following the preliminary studies and subsequent adaptations, the inclusions in the MaHeVi study took place from 2018 to 2019. Enrollment of participants on both sides of the Maroni was completed as planned. The results of the seroprevalence and risk factors study will be published and communicated to the communities concerned.

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

All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1162705/full#supplementary-material

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Rapid, sensitive, and highly specific detection of monkeypox virus by CRISPR-based diagnostic platform

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Background: Monkeypox (MPX), caused by the Monkeypox virus (MPXV), has incurred global attention since it broke out in many countries in recent times, which highlights the need for rapid and reliable diagnosis of MPXV infection.

Methods: We combined recombinase polymerase amplification (RPA) with CRISPR/Cas12a-based detection to devise a diagnostic test for detection of MPXV and differentiation of its two clades [Central Africa clade (MPXV-CA) and West Africa clade (MPXV-WA)], and called it MPXV-RCC. The sensitivity, specificity and practicability of this method have been analyzed.

Results: The optimal conditions of MPXV-RCC assay include two RPA reactions at 38°C for 25 min and a CRISPR/Cas12a-gRNA detection at 37°C for 10 min. The results of MPXV-RCC assay were indicated by a real-time fluorescence analysis software. Thus, the whole detection process, including rapid template preparation (20 min), RPA reaction (25 min) and CRISPR-based detection (10 min), could be finished within 1 hour. The sensitivity of MPXV-RCC for MPXV-CA and MPXV-WA detection was down to 5∼10 copies of recombination plasmids and pseudovirus per reaction. Particularly, MPXV-RCC assay could clearly differentiate MPXV-CA from MPXV-WA, and had no cross-reactivity with other pathogens. In addition, the feasibility of MPXV-RCC assay was further validated by using spiked clinical samples.

Conclusion: The MPXV-RCC assay developed here is a promising tool for quick and reliable diagnosis of MPXV infection.

KEYWORDS

monkeypox, monkeypox virus, RPA, CRISPR/Cas12a, MPXV-RCC

Introduction

As the ongoing COVID-19 pandemic is still challenging the world, a new threat to public health caused by a global surge of monkeypox (MPX) cases has emerged (1). MPX, as a re-emerging zoonotic disease, is caused by the monkeypox virus (MPXV), which is a double-stranded DNA virus with typical virus sizes varying from 200 to 250 nm (2). MPXV is a member of the *orthopoxvirus* genus in the *poxviridae* family and has two genetic clades: Central Africa (MPXV-CA) and West Africa (MPXV-WA). Particularly, MPXV-CA strains exhibited more virulent effects (2). Monkeypox could spread among populations through close contact *via* respiratory droplets, body fluids, and lesions of infected people or animals (3). Until now this year, a total of more than 80,000 MPX cases have been reported in 110 member states of the WHO (4). The primary affected countries included the United States of America, Spain, Germany, the United Kingdom, and France (4). Hence, a rapid and reliable tool for MPXV detection will be helpful to control the dissemination of this virus.

Although antigen and serology-based methods have been used for the diagnosis of MPXV in the early days (5), they have cross-reaction with other *orthopoxviruses* (6). Currently, the definitive confirmation of MPXV infection relies on polymerase chain reaction (PCR) and real-time PCR according to WHO guidelines (6, 7). However, these PCR-based methods are time-consuming and strongly rely on skilled personnel, complex instruments, and a stable power supply. Hence, further development of easy-to-use, simple, and more rapid techniques to diagnose MPXV infection is still needed.

Clustered regularly interspaced short palindromic repeat (CRISPR) and CRISPR-associated protein (Cas) techniques known as "molecular scissors" are used to edit the genome for its efficient and accurate capacity (8). Particularly, the CRISPR/Cas system has been applied as an appropriate tool for nucleic acid detection due to its sensitivity and specificity (9). Various Cas enzymes, such as Cas12a, Cas13, and Cas14, have been developed successfully and show an excellent prospect in nucleic acid diagnosis (10–12). Cas12a, Cas13, and Cas14 enzymes target double-stranded DNA (dsDNA), single-stranded RNA (ssRNA), and single-stranded DNA (ssDNA), respectively (8). Under the direction of guide RNA (gRNA), the aforementioned enzymes could specifically recognize and cleave the targeted nucleic acid sequence. Simultaneously, the non-specific cut capacity of the Cas enzymes is activated to cleave the surrounding ssDNA (Cas12a and Cas14) and ssRNA (Cas13) (8, 13). In this connection, ssDNA and ssRNA could be prepared into fluorescent probes; thus, the produced fluorescent signal will be detected by molecular sensors when cleavage occurs (14).

Owing to omitting sophisticated equipment and saving time with isothermal amplification techniques, the CRISPR/Cas system usually couples with them on the pre-augmented phase to improve the detection sensitivity (14). Until now, several CRISPR-based methods have been implemented in microbe surveillance. For example, multiple cross displacement amplification with CRISPR/Cas12a-based detection (MCCD) was developed for the diagnosis of SARS-CoV-2 (13); the CRISPR/Cas12a-based RPA method was devised to detect severe fever with thrombocytopenia syndrome virus (SFTSV), influenza A virus, and *Mycoplasma hominis* (15–17); similarly, CRISPR/Cas13a coupled with the loop-mediated isothermal amplification (LAMP) assay was contrived for monitoring circular RNA and SARS-CoV-2 (18, 19).

In this study, we coupled recombinase polymerase amplification (**R**PA) with **C**RISPR/**C**as12a-based detection to establish a sensitive and specific diagnostic test for rapid detection of MPXV and accurate differentiation of MPXV-CA and MPXV-WA strains and named it MPXV-RCC. The whole detection procedure could be finished within 60 min, which is thus suitable for point-of-care detection. We illustrated the basic principle and operational process of the MPXV-RCC assay and verified the feasibility by using spiked specimens.

Materials and methods

Target DNA and clinical specimens

In this study, two kinds of plasmids (MPXV-CA recombination plasmid and MPXV-WA recombination plasmid) and MPXV pseudovirus were constructed and used as positive controls. The construction of plasmids and pseudovirus has been expounded as follows. The gene sequence of the MPXV D14L gene (651 bp in length, GenBank KJ642613.1) was synthesized and cloned into *E. coli* pUC57 vector (2,710 bp in length) to specifically construct an MPXV-CA recombination plasmid (3 ng/μl), and the copies of plasmid were calculated using a formula: copies/μl = concentration(ng/μl) × 10^{-9} × 6.02 × 10^{23} (copies/mol)/(sequence length × 660). Thus, the concentration of the MPXV-CA recombination plasmid was 8.1 × 10^8 copies/μl according to the calculation. Similarly, the MPXV-WA recombination plasmid (8.8 × 10^8 copies/μl) was synthesized by inserting the specific sequence of the ATI gene (392 bp in length, GenBank DQ011156.1) into *E. coli* pUC57 vector as well. Two plasmids were constructed by Tianyi-Huiyuan Biotechnology (Beijing, China), and they acted as a positive template to explore the optimal reaction condition of the MPXV-RCC assay. MPXV pseudovirus was purchased from Sangon Biotechnology (Shanghai, China). MPXV partial sequences containing both the D14L and ATI genes were cloned *in vitro* and constructed into adenovirus vector Ad5, which was used to induce pseudovirus in 293A cells. After purification by chromatography column, the pseudovirus was obtained in the form of a DNA sequence encapsulated by an adenovirus capsid. Pseudovirus concentration (1.0 × 10^5 copies/μl) was quantified according to the standard curve of qPCR.

In addition, a total of 35 nucleic acid samples extracted from non-MPXV strains were employed in this study (Supplementary Table S1), and each microorganism was verified using real-time PCR. A recombinase-mediated isothermal amplification kit for RPA reaction was obtained from HuiDeXin Biotechnology Development (Tianjin, China). A LbaCas12a protein kit applied to CRISPR/Cas12a cleavage was purchased from Magigen Biotechnology (Guangzhou, China). The emitted fluorescence signals could be captured by a real-time PCR thermocycler instrument (LightCycler 480, Roche, Basel, Switzerland).

Primers and CRISPR gRNA design

The details of RPA primers and gRNA are listed in Table 1 and Figure 1C. The specificity of two RPA primer sets was verified using NCBI BLAST analysis. Two gRNA strands for MPXV-CA and MPXV-WA were designed based on the MPXV-RCC principle. Furthermore, the probe used for fluorescence detection was labeled at the 5′ end with FAM fluorophore and at the 3′ end with a BHQ1 quencher. The oligonucleotides were synthesized by Sangon Biotechnology (Shanghai, China).

The standard RPA reaction

The RPA pre-amplification process was carried out in a 25 μl reaction mixture according to the manual instruction. In brief, 13 μl of buffer I, 2.5 μl of enzyme mixture, 1.2 μl of forward primer (10 μM) and reverse primer (10 μM), 1.25 μl of buffer II, and 3.6 μl of distilled water were first added into a tube to

TABLE 1 Primers, gRNA, and probe of the MPXV-RCC assay.

Pathogens	Objects	Sequences and modifications (5′-3′)	Length[b]
MPXV-CA	Primer-F1	GATATTGGCGGAGTAGACTTTGGCTCTAGTATA	33 nt
	Primer-R1	TAGATGGAGGTGATTGGCATTTAACAGATTCG	32 nt
	gRNA-1	UAAUUUCUACUAAGUGUAGAUAUCGGUGAAUCUAAAUCGUA	41 mer
MPXV-WA	Primer-F2	GAGCATCTTCTGAGGAGGTAAATAGGCTAA	30 nt
	Primer-R2	GCATTACCGAGTTCAGTTTTATATGCCGAA	30 nt
	gRNA-2	UAAUUUCUACUAAGUGUAGAUAACGAUCGCUAGAGAUCUUC	41 mer
	Probe[a]	FAM-TATTAT-BHQ1	6 mer

[a]probe, 5′ and 3′ ends were labeled with FAM and BHQ1. The probe was shared in both clade detection. [b]nt, nucleotide; mer, monomeric unit.

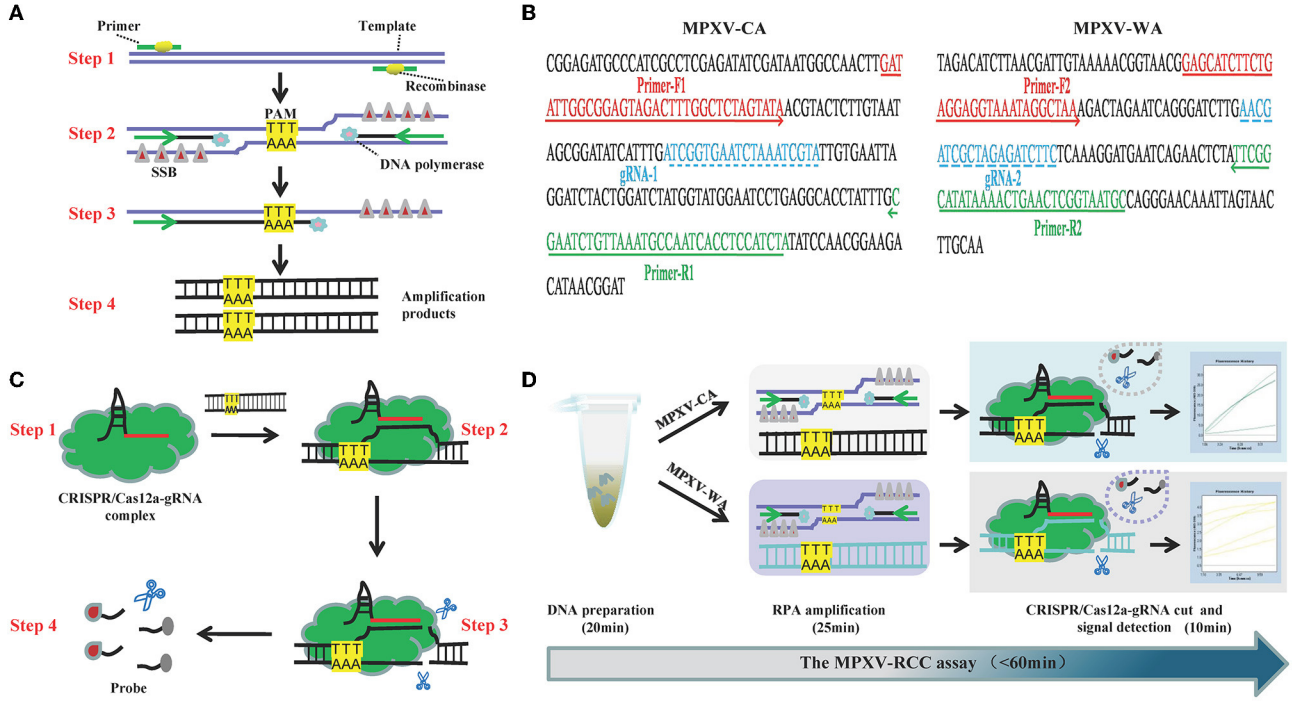

FIGURE 1
Schematic illustration of the MPXV-RCC technique. **(A)** Outline of RPA amplification for MPXV. Step 1: recombinase–primer complex specifically recognizes the target sequence containing a PAM site. Step 2: a D-loop is formed by the combination of SSB and DNA chain, and then, DNA polymerase activates the isothermal amplification. Step 3: displaced D-loop keeps the DNA open when the amplification continues. Step 4: products are generated exponentially. **(B)** Schematic illustration of CRISPR/Cas12a-gRNA detection. Step 1: Cas12a enzyme couples with gRNA to constitute CRISPR/Cas12a-gRNA complex. Step 2: gRNA sequence combines with target DNA near the PAM site. Step 3: Cas12a enzyme is activated to cut the template chain. Step 4: ssDNA is cleaved non-specifically by the Cas12a enzyme, and it could be available as a fluorescent probe. **(C)** Primer and gRNA sequence alignment on the MPXV segment. Nucleotide sequences of MPXV-CA and MPXV-WA are indicated. The locations of primers and gRNA are underlined, and the arrowheads show the amplified direction of primers. **(D)** Outline of MPXV-RCC assay workflow. MPXV-RCC diagnosis platform employs three steps: DNA preparation, RPA amplification, and CRISPR/Cas12a-gRNA cut and signal detection.

construct the reaction system. Then 1 μl DNA template (1.0 × 10^3 copies) and 1.25 μl reaction trigger were added to activate the reaction at 38°C for 25 min. Enterovirus (JACDC-1) (1.0 × 10^3 copies/μl) and distilled water acted as negative and blank controls. The cross-reaction test of primers was carried out between MPXV-CA and MPXV-WA to verify the discernibility of this assay. The RPA amplicons were analyzed using electrophoresis on the 2% agarose gel. The RPA amplification was implemented at a constant temperature from 35 to 42°C with a 1°C increase to optimize the experimental condition.

CRISPR/Cas12a-based assay

The CRISPR/Cas12a-based assay was carried out using Cas12a protein mixing with gRNA, which was implemented similarly to a previous study (14). The reaction system contained 0.5 μl of gRNA (10 μM), 0.5 μl of fluorescence reporter (5′-FAM-TATTAT-BHQ1-3′, 10 μM), 5 μl of reaction buffer (10×), 2 μl of RPA amplicon, 0.3 μl of Cas12a enzyme (20 μM), and 41.7 μl of distilled water to make a final volume of 50 μl. The released fluorescence signals were detected by a fluorescence detector.

Sensitivity analysis of the MPXV-RCC assay

Recombination plasmids and pseudovirus were used to assess the limit of detection (LoD) of the MPXV-RCC assay. MPXV-CA and MPXV-WA recombination plasmids were serially diluted from 1.0×10^5 to 1.0×10^2 copies/μl with 10-fold intervals, and then, six dilutions with concentrations of 50 copies/μl, 25 copies/μl, 10 copies/μl, 5 copies/μl, 2.5 copies/μl, and 1 copy/μl were acquired successively. All the dilutions were tested in triplicate to validate their consistency with distilled water as the blank control. Similarly, serial dilutions of MPXV pseudovirus ranging from 1.0×10^5 to 1 copy/μl were obtained and examined following the settlement process of the plasmid. Similarly, the LoD of MPXV-RCC in pseudovirus was also confirmed.

MPXV-RCC detection in spiked blood specimens

The practicability of the MPXV-RCC assay was evaluated with spiked human blood samples. The spiked blood samples were prepared by inoculating the serially diluted pseudovirus solutions into blood samples. The DNA of the simulated specimen was extracted by the EasyPure Viral DNA/RNA kit (TransGen biotechnology, Beijing, China) and eluted in a 50 μl of buffer solution. Finally, 1 μl of obtained nucleic acid acted as a template for the MPXV-RCC assay. According to the conversion, the final concentration of pseudovirus in the spiked blood samples for MPXV-RCC detection was 1.0×10^5 1 copies/reaction. Distilled water and non-spiked blood samples were taken for quality control of the experiment. Three parallel trials were performed to explore the detection threshold of the MPXV-RCC diagnosis platform in artificial specimens.

Specificity evaluation of the MPXV-RCC assay

The specificity of the MPXV-RCC assay was explored with non-MPXV pathogens and MPXV-positive specimens. Negative samples are displayed in Supplementary Table S1. Due to the lack of proper experimental resources, positive samples were simulated by blending MPXV pseudovirus and human blood. The concentration of MPXV pseudovirus used here was 1.0×10^2 copies/μl, and the strains were singly mixed with blood samples collected from 10 human subjects. The operation procedures of the specimen mixing and genomic DNA extraction referred to that of the spiked sample. Two repeated tests were conducted to determine the accuracy of this method.

Result

Overview of the MPXV-RCC technique

The principle of the MPXV-RCC technique is illustrated in Figure 1. In brief, after the genomic DNA is added to the RPA reaction mixture, the target sequence will be amplified at 38°C according to operating instructions (Figure 1A). In the RPA method, the dissociation of dsDNA relies on the activity of recombinase instead of a denaturation step. Initially, the recombinase–primer complex is constituted by binding recombinase to primers, which then seeks homologous sequences of primers on the target dsDNA segment. Following the displaced DNA strand stabilized by the SSB (single-stranded binding proteins) which formed a D-loop, the recombinase disassociated with the primers and the DNA polymerase binds to the 3′ end of the primer to initiate the amplification of target sequences. Thus, numerous target DNA sequences will be produced within 25 min. Particularly, in this study, the target sequences contained a specific protospacer adjacent motif (PAM) site (TTT), which could be employed for the following CRISPR detection.

During the detection stage, first, the Cas12a protein integrates with gRNA to constitute a CRISPR/Cas12a-gRNA complex, which can recognize the specific sequence adjoining the PAM site under the guidance of gRNA (Figure 1B). The Cas12a is therewith activated to cleave the template chain and simultaneously trim the ssDNA molecule due to its non-specific trans-cleavage capacity, which results in the release of fluorescent signals. The entire detection process of the MPXV-RCC technique consists of three steps, including rapid DNA preparation (20 min), RPA amplification (25 min), and CRISPR-based detection (10 min) (Figure 1D), which can be completed within 60 min.

Confirmation of the MPXV-RCC method

To verify the reliability of the MPXV-RCC method, the RPA primers of MPXV-CA and MPXV-WA were screened based on the images of the specific target band on 2% agarose gel, and each gRNA for CRISPR/Cas12a-based detection was validated according to the fluorescence intensity monitored by a fluorescence detector. As shown in Figure 2, the primers and gRNA of both clades of MPXV showed excellent performance in testing each target. The strong brightness and fluorescence signals could only be observed in the reactions loaded with positive plasmids DNA, while other reactions spiked with non-MPXV templates (enterovirus and distilled water) did not show the corresponding experimental characteristics. Furthermore, each primer set specially amplified its target template, and no cross-reaction was observed. Thus, the primer sets and gRNAs were suitable candidates for the development of MPXV-RCC assay to diagnose MPXV infection.

Optimal temperature for the MPXV-RCC method

To determine the optimal reaction temperature of MPXV-RCC at the amplification step, MPXV-CA- and MPXV-WA-RPA reactions were performed at temperatures from 35 to 42°C (interval of 1°C) using MPXV-CA and MPXV-WA recombination

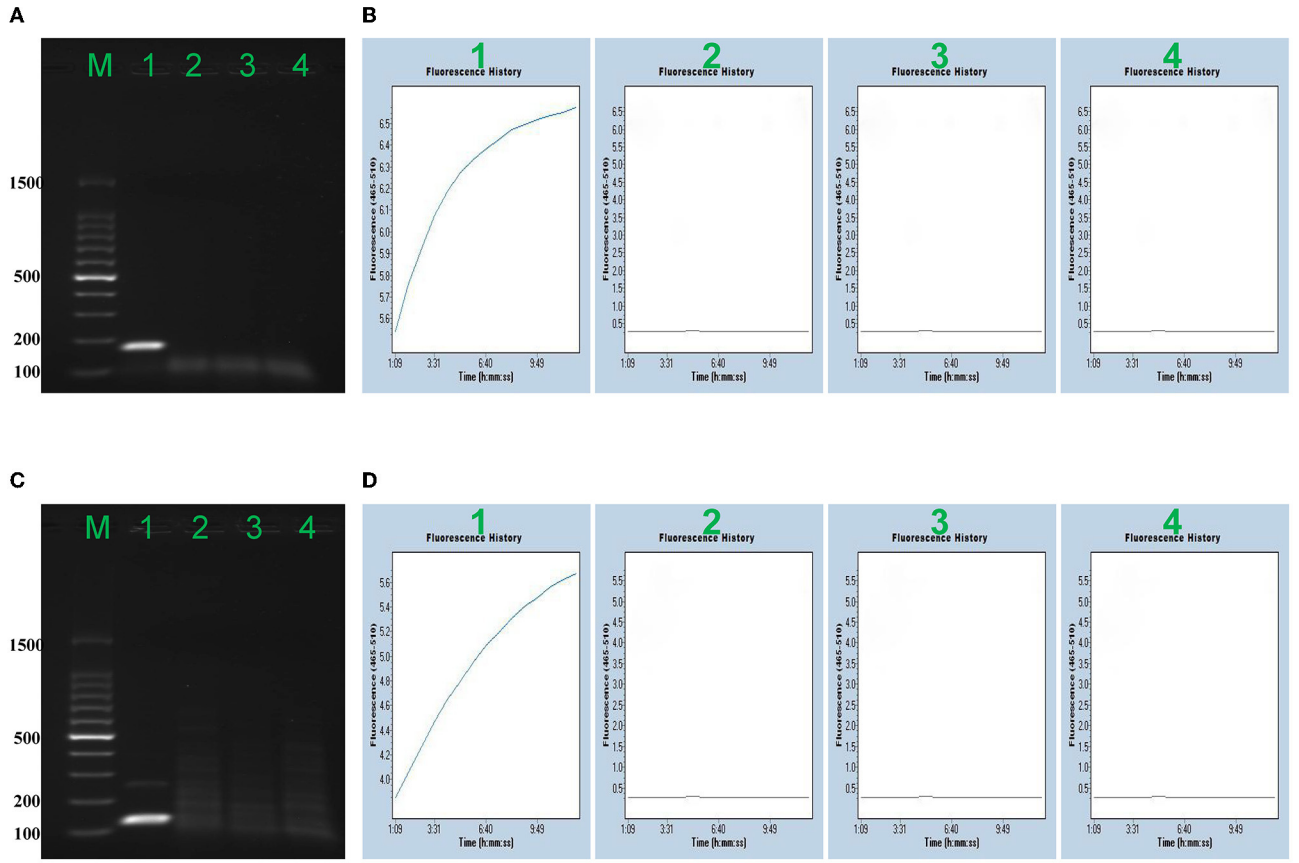

FIGURE 2
Confirmation of the MPXV-RCC assay. Agarose gel electrophoresis (AAG) indicated the RPA results, and fluorescence signal (FS) detection showed further CRISPR results. AAG **(A)** and FS **(B)** exhibited the detection of MPXV-CA. AAG **(A)**/FS **(B)** 1–4 represented MPXV-CA plasmid, MPXV-WA plasmid, enterovirus (JACDC-1), and distilled water, respectively. AAG **(C)** and FS **(D)** exhibited the detection of MPXV-WA. AAG **(C)**/FS **(D)** 1–4 singly represented MPXV-WA plasmid, MPXV-CA plasmid, enterovirus (JACDC-1), and distilled water.

plasmids (1.0×10^2 copies/μl), respectively. The results from electrophoresis (Supplementary Figure S1) illustrated that 37 to 40°C were the better options for the MPXV-CA-RPA reaction and 37 to 39°C for MPXV-WA. As a result, a temperature of 38°C was selected to implement the MPXV-RCC method at the isothermal reaction stage.

Sensitivity estimation of the MPXV-RCC assay

We evaluated the LoD of the MPXV-RCC assay using recombination plasmids and pseudovirus, and various dilutions of MPXV-CA and MPXV-WA plasmids (ranging from 1.0×10^5 1 copies per reaction) were tested. Using the plasmid as a template, the LoD of the MPXV-RCC assay was down to five copies per reaction for both MPXV-CA and MPXV-WA detection (Figure 3). These tests were performed with three duplicates. Using the DNA templates extracted from pseudovirus, the MPXV-RCC assay could detect down to five copies of pseudovirus for MPXV-CA but 10 copies for MPXV-WA (Supplementary Figure S2).

Validation of the MPXV-RCC detection system using MPXV-spiked clinical samples

To examine the feasibility of the MPXV-RCC assay in clinical settings, the spiked human blood samples with MPXV pseudovirus were applied due to the unavailability of clinically positive patients. Similar to the pure templates, the LoD of the MPXV-RCC assay for CA and WA clades of MPXV were 5 and 10 copies per reaction in spiked blood samples, respectively, as well (Supplementary Figure S3), while the lower concentrations, non-spiked sample, and distilled water gave negative results.

Specificity of the MPXV-RCC method

To further determine the specificity of our assay, we extracted DNA templates from spiked MPXV pseudovirus samples and various non-MPXV strains. Positive signals were only observed in MPXV-spiked samples but not in the non-MPXV templates, suggesting their accuracy for both clades of MPXV detection (Figure 4; Supplementary Figure S4). These

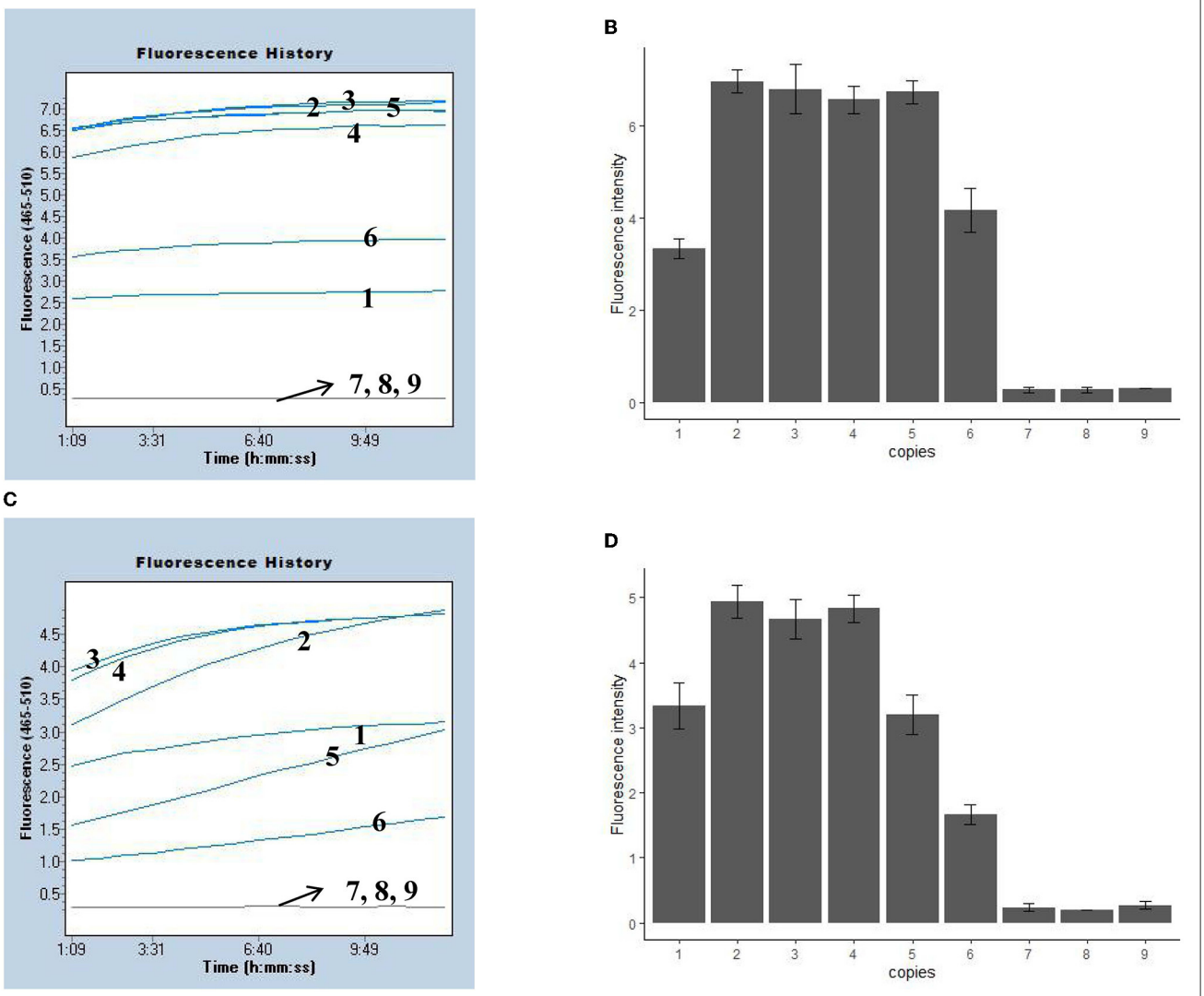

FIGURE 3
Sensitivity of the MPXV-RCC assay in recombinant plasmids. **(A, C)** Indicate the fluorescence results of the MPXV-RCC assay for MPXV-CA and MPXV-WA detection with recombinant plasmids, respectively. **(B, D)** Show the statistical results of fluorescence intensity for two clades. Lines/bars 1–9 represent the results with plasmid levels of 1.0×10^3, 1.0×10^2, 50, 25, 10, 5, 2.5, 1 copies/reaction, and blank control, respectively.

results suggested that the assay developed here could specifically detect MPXV.

Discussion

The outbreak of monkeypox has caused another public health emergency during the COVID-19 pandemic. To control the further spread of this disease, simple and efficient methods for MPXV detection are increasingly in demand. In this study, a rapid, accurate, and sensitive MPXV diagnosis platform coupling RPA amplification with CRISPR/Cas12a-gRNA detection has been devised and named MPXV-RCC. In the MPXV-RCC system, the target sequences were pre-amplified by RPA assay at a constant temperature. Then, the trans-cleavage characteristic of the Cas12a protein is activated to cut the RPA products and digest the detection probe in the mixtures. Finally, the diagnostic result could be judged by observing fluorescence intensity.

In this study, the reaction conditions of the MPXV-RCC assay, including RPA reaction and CRISPR/Cas12a-gRNA detection, were optimized. The isothermal RPA reaction only needed a simple heating instrument, such as a water bath, to maintain the reaction at a constant temperature (38°C), and the MPXV products could be amplified exponentially within 25 min. CRISPR/Cas12a-gRNA detection was carried out at 37°C for 10 min by employing a real-time fluorescence instrument, and the procedure of detection was simple and rapid. The whole process of the MPXV-RCC assay, including DNA preparation (20 min), RPA isothermal amplification (25 min), and CRISPR/Cas12a-gRNA detection (10 min), could be performed within 1 h. Therefore, the established method could be applied as a rapid tool for the diagnosis

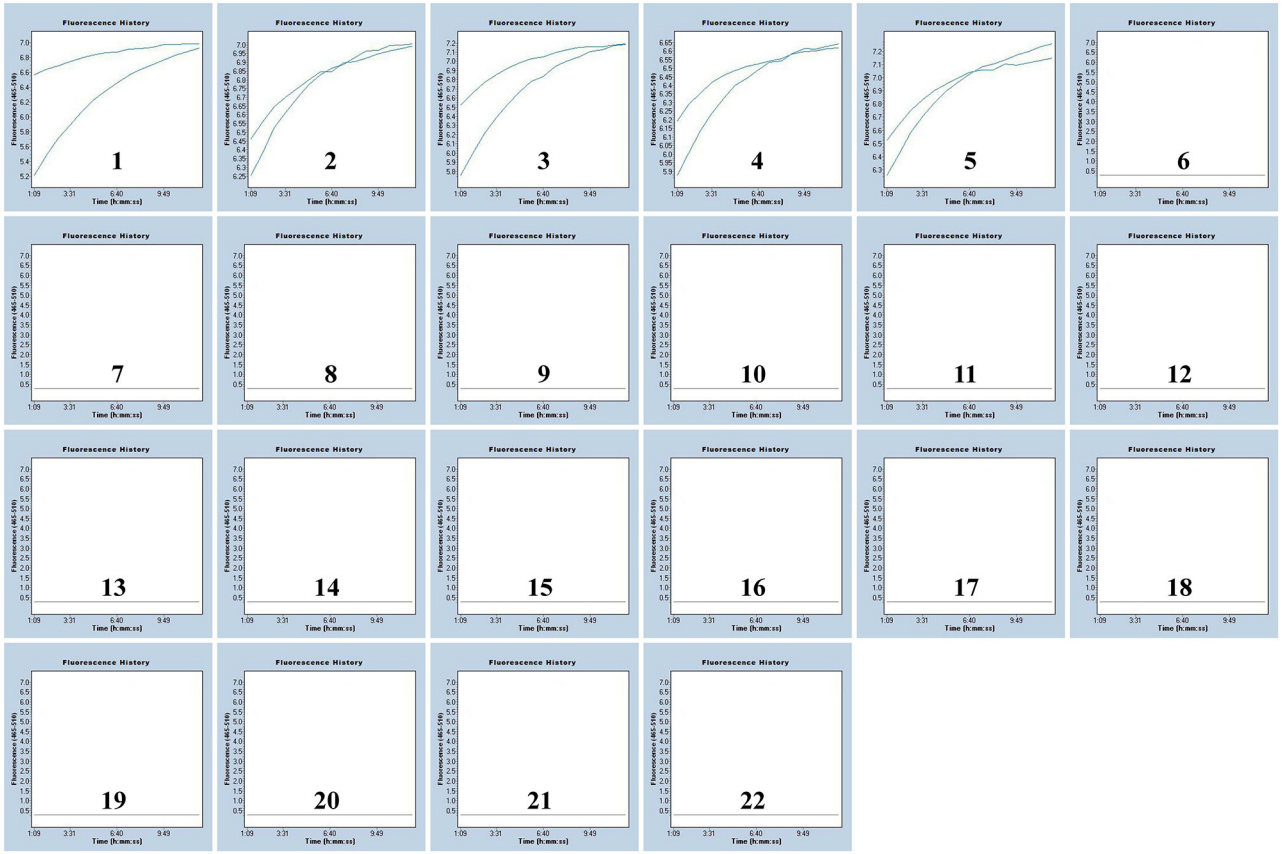

FIGURE 4
Specificity of the MPXV-RCC assay for MPXV-CA detection. The results of two repeated tests are shown in the figure. Graphs 1–5: MPXV-CA agents (spiked clinical samples). Graphs 6–21: influenza A virus, enterovirus, adenovirus, coronavirus, dengue virus, Epstein-Barr virus, hepatitis B virus, human rhinovirus, herpes simplex virus-1, influenza B virus, measles virus, parainfluenza virus, rubella virus, respiratory syncytial virus, Visna virus, and vesicular stomatitis virus, separately. Graph 22, blank control.

of MPXV infection, which might be especially suitable for the point-of-care (POC) test.

The recombination plasmids and pseudovirus were used to evaluate the sensitivity of the MPXV-RCC method, and an excellent result was obtained. This assay could detect plasmid templates as low as 5–10 copies/reaction when identified for MPXV-CA and MPXV-WA, which is >100 times more sensitive than the conventional PCR method and has a better sensitivity than the real-time PCR assay (20, 21). Particularly, PCR-based assays need more reaction time. In addition, due to no available clinically positive MPXV-infected patients in our country, the clinical feasibility of the MPXV-RCC assay was further validated using spiked samples. This technique was able to detect <10 copies of pseudovirus in simulated blood specimens, and it demonstrated that the established diagnostic platform was supersensitive in clinical settings. In addition to the low LoD, the novel-designed assay appeared specific. The identification ability of nucleic acid extracted from MPXV-positive samples and negative species could explain the specificity of the MPXV-RCC method. Particularly, the specificity was guaranteed by combining gRNA with primers, which recognized the MPXV sequence precisely. The results displayed that the MPXV-RCC assay could differentiate MPXV-CA and MPXV-WA and had no cross-reactivity with other pathogenic microorganisms. Hence, the diagnostic method relied on CRISPR/Cas12a-gRNA detection exhibited excellent accuracy.

Some isothermal amplification methods including LAMP and RPA have been exploited to monitor MPXV for the past few years (21, 22). Compared with LAMP, the MPXV-RCC method possesses a slightly higher sensitivity. Primer design and screening of this assay are easier than that of LAMP, which needs three pairs of primers (21). Similarly, the MPXV-RCC platform reveals a preferable sensitivity in comparison with the RPA-only detection (23), and a previous study also indicated that the CRISPR/Cas12a-gRNA detection could enhance the sensitivity of the diagnosis assay (24). In addition, non-specific amplification is an inherent defect of RPA reaction, whereas the CRISPR/Cas12a-gRNA detection can solve this problem and make the method more specific. For result judgment, fluorescence signal detection used in the MPXV-RCC method is more exact than the colorimetric indicator, which was usually used in isothermal amplification tests. Those tests obtain the result based on color change, and it will face trouble when the content of the target sequence is very low (25, 26).

In summary, we chose the target sequence elaborately, devised the primers and gRNA to establish a reliable CRISPR/Cas12a-based method for the diagnosis of MPXV infection, and subsequently verified the practicability of the proposed assay in this study. The MPXV-RCC diagnosis platform developed here shows merits in simplicity, rapidity, specificity, and sensitivity, which could detect 5–10 copies of DNA per reaction in recombination plasmids, pseudovirus, and spiked clinical samples, and the detection time only needs 60 min. Thus, the MPXV-RCC assay provides a useful diagnostic tool for the timely diagnosis of monkeypox cases in clinical settings.

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 study involving the samples of human origin was approved by the Institutional Review Board of Wuhan Center for Disease Control and Prevention (WHCDCIRB-K-2021038). The blood specimens were extracted from 10 healthy people who had consented the experiments and signed the ICF (Informed Consent Form).

Author contributions

LG, XL, JL, and YiW devised the experimental scheme. LG, XC, and YiW wrote and reviewed the manuscript. LG performed the experiments. YimW and YiW collected partial samples and reagents. LG and XL analyzed the experimental data. 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.2023.1137968/full#supplementary-material

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Knowledge, attitudes, and practices toward COVID-19 prevention in Yemen: a community-based cross-sectional study

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Background: Pandemics, especially in fragile war-torn countries like Yemen, challenge their already strained health systems. Community adherence to pandemic prevention measures is necessary to curb the severity and spread of emerging pandemics – which is influenced by factors, such as people's knowledge and attitudes toward the pandemic. No studies in Aden have been published on the communities' knowledge, attitudes, and practices (KAP) toward COVID-19 prevention to date. To understand adherence to pandemic prevention measures in contexts with fragile health systems, this study investigated KAP of Yemeni participants toward the COVID-19 pandemic.

Methods: We conducted face-to-face semi-structured questionnaires among 400 eligible participants whom were identified for participation in this study through systematic household sampling from eight districts in Aden, Yemen. Eligible participants were Yemeni community members who were ≥18 years, living for more than 10 years in Yemen, and were willing to voluntarily participate in the study. The questionnaire included questions surrounding the participants' COVID-19 knowledge (e.g., awareness of spread and prevention), attitudes (e.g., willingness to accept the vaccine or other prevention measures), and prevention practices during the pandemic (e.g., mask wearing, social distancing, vaccine uptake). Total KAP scores were calculated. Univariate and bivariate statistical analyses were conducted using STATA 13 software.

Results: From January to May 2021 we conducted 400 questionnaires with Yemeni community members. The average age was 41.5 ± 14.5 years (range 18–86 years). The results demonstrated that the participants in this study had an intermediate knowledge (53%) and fair attitude (58%) scores. However, participants reported very poor COVID-19 prevention practices- with only 11% demonstrating these practices. Only 25% (100/400) practiced social distancing, 25% (98/400) wore a mask, and only 6% (27/400) of participants accepted (at least one dose of) the COVID-19 vaccine. Factors associated with increased knowledge were being male, married, and surprisingly those having a primary and middle school education levels ($p<0.05$). Also participants who were diagnosed with COVID-19 or had a family member diagnosed with COVID-19 (vs. those not diagnosed OR=2.08, 95% CI 1.07–3.78, $p<0.05$) were more likely to know that

the vaccine protects against severe COVID-19 infection and were more likely to apply good practices such as accepting the vaccine (OR=2.65, 95% CI 1.17–6.00, $p<0.05$) compared to those who were not.

Conclusion: These findings raise awareness for the need of community-oriented education programs for COVID-19 which considers associated factors to improve the level of public knowledge, attitudes, and practices.

KEYWORDS

attitudes, COVID-19 prevention, knowledge, practices, Yemen, emerging diseases

1. Introduction

Coronavirus disease (COVID-19) is a threat to global health and was declared a public health emergency of international concern as well as a global pandemic in March 2020 (1). COVID-19 is a respiratory disease that is highly transmissible and has been recorded among 627 million people globally and has attributed to more than 6,578,440 deaths as of November 1st, 2022 (2). COVID-19 is caused by infection with a novel and highly contagious coronavirus strain (SARS-CoV-2) that spreads predominately though respiratory droplets. It was first identified in Wuhan, China in 2019 (3, 4). The frequent modes of COVID-19 transmission include (1) contact with droplets produced when an infected person coughs or sneezes and/or (2) when in contact with contaminated surfaces (5). While many infections are asymptomatic, COVID-19 can progress to severe illness such as pneumonia, acute respiratory distress syndrome (ARDS), multi-organ dysfunction, and ultimately death (6). People who are of older age and those with co-morbid chronic disease are at higher risk of serious and critical illness (1, 7).

Strategies to control the spread of COVID-19 include following certain preventative measures such as early screening, early diagnosis, isolation, and treatment of cases (8). Moreover, additional strategies to control infections at community level are through frequent hand washing, hand sanitizing, and social distancing (8). To guide these prevention strategies, the World Health Organization (WHO) adopted a one year Strategic Preparedness and Response Plan (SPRP) which intended to help direct the public health response to the pandemic at different phases and to prioritize the worldwide strategic response to COVID-19 (9).

Pandemic diseases pose a challenge to health systems, especially in war-torn countries like Yemen which was already strained by more than seven years of civil conflict, mass human displacement, natural disasters, and several disease outbreaks (e.g., cholera, diphtheria, and measles) (10–12). In Yemen, the first confirmed case of COVID-19 was on April 10th, 2020 in Hadhramaut (12). Recent data shows that Yemen has since then registered 11,939 confirmed cases and 2,158 deaths attributed to COVID-19 (13). However, the real burden and trajectory of the disease and its spread are vague, due to the absence of well-equipped labs, testing tools, human capacity, and poor infrastructure challenging effective monitoring and evaluation of the COVID-19 pandemic (14). Similarly, limited human capacity and medical supplies may have attributed to inconsistencies in the numbers of confirmed COVID-19 cases and deaths reported. For example, one geospatial analysis of burial activity in Aden during the pandemic, suggested the extensive (and under reported) impact of COVID-19 mortalities (15). The findings in the study estimated ~1,500 excess burials across Aden by July 6th, 2020 and up to 2,120 excess burials by September 19th, 2021. At the peak of the pandemic, they found an increase in burials of nearly 230% compared to the previously reported numbers (15).

For effective control and mitigation of further COVID-19 infections, adherence to protective and preventive measures are necessary. Specifically, the compliance with protective health measures is often influenced by the population's knowledge, attitude, and practice (KAP) (16, 17). For example, studies conducted during the severe acute respiratory syndrome (SARS) outbreak in 2003 in China and the US found that population attitudes and practices toward the SARS virus prevention were influenced by negatively held emotions and assumptions (18–20). Moreover, COVID-19 knowledge gaps have been associated with multiple socio-economic patterns (e.g, limited education, and low income)- and individuals who demonstrated both were found to undermined the risk of COVID-19 spread and symptoms (20).

This study is the first to identify the knowledge gaps and practices toward COVID-19 prevention among a community in Yemen. We aim to generate actionable and timely data from this population to inform tools that are needed to gauge the public's awareness of and attitudes toward the disease. This study will also help inform health authorities to craft robust interventions and effective policies regarding the management of COVID-19 that are relevant and appropriate to the Yemeni situation.

2. Materials and methods

2.1. Setting

Yemen has 22 governorates, this study was conducted in the Aden governorate which has eight districts: Sira, Khormaksar, Al Mualla, Al Tawahi, Sheikh Othman, Mansoura, Dar Saad, and Brega. Each district has its own focal health point, which reports to the central Ministry of Public Health and Population (MoPHP) through the Governorate Health Office (GHO). In this study, the Aden governorate was selected for the following reasons: Aden has a large catchment area covering 1,114 km^2, a population of around one million people, and it was the first governorate in Yemen to register COVID-19 cases (Figure 1).

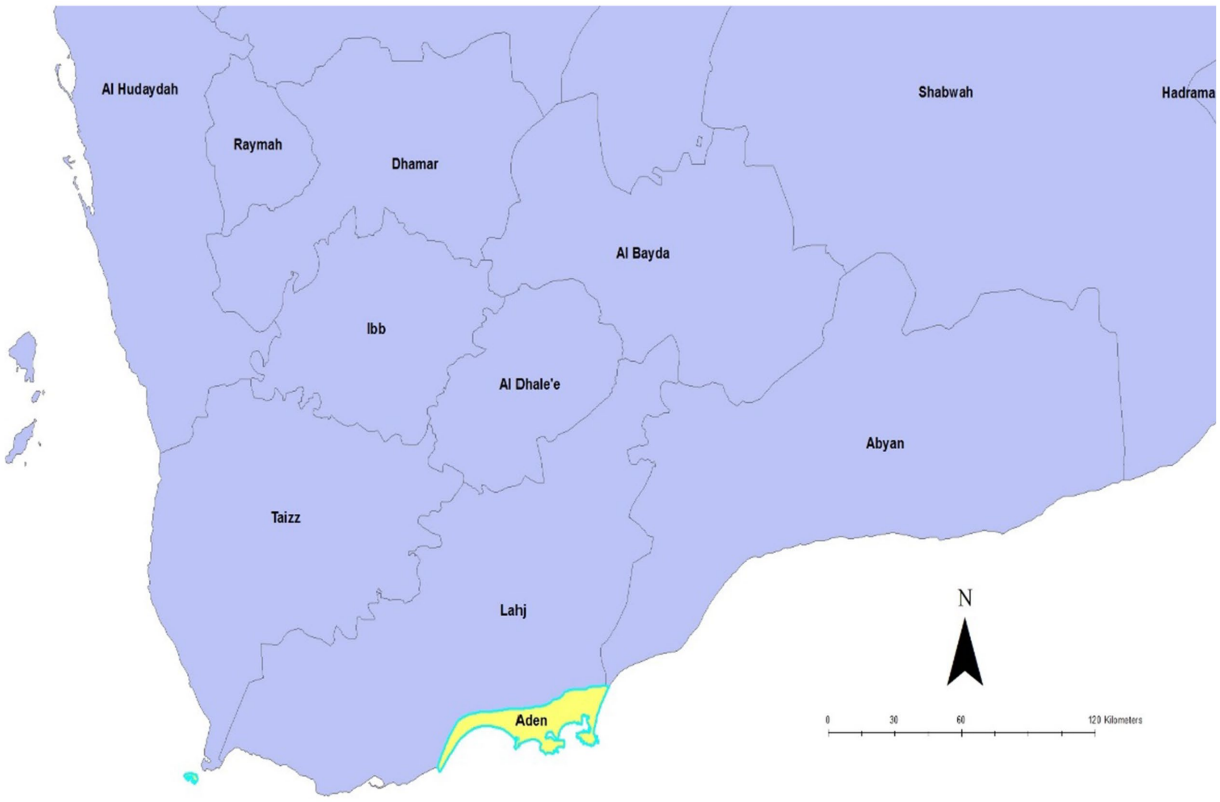

FIGURE 1
Source: Dureab F et al. Map showing location of Aden, Yemen. Aden; 2019 (21).

2.2. Study design and sampling

A cross-sectional study was conducted between January and May 2021. Four of the eight districts were selected by simple random sampling. Participants were randomly selected based on cluster sampling. The sample size (N) needed for the study was calculated using the formula $N = Z2P\ (1–P)/d2$ with the assumption that the proportion of Yemeni women with health (maternal and neonatal) knowledge (P) was 50% with a 5% margin of error (d) and a 95% confidence interval (CI; Z) equal to 1.96 as based on previous literature (22).

A simple systematic sampling method was used to recruit participants- 100 from each district (total of 400 participants). Of households selected to participate in this study, the first and third household respondents were randomly selected. The respondents who met the inclusion criteria and agreed to participate in the study were recruited and this process was continued until the calculated sample size was reached.

2.3. Data collection tool

The data was collected by eight trained research assistants using a face-to-face semi-structured questionnaire in the local language (Arabic). The questionnaire was adapted from a similar KAP study on COVID-19 conducted by Zhong et al. (2020) in China. We piloted the questionnaire among experts at the University of Aden and Heidelberg University before being implemented. A three-day training course was provided to eight research assistants by Aden's research team to develop their interviewing skills and ensure consistency in the data collection. The questionnaire collected information on (1) socio-demographic characteristics; (2) knowledge of COVID-19 transmission and prevention measures; (3) attitudes toward COVID-19 prevention measures; and (4) practices regarding COVID-19 prevention. Participant's answered most knowledge questions with "Yes/No," or "I do not know." Other knowledge questions were open-ended which included asking participants to list forms of COVID-19 transmission and prevention methods they utilized. For both types of questions (closed and open-ended) those who gave an incorrect or an incomplete answer was scored "0," and the correct answer was scored "1." Similarly, in the section on attitudes and practices, a score of "1" was given to answers that reflected positive attitudes and/or good prevention practices toward COVID-19 and a score of "0" was given to answers that reflected negative attitudes and/or poor COVID-19 prevention practices. Practices were deemed good/poor through comparison with guidelines and literature on COVID-19 prevention practices. Two researchers, both with graduate-level quantitative training, agreed on categorizing answers as good/poor via consensus. Thereafter, an average score was calculated for each section and compared with a predetermined scale: 0–40% = poor knowledge/negative attitudes/ poor practices; 41–70% = intermediate knowledge/fair attitudes/fair practices and 71–100% = good knowledge/positive attitudes/ good practices.

2.4. Validity and reliability of the data collected

The validity of this study was assured by the following actions. The semi-structured questionnaire demonstrated both content validity and face validity through its adaptation from a recent study (23). Furthermore, face validity was established by having four researchers – two from each university (with clinical and/or academic backgrounds) review the questionnaire and confirm that it measured the variables of interest. Thereafter, the questionnaire was reviewed by a German international health expert (study supervisor), three additional Yemeni researchers, and two medical doctors before implementation. To assure efficacy of the questionnaire, it was piloted with 20 Yemeni community members selected with the same age criteria (≥18 years) and who maintained similar socio-demographic characteristics to the participants in the final study. Based on this pilot study, two questions were modified.

2.5. Statistical analysis

All data was translated from Arabic to English by an experienced translator, cleaned, and coded using Excel. Descriptive analyses, standard deviations for continuous variables, and the count/percentages for the dichotomous or categorical variables were analyzed in Stata® 13 software. Descriptive statistics were presented in four main sections: (1) socio-demographic characteristics; (2) knowledge of COVID-19 transmission and prevention methods; (3) attitudes toward adherence to COVID-19 prevention measures; and (4) practices in regard to COVID-19 prevention and vaccination uptake.

Inductive statistics using chi-squared tests, p-values, and linear logistic models (odds ratios) were conducted to analyze the association between all socio-demographic variables (age, education, marital status, and occupation, time to reach health facility) and variables reflecting participant knowledge, attitudes and practices toward COVID-19 prevention was presented using tables. Analysis of variance and chi-squared tests were used to measure significance. The level of significance was set at $p < 0.05$.

3. Results

A total of 400 participants completed the questionnaire (response rate = 100%) and participated in this study with a mean age of 41 (SD 15) years and a range of 18 to 86 years. More than half the participants were males ($n = 205$, 51%) and most participants were married ($n = 267$, 67%). The majority of participants ($n = 265$, 66%) had either completed a high school education or had a university degree. Fewer participants had either completed middle school ($n = 42$, 10%), elementary school ($n = 47$, 12%), or had had no formal education ($n = 37$, 9%). About half of the participants were employed ($n = 203$, 50.75%) and only about a quarter of participants ($n = 106$, 26%) reported that they or one of their family members were diagnosed with COVID-19. Further, the majority of participants ($n = 267$, 66%) said that they need less than 15 min to reach the closest health facility. Table 1 presents the detailed socio-demographic characteristics of the participants.

TABLE 1 Respondents socio-demographic and health characteristics in Aden (N=400).

Characteristics	Frequency (N)	Percentage (%)
Age		
≤24	54	13.5
25–49	218	54.5
≥50	128	32.0
Gender		
Female	205	51.3
Male	195	48.7
Marital Status		
Married	267	66.8
Other	133	33.3
Occupation		
Employed	203	50.7
Unemployed	197	49.3
Education		
Elementary school	46	11.5
Up to middle School	89	22.3
High School/University	265	66.3
Time to reach health facility		
Less than 15 min	267	66.8
15 to 30 min	115	28.8
More than 30 min	18	4.50
Has been diagnosed with COVID-19 (participant or one close family member)		
No	286	71.5
Yes	106	26.5
I do not know	8	2.00

3.1. Participant knowledge on COVID-19

Table 2 shows results of participants' knowledge on COVID-19 cause of infection, onset, transmission modes, and prevention methods. The majority of participants ($n = 286$, 72%) in this study reported that they received information on COVID-19, mainly from media/news ($n = 288$, 72%) and family/friends ($n = 195$, 49%). The majority of participants ($n = 320$, 80%) correctly reported that COVID-19 is a disease caused by a virus. Around 64% ($n = 255$) of participants correctly answered that symptoms appear between 2–14 days and 79% ($n = 314$) of participants understood the disease can be fatal. Moreover, the majority of participants ($n = 345$, 86%) reported that they knew how COVID-19 is transmitted, however, only the minority of respondents identified that contact with other individuals ($n = 81$, 20%), touching surfaces ($n = 90$, 22%), coughing ($n = 75$, 18%), sneezing ($n = 118$, 29%) and droplets in the air ($n = 77$, 19%) as routes of transmission. When asked whether they knew about COVID-19 prevention methods, 91% ($n = 364$) responded "Yes." Nevertheless, only 62% ($n = 248$) identified covering mouth and nose, 65% ($n = 259$) reported hand washing with soap and water, and 64% ($n = 256$) said social distancing were prevention methods. It is worth mentioning that only 13% ($n = 51$) of participants mentioned

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TABLE 2 Knowledge of COVID-19, transmission, and prevention (N=400).

Knowledge items	Correct response *N* (%)	Incorrect response *N* (%)
Ever received information on COVID-19*	286 (71.50)	106 (26.50)
Causative Agent of Covid-19	320 (80.00)	80 (20.00)
COVID-19 symptoms appear in 2–14 days	255 (63.75)	145 (36.25)
COVID-19 can be fatal	314 (78.50)	62 (15.50)
Know tramission routes of COVID-19	345 (86)	55 (14)
Contact with others	81 (20.25)	319 (79.75)
Touching surfaces	90 (22.50)	310 (77.50)
Coughing	75 (18.75)	325 (81.25)
Sneezing	118 (29.50)	282 (70.50)
Through the air	77 (19.25)	323 (80.75)
Know main prevention methods	364 (91)	36 (9)
Cover mouth and nose	248 (62.00)	152 (38.00)
Hand washing with water and soap	259 (64.75)	141 (35.25)
Social Distancing	256 (64.00)	144 (36.00)
Vaccination	51 (12.75)	349 (87.25)
Believe protective measures are effective*	293 (73.25)	59 (14.75)
Believe COVID-19 vaccine is protective	130 (32.50)	270 (67.50)

*Numbers for each item may not add up to the total number of study population due to missing values.

vaccination as methods of preventing and protecting oneself from becoming infected with COVID-19. When we asked the participants whether they think the COVID-19 vaccine is effective, 33% (n = 130) responded positively and the other participants either responded that the vaccination is not effective (n = 139, 35%) or they were unsure (n = 131, 33%). Participants mean score reflecting knowledge was calculated to be 53% (Table 3). Regression analysis showed that males (vs. females OR = 1.78%, 95CI 1.13–2.84, p < 0.05) and those ≤24 (vs. other age groups OR = 2.14%, 95CI 0.99–4.56, p < 0.05) were more likely to know about hand washing as a preventive measure toward COVID-19. Also, married men (vs. those having other marital statuses OR = 2.07, % 95CI 1.03–4.16, p < 0.05) were more likely to mention vaccination as a preventive measure. Surprisingly, those participants having lower education level (primary school and middle school) were more likely to have adequate knowledge on preventive measures such as covering their mouth and nose (OR = 3.06, 95% CI 1.52–6.13, p < 0.05,OR = 3.23, 95% CI 1.92–5.43, p < 0.01), hand washing (OR = 6.85, 95% CI 3.3–14.2, p < 0.001, OR = 1.70, 95% CI 1.01–2.89, p < 0.05), social distancing (OR = 5.46, 95% CI 2.66–11.24, p < 0.01, OR = 2.03, 95% CI 1.21–3.42, p < 0.01), and vaccination (OR = 4.01, 95% CI 1.09–14.70, p < 0.05, OR = 18.59, 95% CI 2.49–138.5, p < 0.01) compared to those with higher education level (high school and university degree participants). Additionally, those needing less than 15 min to reach the closest health facilities (vs. those that need more than 15 min OR = 0.43, 95% CI 0.27–0.68, p < 0.01, OR = 0.62, 95% CI 0.39–0.98, p < 0.05, OR = 0.56, 95% CI 0.35–0.88, p < 0.01) were less likely to mention covering mouth and nose, hand washing and social distancing as methods of prevention toward COVID-19. Finally, those who were diagnosed with having COVID-19 or had a family member diagnosed with COVID-19 (vs. those not diagnosed OR = 2.08, 95% CI 1.07–3.78, p < 0.05) were more likely to know that the vaccine protects against severe COVID-19 infection (Table 4).

3.2. Participant's attitudes toward COVID-19 prevention and vaccination

Only 44% (n = 177) of the participants in this study reported interest in receiving more information on COVID-19. However, the majority of participants (n = 366, 92%) showed that they were willing to comply with the COVID-19 restrictions such as lockdowns, if established. Interestingly, around 61% (n = 243) of the respondents were not willing to accept the COVID-19 mainly for the following reasons: there were many rumors about the vaccine (n = 55.13%), the vaccine was believed to cause other health problems (n = 53, 13%); participants were unwilling to try a novel vaccine (n = 48, 12%); the

TABLE 3 Mean score of grouped variables reflecting knowledge, attitudes, and practices.

Sections	Correct/Positive answers	Number of total participant answers	Score (%)	Interpretation
Knowledge	3,562	6,720	53	Intermediate
Attitudes	700	1,200	58	Fair
Practices	581	5,200	11	Poor

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TABLE 4 Attitudes toward COVID-19 prevention and vaccination (*N*=400).

Attitude items	Correct response *N* (%)	Incorrect response *N* (%)
Needs more information on COVID-19	177 (44.25)	223 (55.75)
Would take the vaccine	157 (39.25)	243 (60.75)
Would commit to prevention methods	366 (91.50)	34 (8.50)

vaccine was considered to be a conspiracy ($n=32$, 8%); or the belief that the vaccine would not prevent infection ($n=28$, 7%). Participants' mean attitude score was 58% (Table 3). Regression analysis showed that males (vs. females OR = 1.62, 95% CI 1.03–2.54, $p<0.05$), participants between 24–49 years of age (vs. other age groups OR = 2.32, 95% CI 1.39–3.89, $p<0.001$) were more likely accept to take the vaccine if it was offered to them. However, those with low education levels such as elementary school OR = 0.31, 95% CI 0.13–0.74, $p<0.01$) and those with middle school education (vs. High education OR = 0.51, 95% CI 0.29–0.89, $p<0.05$) were less likely to accept to take the vaccine if offered to them (Table 5).

3.3. Participant's practices taken for the prevention of infection

Table 6 describes the protection measures taken by participants to prevent COVID-19 infection. A quarter of the participants applied

TABLE 5 Results of logistic regression analysis on factors associated with adequate knowledge, positive attitudes, and good practices toward COVID-19.

Variables	Good knowledge			Positive attitudes		Good practice
	Cover Mouth and Nose	Hand washing	Social Distancing	Vaccination	Would you take the Vaccine?	Did you take the vaccine?
	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)	OR (95% CI)
Sex						
Male	1.12 (0.72–1.77)	1.78 (1.13–2.84)*	1.32 (0.83–2.09)	1.48 (0.78–2.79)	1.62 (1.03–2.54)*	1.11 (0.49–2.54)
Female	Ref	Ref	Ref	Ref	Ref	Ref
Age						
<24	1.10 (0.51–2.38)	2.14 (0.99–4.56)*	1.22 (0.57–2.62)	2.66 (0.87–8.14)	1.29 (0.58–2.87)	2.46 (0.54–11.19)
24–49	1.03 (0.63–1.67)	0.88 (0.64–1.44)	0.96 (0.59–1.57)	1.40 (0.69–2.85)	2.32 (1.39–3.89)***	1.60 (0.66–3.86)
>50	Ref	Ref	Ref	Ref	Ref	Ref
Marital status						
Married	0.76 (0.46–1.25)	0.931 (0.56–1.56)	0.66 (0.39–1.08)	2.07 (1.03–4.16)*	0.75 (0.45–1.26)	1.88 (0.78–4.49)
Others		Ref	Ref	Ref	Ref	Ref
Occupation						
Employed	0.79 (0.51–1.24)	0.99 (0.63–1.56)	0.82 (0.53–1.30)	0.71 (0.37–1.37)	1.16 (0.74–1.83)	1.34 (0.57–3.11)
Unemployed	Ref	Ref	Ref	Ref	Ref	Ref
Educational level						
Elementary School	3.06 (1.52–6.13)*	6.85 (3.3–14.2)***	5.46 (2.66–11.24)***	4.01 (1.09–14.70)*	0.31 (0.13–0.74)**	0.89 (0.26–3.08)
Middle school	3.23 (1.92–5.43)**	1.70 (1.01–2.89)*	2.03 (1.21–3.42)**	18.59 (2.49–138.55)**	0.51 (0.29–0.89)*	2.17 (0.61–7.7)
High education level	Ref	Ref	Ref	Ref	Ref	Ref
Health facility access						
<15 min	0.43 (0.27–0.68)**	0.62 (0.39–0.98)*	0.56 (0.35–0.88)**	1.49 (0.78–2.88)	0.65 (0.41–1.04)	0.45 (0.17–1.21)
>15 min	Ref	Ref	Ref	Ref	Ref	Ref
Diagnosed with COVID-19						
Yes	0.99 (0.61–1.61)	1.10 (0.67–1.79)	1.48 (0.89–2.44)	2.08 (1.07–3.78)*	1.08 (0.67–1.74)	2.65 (1.17–6.00)*
No	Ref	Ref	Ref	Ref	Ref	Ref

OR, odds ratio. CI, confidence interval. *$p<0.05$, **$p<0.01$, ***$p<0.001$.

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TABLE 6 Preventive practices taken by respondents during the COVID-19 pandemic (*N*=400).

Practice items	Correct response *N* (%)	Incorrect response *N* (%)
Practice social distancing	100 (25.00)	300 (75.00)
Wear a mask	98 (24.50)	302 (75.50)
Eat healthy foods	40 (10.00)	360 (90.00)
Stay home	61 (15.25)	339 (84.75)
Avoid contact with sick people	17 (4.25)	383 (95.75)
Avoid handshaking	6 (1.50)	394 (98.50)
Avoid close contact with others	34 (8.50)	366 (91.50)
Avoid using others' utensils	7 (1.75)	393 (98.25)
Apply hand washing	93 (23.25)	307 (76.75)
Apply hand sterilization	76 (19.00)	324 (81.00)
Take vitamins	17 (4.25)	383 (95.75)
Fully immunized	5 (1.25)	395 (98.75)
Received any dose of the vaccine	27 (6.75)	373 (93.25)

social distancing ($n=100$, 25%); covered their mouth and nose ($n=98$, 24.5%); ($n=93$, 23%); followed hand washing etiquette; used sterilization ($n=76$, 19%); or avoided close contact with others ($n=34$, 9%), as methods to prevent infection. Some participants mentioned additional methods they employed to prevent infection such as eating healthy foods; drinking herbal teas and juices; inhaling steam, and taking vitamins. Interestingly, only around 1% ($n=5$) of participants said that they were fully vaccinated against COVID-19. However, when asked in a separate question, only 6% ($n=27$) of the participants took at least one dose of the COVID-19 vaccine. Participants' mean practice score was 19% (Table 3). Regression analysis showed that those who were personally diagnosed or had a family member diagnosed with COVID-19 (vs. those who were not diagnosed OR = 2.65, 95% CI 1.17–6.00, $p<0.05$) were more likely to have accepted the COVID-19 vaccine (Table 5).

4. Discussion

Intermediate knowledge levels, fair attitudes, and poor practice of COVID-19 prevention methods by the public were identified in our study which may hinder proper Infection and Prevention Control (IPC) measures to mitigate COVID-19 at all levels. This is the first epidemiological study in Aden, Yemen aiming to assess the knowledge, attitudes, and practices of community members toward coronavirus and its prevention. Generating such information is crucial for promoting correct preventative actions and behaviors as a strategy to manage its spread. The findings from our study demonstrated that the study participants achieved a mean score of 53% on the correct knowledge items thereby showing intermediate levels of knowledge. The findings of this study are similar to previous studies conducted in Liberia (51.0%) (24) and Syria (60%) (25), however it is higher than that in Cameroon (21.9%) (26) but lower than that reported in some other countries such as Bangladesh (85.0%) (27) Saudi Arabia (89.9%) (28) and China (91.2%) (29). One interesting finding in our study was that the majority of participants (86%) reported that they knew about modes of COVID-19 transmission, however, only the minority gave correct answers such as contact with others (20%), touching surfaces (23%) or coughing (19%). In our sample, most participants said that they received information mainly from the media and news (67%) as opposed to social media as was found in studies conducted in Oman, Saudi Arabia, and Egypt (72, 85.8, and 80.8%). The gap in knowledge in our findings might be attributable to the fact that the population may have gained false or insufficient information about the disease and its transmission from the news, social media, and TV as daily information on COVID-19 was published during the pandemic and this study was conducted in the middle of the outbreak (14, 30, 31). Another factor that might have contributed to this difference in knowledge may be due to the difference in age distribution, as compared to other studies. For example, in Saudi Arabia more than 92% of the sample of study were under the age of 49 whereas in our study 67% of the participants were above the age of 49 (32). Our findings showed that level of knowledge on different COVID-19 topics were varied. The majority of participants in this study had information about the cause of infection, onset, and prognosis. However, the information about COVID-19 transmission and prevention was limited. Similarly, in a recent study conducted in Saudi Arabia, they found that almost half the participants did not know when and who should wear a mask to prevent infection and were unaware that COVID-19 can spread from one person to another in close proximity (30). This highlights the need for increased awareness on modes of COVID-19 transmission for preventing the spread of the virus. Surprisingly, our findings showed that those with lower education levels were more likely to know about COVID-19 preventative measures compared to those with higher education levels. This could be attributable to the fact that those with higher education levels may have critically questioned information they received from social media and other news sources (33).

Furthermore, this study highlights that less than half participants wanted to have additional COVID-19 information. Yet, the majority were willing to comply with preventive measures if established and enforced by the government such as mandatory mask wearing, social distancing, and mandatory lockdowns. Our findings are in line with attitudes from Saudi Arabia which showed that most of the community was willing to stay at home (i.e., lock down) or avoid shaking hands to mitigate the spread of the virus, if enforced by the government (32). Perhaps most importantly from our findings is that, very few respondents mentioned vaccination as a preventative and disease control measure and few participants found the vaccine effective and protective, 12.5 and 32%, respectively. Conversely, in a recent study in Oman, 52% of the participants believed that vaccines could protect them from contracting the virus and 42% believed that patients will not contract COVID-19 after vaccination (34). These differing findings may reflect the contextual factors, Oman is politically stable and is globally acknowledged for its well-structured and high coverage immunization programs while Yemen is challenged by a fragile political and healthcare system and thereby has a weak vaccination strategy (15).

In regard to vaccination, most participants (61%) in this study were not willing to accept the vaccine (61%) at the time of this study for varying reasons. The main reasons for hesitancy toward the vaccine were the presence of rumors round it (14%) and that it is a conspiracy (8%). Some participants (12%) claimed that they were not willing to try a vaccine that was newly developed and need to know its long-term effects. A systematic review conducted by Wang et al. (2021), showed that the overall acceptance rate of the COVID-19 vaccination rate was 64.1% globally ranging between 19.9 and 92.1% across countries (35, 36). Factors such as trust in the health care system and government, personal history of vaccination, concerns surrounding vaccine safety and effectiveness, concerns over rapid vaccine development, and knowledge of COVID-19 were associated with COVID-19 vaccine hesitancy (35). Our findings are in line with those from another study conducted by Nguyen et al. (2021) highlighting that *"understanding of how the vaccine works will reduce the pandemic's consequences, especially if that understanding is shared between people."* Confidence toward vaccine uptake may be attained when rumors are neutralized and only official sources share correct and evidence-based information (34, 37). Further, a study conducted on acceptability of COVID-19 vaccines in the Arab world, mirrored our findings in which they also found a high rate of COVID-19 vaccine hesitancy in Yemen (54%) (38). In contrast, Oman showed good level of COVID-19 vaccine acceptance in which around 59% of participants would advise others to accept it, 56.8% would take it themselves, and 47.5% were willing take a second dose (34).

When sharing their practices, only a minority of participants followed any preventative measures since the beginning of the pandemic. Only a quarter, applied social distancing, covered their mouth and nose, followed hand washing etiquette, sterilization, and/or avoided contact with others. Our findings may also reflect contextual factors in which vaccine rollout in Yemen was limited only to southern governments, and only around 2% of the Yemenis received both COVID-19 vaccine doses and around 3% received at least one dose (39, 40). Those who were personally diagnosed or had a family member diagnosed with COVID-19 (vs. those who were not diagnosed OR = 2.65, 95% CI 1.17–6.00, $p < 0.05$) were more likely to have accepted the vaccine. Yemenis' perceived risk of accepting the vaccine largely outweighed its perceived benefits and led to limited uptake. This was due not only to personal perceptions (as found in this study) but also to external factors such limited vaccine availability and access.

This study had some limitations. The study is a cross sectional study which is a single time point study and therefore only measured KAP regarding COVID-19 at a singular time point and did not measure changing KAP over the course of the pandemic. Another limitation to this study might be through the use of dichotomous Yes/No questions which may be influenced by social desirability bias in which the participants might have provided a socially desired answer regarding their own COVID-19 prevention behaviors. While there were limitations in this study, strengths included that this is the first study conducted in Aden on COVID-19 KAP and it adds to the body of evidence on practices of COVID-19 prevention. Specifically, while current practices in Aden are poor, the community is willing to enhance and commit to prevention methods. Results of this study have significant implications for infectious disease health promotion including COVID-19 and other diseases that may spread in the future. For this reason, it is important that public health authorities set up strategies to indicate vulnerable subpopulations and prioritize policies as well as communication efforts (in collaboration with the media) to enhance public knowledge about infectious diseases. For example, the Ministry of Health can implement regular community workshops to enhance information on different emerging diseases and methods of prevention. While this is the first study to be conducted on the community in Aden regarding knowledge, attitudes, and practices surrounding COVID-19 prevention; it is important to recognize that Aden is an urban area with likely higher access to education, and thus, may not be generalizable to more rural Yemeni areas. As a result, conducting follow-up studies is recommended to understand a broader KAP toward COVID-19 in Yemen.

5. Conclusion

With intermediate knowledge and fair attitudes toward COVID-19 prevention, Yemen's community has a promising chance to expand their practices on the topic of COVID-19 prevention methods. However, practice (or adoption of) these prevention methods, including vaccination rates, remains poor. This study highlights the need for increased awareness of COVID-19 modes of transmission for preventing the spread of the virus as well as the need for comprehensive and accessible vaccination campaigns. Yemen's government and health system must establish awareness campaigns and adopt innovative communication methods to enhance the community's knowledge of and resulting practices toward COVID-19 prevention.

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 Ethical Review Committee of Heidelberg University (protocol code: S-887/2020 and date of approval: 15.02.2021) and from the Ethical Review Committee of University of Aden (protocol code: REC-96-2021 and date of approval: 23.03.2021). The patients/participants provided their written informed consent to participate in this study.

Author contributions

OH, FD, and AJ worked on study conceptualization and methodology. FD and AA-A analyzed and interpreted data. HB, KA-S, and DH contributed to the interpretation of results and reviewed the manuscript. OH wrote the original draft. AJ supervised and contributed to writing the manuscript. AA-A, MM, and MDI reviewed and edited the manuscript. FD supervised and gave the final approval. All authors read and approved of the final manuscript.

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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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Understanding the challenges and gaps in community engagement interventions for COVID-19 prevention strategies in Rohingya refugees: a qualitative study with frontline workers and community representatives

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Background: Rohingya refugees in Bangladesh are vulnerable to infectious diseases such as COVID-19 due to the crowded living conditions with fragile shelters, and limited water, sanitation and hygiene facilities and practices. While risk communication and community engagement (RCCE) is the cornerstone of outbreak control, there is limited evidence available on the effectiveness of the RCCE strategies in this setting.

Objectives: The goal of this study is to evaluate the effectiveness of RCCE strategies and to explore the challenges and community recommendations in relation to COVID-19 preventive measures in the context of Rohingya refugee camps in Bangladesh.

Materials and methods: It was a qualitative study. Methods used were (a) observation of RCCE intervention by 3 clinical supervisors accompanying 25 Community Health Workers (CHWs) and (b) 5 focus group discussions engaging 60 community representatives. Data were analyzed using a thematic analysis approach, separately for observation and focus group discussions.

Results: The study identified a number of good practices of RCCE, including selecting CHWs from the local community, engaging female CHWs, using local dialect, and collaborating with community/religious leaders. Certain good practices need scaling up, such as utilization of multiple communication methods and interpersonal communication skills. Some areas need improvement, such as CHWs being overburdened with multiple tasks, less effort to active listening, repeated delivery of same messages, inadequate linkage to culture, context, and resources, and less effort to empower the community. Engaging the community, five critical themes were identified in relation to poor COVID-19 preventive practices: culture, religion, and language; local context and resources; community trust and interaction with aid workers; communication methods; and gender and social inclusion. Religious misinterpretation, cultural barriers, physical barriers, lack of resources, breach of trust between the community and aid workers, inconsistent/complex messages, lack of gender and social inclusion, and stigmatization are among some key factors. Some key actions were recommended to improve COVID-19 RCCE strategy.

Conclusion: We urge the RCCE partners to make use of the findings and recommendations to develop a robust RCCE strategy relevant to local culture and context, responsive to people's concerns and needs, and inclusive of gender, age and social vulnerabilities.

KEYWORDS

COVID-19, Rohingya, knowledge, attitude and practice, community health worker, Cox's Bazar, risk communication, community engagement

Introduction

Following the massive displacement from Myanmar in 2017, about 883,600 Rohingya refugees are currently living in 34 overcrowded camps in Ukhiya and Teknaf Upazilas of Cox's Bazar (1). This was preceded by decades of influxes driven by systematic discrimination and deliberate violence against the Rohingya community (2). The refugees are especially vulnerable to natural and man-made disasters, including outbreaks of infectious diseases since they live in crowded bamboo-made settlements on hilly slopes and basins with limited access to essential livelihood and entitlements (3). The infectious disease epidemics are predisposed by the crowded living conditions with fragile shelters, a lack of adequate water, sanitation, and hygiene (WASH) facilities and practices, and intense monsoons in the refugee camps and surrounding host community (4). Since 2017, the Rohingya camps have experienced epidemic or upsurge of a number of infectious diseases, including diphtheria, measles, acute watery diarrhea, and dengue (4). COVID-19 was a new threat to this community that appeared to be superimposed on the existing susceptibility of the community to different disease outbreaks. As a cluster of viral pneumonia, the disease was first reported in Wuhan in December 2019 and since has spread widely over the world, with more than 500 million confirmed cases and 6 million fatalities reported in 200 countries (5, 6). World Health Organization (WHO) declared COVID-19 outbreak as a global pandemic on March 11, 2020 (7). The first confirmed case of COVID-19 was detected in Bangladesh on March 8, 2020, and the first confirmed case from Rohingya refugee camps was reported in the month of May of the same year (8, 9). As of the June 30, 2022, there were a total of 103,352 tests conducted in the refugee camps, which resulted in the confirmation of 14,731 instances of COVID-19, along with 42 fatalities (10).

There has been a number of public health recommendations issued for the prevention and control of COVID-19, which includes social distancing, use of mask, hand washing, cough etiquette getting vaccinated, staying home while unwell, and seeking medical attention when necessary (11, 12). Public health and social measures remained the most essential instrument for preventing the spread of disease before vaccines became widely available to the general population (13). Therefore, risk communication and community engagement (RCCE) was considered as one of the primary pillars of the COVID-19 response strategy (13).

The International Organization for Migration (IOM) carried out a wide range of RCCE interventions in Cox's Bazar aiming to reduce the COVID-19 disease transmission through strengthening the capacity of the community to practice public health measures. This included household visits and community meetings facilitated by the community health workers (CHWs); the production and dissemination of information, education and communication materials (e.g., audio-visual clips and printed materials); social advocacy conducted by social leaders and community groups; and "go and see visits" to the service sites. The majority of the communication, both its messages and its contents, was based on materials generated by the Communication with Community (CWC) working group.

The successful implementation of public health measures is largely dependent on what people know about those measures (knowledge), how they think or believe in those measures (attitude), and how they do or experience those measures (practice) (14). Prior to this qualitative study, a quantitative study was carried out by the same study team to assess the level of knowledge, attitude and practice of COVID-19 preventive measures among the community following the RCCE interventions carried out (15). It was cross-sectional study, 500 Rohingya individuals were surveyed using a structured questionnaire. The study found that the mean scores for knowledge, attitude and practice were, respectively, 9.93 (out of 14), 7.55, respectively, (out of 11) and 2.71 (out of 7) indicating that the Rohingya refugee community in Cox's Bazar had improved knowledge and attitude toward COVID-19 preventive measures, however, the practice level of these measures remained low compared to the knowledge and positive attitude (15). Also, different forums and reports have highlighted the issue of noncompliance among the population with COVID-19 measures; however, there is no evidence as to why the public health measures are not accepted or practiced by the community and/or how the issue can be addressed using local knowledge and resources. Moreover, there is no evidence available on how effective the current communication and community engagement approaches are in terms of inter-activeness, acceptability and comprehensibility. This assessment was carried out through the active involvement of the community and frontline volunteers from November 2021 to January 2022 in order to address these gaps in information and evidence on the COVID-19 practice and RCCE strategy. The findings of the study could

Abbreviations: AWD, Acute watery diarrhea; CHW, Community health worker; CI, Confidence interval; CWC, Communication with community; ERC, Ethical review committee; FDMN, Forcefully displaced Myanmar nationals; FGD, Focus group discussion; IEC, Information education and communication; IOM, International organization for migration; IRB, Institutional review board; ISCG, Inter sector coordination group (ISCG); ITC, Isolation treatment center; KAP, Knowledge, attitude, and practice; MERS-CoV, Middle east respiratory syndrome coronavirus; NSU, North South University; RCCE, Risk communication and community engagement; SARI, Severe acute respiratory infection (SARI); SARS-CoV-2, Severe acute respiratory syndrome coronavirus 2; WASH, Water, sanitation, and hygiene; WG, Working group; WHO, World Health Organization.

support the development of a robust strategy on RCCE for the ongoing pandemic as well as future outbreaks of infectious diseases.

Materials and methods

Study area

The research was conducted in Rohingya refugee settlements in Cox's Bazar, Bangladesh, where IOM Health implemented community health interventions. In Cox's Bazar refugee camps, there are around 883,600 Rohingya people.

Study design and participant selection

It was a qualitative study, which used phenomenological approaches. The study was conducted followed by a quantitative study conducted earlier which explored the level of knowledge, attitude and practice (KAP) among the Rohingya refugees in Cox's Bazar. This qualitative study was explanatory in design to understand the status of RCCE interventions and perception of the community regarding the current status of COVID-19 knowledge, attitude and practice and how these can be further improved.

Methods used in the study were (a) observation of RCCE intervention and (b) focus group discussions (FGD) with community representatives. Three clinical supervisors were selected and trained for both observation and moderation of the FGD. Five Rohingya refugee camps, selected randomly in the earlier KAP survey were selected both for the observation and FGD. CHWs are the frontline workers in the Rohingya refugee camps responsible for the RCCE activities. For observation, five CHWs were randomly selected from each of the selected five camps from the camp-wise CHW list.

The study included five Focus Group Discussions (FGD) facilitated by the Principal Investigator and Clinical supervisors, one for each camp with the view to document recommendations from the community representatives to improve compliance and practice on COVID-19 preventive measures. The participants in the FGDs were recruited from four community groups residing in five different camp locations, using a network of CHWs. Each FGD consisted of 12 representatives from the community. The selection process for study participants was purposive, at each FGD representation of a cross-section of community perspectives was ensured based on socio-demographic characteristics, economic condition, and level of vulnerabilities, including women and men, adolescents and youths, older persons, persons with disability, community leaders (*Majhi*), and religious representatives. This process ensured that the study included a diverse and representative sample of participants relevant to the research question being studied. Exclusion criteria used for the study were those having cognitive impairment or those who faced severe challenges to group participation and discussion. Table 1 showed the breakdown of different representative groups in the FGD process.

TABLE 1 Different representative groups in the FGD process.

Representative type	Men	Women
Adolescent	5	5
Youth	5	5
Older person	4	6
Person with disability	5	3
Other general community member	5	5
Community leaders	5	2
Religious representatives	5	0
Total	34	26

Sample size

Although the sample size for qualitative research is determined by the theoretical saturation, empirical studies suggest that the recommended number of observations be between 5 and 15 and the recommended number of focus group discussions (FGDs) be between 3 and 6 with between 8 and 12 participants in each FGD (16–19). In order to conduct this study, we observed 25 CHWs and held 5 FGDs with a total of 12 participants.

Data collection

For observation, at each of the five camps, a Clinical Supervisor was accompanied by the selected five CHWs and observed the approach of their communication, interaction between CHWs and beneficiaries, and acceptability/comprehensibility of the messages among the beneficiaries during COVID-19 risk communication. They also observed the availability and use of existing RCCE tools and materials. An open-ended observation template with a range of indicators was used for the observation, which was developed based on the recommendations from different RCCE guidelines developed by WHO and partners (20, 21).

A focus group discussion was held at each of the five camps, moderated by a Clinical Supervisor. Though the clinical supervisors were fluent in local Rohingya/Chitagongian language, a translator from local Rohingya community was engaged at each FGD to eliminate any sort of linguistic barrier between the community representatives and the moderators. Participants were invited to participate in an FGD session in a camp location familiar to them, seated in a circle or semicircle adjacent to the FGD facilitator and translator to encourage interaction and engagement. After introductions, the camp-based findings of the KAP survey conducted earlier were presented in the discussion so that the group could better understand the level of knowledge, attitude and practice and could recommend the improvement measures accordingly. Further, open-ended questions were asked using a focus group discussion guidance note (see Supplementary Annex S2) to generate discussion and comment. FGD participants were informed that there were no right or wrong answers and were encouraged to ask questions if they did not understand the session content. Average duration of an FGD was around 90 min.

The clinical supervisors were responsible for data collection both for observation and FGDs. A note taker assisted in writing up the notes as well as ensuring proper audio-recording of the discussion.

The clinical supervisors were trained on the overview of the study, the data collection instrument, and data collection procedures. They were also trained on how to obtain informed consent, how to ensure the confidentiality and privacy of the participants, and how to handle sensitive information. Both the observation template and the FGD instrument was pre-tested, respectively, among a small sample of 5 CHWs and 5 community representatives to ensure its validity and reliability. All data were stored and regularly backed up in a secure location to ensure its confidentiality and privacy. Written informed consent was obtained from all participants before the survey was administered. Participants were informed of the purpose of the study, the procedures involved, and the potential risks and benefits. They were also informed that their participation was voluntary and that they could withdraw at any time without any consequences. Overall, strict measures were taken to ensure the protection of participants' rights and the confidentiality and privacy of their data throughout the study.

Data analysis

The findings of the observation by the researchers were immediately noted by the clinical supervisors during the time of observation and transcribed and translated into English at the same day of the observation. The focus group discussions were recorded using audio devices and the audio recordings were transcribed verbatim (word-to-word) in English. The quality of translation was ensured by the principal investigator by comparing the translated transcript with original recording in Rohingya. Data were analyzed using a thematic analysis approach, separately for observation and focus group discussions. Software NVivo v12.0 was used to organize the data into themes. The steps of the thematic analysis included familiarizing with all the data, coding key features, grouping codes into potential themes, reviewing themes against the codes and dataset, defining and naming the themes, and writing a narrative of the analysis. To ensure the validity of the data, low relevance items (i.e., statements irrelevant to the research objectives) were eliminated, and similar concepts were merged during the analysis process.

Ethical consideration

All respondents choose to participate voluntarily. Written informed consent was obtained from the participants for the publication of any potentially identifiable images or data included in this article. The data protection policy of the IOM is rigorously followed at every stage of the study. All recordings were temporarily stored in IOM devices and deleted after transcription. Ethical issues in the study were reviewed and approved by the Institutional Review Board of North South University (2021/OR-NSU/IRB/0401). The study adhered to the "no-harm" principle, and no intervention in the project caused significant harm to the subject population or endangered their health or lives. There was no legal risk associated with the participation of the beneficiaries in this study. Local rules/regulations were respected during interaction with the beneficiaries.

TABLE 2 Practices and gaps in RCCE approaches.

Good practices
Selection of CHWs from the local community
Established longstanding relationship between CHW and community
Deployment of female CHWs having better accessibility
Speaking in the local dialect
Linkage with community and religious leaders
Established trust, reliability and respect
Multiple methods of communication, posters, billboards, audio-video clips
Visualization of local customs in the promotional materials
Partially implemented good practices that need further scale-up
Use of interpersonal communication skills
Engagement with community stakeholders, e.g., community groups, Majhis, imams, TBAs
Areas need improvement
Over workload for CHWs, burdened with too many households to visit per week
Less time and effort for active listening and engagement
No guidance for CHWs on the delivery of key messages
Frequent delivery of the same messages without appropriate linkage with day-to-day practice and challenges
Messages were not adequately linked with culture, context, and resources
Less effort to link the community with appropriate resources
Cultural modes of communication (e.g., folk theaters, songs) are less explored
Less effort to empower the community/families for decision making

Results

Status and effectiveness of risk communication and community engagement approaches

The findings from the observation of community health activities have been presented in this section. Table 2 summarizes the key findings into good practices and areas needs improvement.

A CHW was assigned to cover around 150–200 households, which made them visit 20–30 households a day. They also needed to facilitate frequent community awareness-raising events, such as courtyard sessions. Most CHWs were working in the refugee camps for some years and had good familiarity with the community. Since a majority of the Rohingya men go to work in the early morning, CHWs mainly meet the female family members during their household visits. Therefore, it is more convenient for female CHWs to communicate and engage with the family members.

CHWs were either selected from the local refugee communities or from the nearby host community. Whatever their nationality, they spoke in the local dialect creating an atmosphere comfortable for the family members to communicate. Due to the long-term relationship between the CHW and the community, the family members mostly respected them and regarded them as a trusted messenger to the community. They acted as the bridge to connect the community to healthcare providers. Most often, they were well accepted and admired within the community. It was found that CHWs who worked in their catchment areas for a long duration were more engaging than those who were newly recruited. Most CHWs greeted the family members

during their household visits politely. However, for some, it was found that they treated it more like routine work instead of having the greetings based on cordiality.

Since they were under pressure to visit each family once a week, the CHWs could barely spend enough time with each family to engage in effective interpersonal communication. Many of the CHWs were found to spend less sufficient time listening to or addressing the concerns of beneficiaries. Some CHWs began their visit to households with a smile on their face, warm regards and engagement, however, after visiting a few households, due to lack of availability of time and exhaustion, they sometimes lost their positive body language, quickened the conversations with the family members and tried to get rid of the questions without adequate explanation.

CHWs were equipped with key messages that they need to deliver in line with the global recommendations and endorsed by local health authorities. However, the messages were not always accompanied with a guidance note on how these should be communicated with the community. In many cases, rather than providing enough context and background, many CHWs often ask some routine questions to collect information and provide routine key messages on COVID-19. CHWs were barely found providing practical examples of risk communication messages they are providing. For example, it was found that a CHW requested the household members to wash their hands and maintain respiratory hygiene and physical distance, but sometimes they did not provide any context and reason behind this measure. Also, due to the repeated delivery of the same message on every visit without any innovative method/mode, it was found many family members were hesitant to heed. There were also good examples, few CHWs were found to be able to connect with the community they were serving because they shared life experiences, listened to the community with empathy and demonstrated a deep desire to help them.

Key messages were mostly adapted from the public health preventive measures recommended globally. In some cases, messages were not enough to address the local culture, context and/or resources; and do not adequately explain how these can be achieved recognizing the limited availability of resources. For example, women were used to wearing the *Burkah* and so did not want to wear a mask underneath it; people were mostly living in very crowded location, where making physical distance maintenance difficult. The RCCE strategy and messages did not adequately address this challenge. Some CHWs were not well-oriented with the updated messages.

Most CHWs were oriented on interpersonal communication skills and tried to apply that. The weekly courtyard sessions provided a good platform for everyone to express their thoughts, fears, and concerns. The attitude of active listening varies from CHW to CHW—while some fully utilized the skill, some did not engage themselves much in active listening. Many of them used printed documents or pre-developed forms to share messages and information instead of generating interactive discussions.

Sometimes, CHWs were not provided with the information to respond to specific challenges from the community. For example, it was found challenging for CHWs to respond to how a beneficiary should access healthcare if she had three or more children and her husband was not at home, because it is difficult for her to care for three children in a health facility. Sometimes, CHWs listened to the impactful persons in the family (e.g., family head) but not to the weak or vulnerable individuals (e.g., older adult, children).

CHWs regularly received training from their agencies as well as the community health working group (CHWG). If there were any questions from the family members, CHWs tried their best to answer from their existing knowledge. However, they struggled to answer some due to lack of updated information in some cases. Some took notes of the questions so that they could communicate with their supervisor and provide the explanation on the next visit. Some CHWs were found providing information but not seeking any feedback from the community if they had any concerns or anything that needed clarification.

Some CHWs tried to explain a list of pre-identified rumor to the community, however, limited initiatives were taken by the CHWs to discover and document other rumors in the catchment area, although there was a rumor tracking system in place. The message CHW delivered focused mostly on the prevention measures, but limited attempts are taken to dispel the stigma associated with COVID-19.

For COVID-19 multiple types of communication tools were available. Flipcharts and posters were utilized by CHWs as a means of communicating risks. Sometimes, they used other forms of communication, such as radios, flyers, and audio-visual materials… It was seen that the flipcharts, posters and audio-video clips used local languages and portrayed local customs. The flip charts and posters visualized local dresses, settlements and traditions. In a few camps, especially in front of SARI ITCs, big billboards were placed by IOM with pictorials along with key messages. Through a favorite means of communication, it was found that many of the families did not have access to radios. Audio and video clips produced on COVID-19 RCCE were extensively spread among the community. However, we found that other culture-friendly media, such as folk songs and traditional theater, remained untapped for risk communication. Majhi and Imam wield considerable influence over the community, but they are typically preoccupied with other responsibilities. However, CHWs are found to involve the Majhis, imams, and traditional birth attendants, who attempted to coach community members on how to improve their health-seeking behavior and persuade them to adhere to strict preventive measures. Yet, there is scope to further enhance this collaboration between CHWs and opinion leaders to empower the community for adherence to COVID-19 preventive measures.

It was observed that most CHWs did not provide any decision-making options to the community members. They were found taking less effort to influence the family heads for taking community or family level action plans to implement the COVID-19 preventive measures. Family heads were not engaged or empowered to utilize or strengthen their leadership role in the family for monitoring and implementing the preventive measures at family level. Even if the CHWs listened to the problems of beneficiaries in terms of the inability to implement any preventive measure, no initiative was taken to link the beneficiaries to appropriate resources or stakeholders for solving the issue.

Reasons of poor compliance to COVID-19 preventive measures and recommendations from the community

Focus group discussions were carried out with community representatives to identify the reasons behind the poor practice of COVID-19 preventive measures and generate recommendations by

TABLE 3 Causes of poor compliance to COVID-19 preventive measures.

Culture, religion, and language
1. Religious misinterpretation and tendency to improperly replace preventive measures with religious practices
2. Cultural norms and perceptions hinder the practice of preventive measures
3. Diversity of language creates difficulty in communication and understanding
4. Rumor and misinterpretation of preventive messages
Local context and resources
1. Poverty and lack of resources affect the prioritization of COVID-19 measures, e.g., isolation, quarantine etc.
2. Lack of supply of preventive resources, e.g., masks, closed bins, handwashing points
3. Concern of security of property in case of facility-based quarantine or isolation
4. Physical challenges in handling with mask, e.g., interruption of communication
5. Congested living arrangement at the shelters
6. Crowding to access common water points and toilets
Trust and interaction
1. Breach of trust on healthcare workers due COVID-19 preventive distancing and isolation measures
2. Fear/rumor of isolation in case of positive COVID-19 symptoms
3. Inadequate compliance with preventive measures by aid workers
Communication
1. Limited access to reliable information; lack of community engagement events during lockdown
2. Inconsistency and unclarity messages; messages are not presented in a form that is understandable to the community
3. Improper use of channel—challenge of distancing, interruption and unclarity
Gender and social inclusion
1. Gender-based violence associated with isolation and treatment
2. Inequality of men and women in terms of use of masks and accessing services
3. Communication materials and messages are not responsive to the special needs of older persons and persons with disability
4. Stigma toward older persons, persons with disabilities, women and COVID-19 positive patients

them to address the reasons. After analysis of the findings five key domains were identified—(a) culture, religion, and langue; (b) local context and resources; (c) community trust and interaction with aid workers; (d) method of communication; and (e) gender and social inclusion (Table 3).

Culture, religion, and language

Religious misinterpretation

The community has a strong perception that everything happens according to the will of God and what will happen cannot be altered by human. People consider COVID-19 as a disease of rich people. There is a belief that because of their religion, or because they routinely visit the mosque or pray, they are immune or protected from the illness. As opined by an Imam "*All things happen according to Allah's will. There is nothing we can do. We will all die in this location if Allah wills it, or we will live for another year if Allah wills it.*"

People also try to correlate hygienic practices recommended for COVID-19 prevention to religious practices. For instance, there are beliefs that Hijabs can replace the use of masks for women; hand washing before prayer time (five times a day) is sufficient as hand hygiene practice. *"We do 'Salah' five times a day, so we do 'wudu' five times a day, and this is sufficient,"* explained by an Imam.

Health messages are not reflective of people's tradition and religious beliefs. As an Imam expressed—*"NGOs have instructed us to avoid namaj in masjid, but we did not follow them. We pray in masjid regularly. Other than going to masjid Allah will never forgive us from this 'beram' (disease)."*

Cultural barriers

There is a perception in the community is that type of mask is related with different social economic status. It is expressed that *"elite people like Majhi use surgical masks and as a result those who cannot afford surgical masks are refraining from wearing any other masks for fear of social stigma."* Sometimes, the use of masks is considered by many unsuitable in their culture. Many people think "*it is rude to talk to elders while wearing a mask*." Some people are comfortable wearing a mask when he is alone, when they face a mentor or a senior, they consider taking off the mask as a courtesy. Many have said that maintaining physical distance with acquaintances is routinely disrespectful.

Language diversity

Language difference often creates difficulty in communication and understanding. Sometimes, healthcare workers are not familiar with the words of the patients, and they are unable to provide appropriate messages about the diseases to them. As expressed by one of the participants, *"doctors and nurses do not understand our language, we do not understand theirs, how will they feel our pain our problems."* There are some audios and videos produced in Rohingya language that became popular among the community. Many IEC materials are translated into Burmese, however, Burmese is not always understandable to the community, since it's not their mother tongue.

Misinterpretation, inadequate understanding

It was found among the community that there is lack of understanding of the disease, its transmission, severity and preventive measures. People often disregard the severity of the disease and try to relate it with the common cold, as expressed by one of the participants, "*We dealt with this condition frequently in our country. We were able to overcome it by using herbal medication, ginger, and warm water*." Many people think that even if they do not wear a mask, nothing will happen to them. There is also a perception that COVID-19 is a "*disease of the rich, the poor will have nothing to do with it*." There is belief that "*there is no corona here (in the camp), none have died*." Some do not understand the purpose of some preventive practices, as stated by one participant, "*we do not deal with dirt, there is no reason to wash hands*!" People have negative perceptions on the SARI isolation and treatment centers, as expressed, *"The isolation centre is a "Jail" being sent to die alone, they do not give tasty food and do not allow phone calls. They confine you for days."*

Recommendations from the participants

In order to improve compliance of the community to COVID-19 preventive measures, the participants expressed to address the concern of their religious belief, traditions and culture. They also urged to have health services and communication inclusive of Rohingya (in some cases Burmese) language for better understanding.

Local context and resources

Lack of resources

One of the reasons behind poor compliance is the lack of resources. Poverty causes the community to set their priority differently than COVID-19 preventive measures. As opined, "*We are poor people, many of us lost their day work in this pandemic and due to the imposed restrictions on movement, we are sometimes unable to gain access to the food distribution place. How long will we keep waiting in our house!*" An older adult participant broke out in agitation. "*We still have bloodshed still in our mind, the struggle of daily life is hard to pass by, what COVID will add on?*." Some did not comply to facility-based isolation and quarantine measures due to lack of support to the family during their isolation/quarantine period. "*If our rations and food are not provided regularly on our house, and family is bit insecure back at home, I would not ponder overstaying there at the hospitals, I cannot sit idle knowing our house is not safe back in the downhill*" a healed COVID fighter participant stated strongly.

Participants reported that there is lack of facilities for handwashing in the camp. The supply of masks from the humanitarian agencies is also reported inadequate and infrequent. As expressed, *"eventually, we are not habituated, and you do not provide basic supplies on a regular basis. We received the cloth mask long days back and after that, there is no follow-up. No health workers coming towards our home with masks, so how they are expecting that we are going to maintain their instruction?."* As per the opinion of community leaders, NGO services became bad during the pandemic because all washing and drain-cleaning services were discontinued. *"There is not enough bin, NGO worker told us to put cough in closed bin, not here and there but where are those? We will go to the hospital to throw it, is it?"* a female participant pointed out toward shortage of closed bin and improper waste management.

Security of shelter and resources was identified as one of the causes behind reluctance for getting isolation and treatment service. As expressed by a woman, "*when we go to your isolation centre our house, our chicken aren't protected, we miss out our cylinder, our ration is not delivered at right time, our neighbors house was attacked by thief in the meanwhile*."

Physical barriers

There are some physical factors that are limiting the practice of COVID-19 preventive measures. Some people do not comfortable wearing a mask, some find it hard to breathe when it is humid outside. Some do not practice using a mask because it interrupts communication, as expressed by one shopkeeper, "*every minute I have to talk to buyers. They do not understand me if I'm wearing a mask. And it's hot, that's why I do not wear a mask*." People wearing glasses also find difficulty in wearing masks because their glass becomes blurred. For these physical factors, even if some people wear masks, they keep it under the chin. The congested living arrangement, 6–8 people in a small shelter, and extreme temperature during summer make it difficult for people to stay at their home. As raised by one representative, "*we cannot maintain social distancing in our shelter and outside as well. Our room becomes very hot during this hot summer due to the roofing materials, insufficient solar fans, and inadequate ventilation. Therefore, we cannot stay at room for long, and cannot stay outside also because of government restrictions, what you guys want us to do?"*

Some of the standard measures are not appropriate to the camp context due to the lack of physical facilities. People are living in a crowded setting and need to use common water points and toilets, which makes the practice of social distancing impossible. As stated by one of the participants, "*where they live in a house of eight to ten people, eight to ten families together use same place to use water and use a same toilet, how 'social distance' cannot be maintained, and what would bes the benefit of using a mask?*"

Recommendations from the participants

The participants urged to improve social support to the families of those who are isolated or quarantined. It should be ensured that the families are delivered with regular foods and supplies and their children are getting enough care instead of absence of one or more family members due to their stay at isolation or quarantine facility. Financial support to compensate for lost income can be considered for those staying in hospital or quarantine facility. Interventions should be explored to improve family togetherness or communication during facility-based quarantine or hospital stay.

It is expressed that regular supplies necessary for maintaining COVID-19 preventive measures should be ensured in line with the preventive messages. This includes masks, soaps, handwashing devices and a proper waste management system. There was also a request to explore adequate space at each shelter enabling home isolation/quarantine when necessary.

It was also raised to consider means of entertainment within the home environment to limit outside gathering.

- "*We are told to maintain social distance, to stay at home but how can we, our only joy come out when we got to bazar and gossip, if there was the options of television, radio we would not have the urge to go there predominantly*" one of the young ladies told.

One of the suggestions from the community to consider a basic literacy program for improving the understanding level of the community.

- "*Education will make us understand your difficult words and orders. We will be able to maintain ourselves more easily*" agreed by two of the men out there.

Trust and interaction

Breach of trust between healthcare workers and the community

Due to rumors, lockdown, and additional precautions taken by the health facilities and healthcare workers, there was mistrust between healthcare workers and community. The regular consultation service was dropped. There was fear of being killed or isolated. One of the Majhis expressed, "*We are family persons, what kind of rudeness it is not to allow us to see our family*." Moreover, sometimes, healthcare workers and facilities were considered as the "spreaders" of the virus.

Complained by one woman, *"These days, the healthcare workers behave rudely …. If we are infected (or suspected to be) by COVID-19. The guard man and other staff got violent. If we do not have fever, they even refuse to see us after keeping us waiting for the whole day and give us only paracetamol. We locally call it 'paracetamol center'."*

There remains some fear among the community about the isolation centers, that is precisely due to the disbelief and mistrust. Some people believe that they will be killed, if they tested as positive for symptoms of COVID-19. However, the groups suggested due to

extensive awareness-raising events, such rumors and fears have greatly reduced.

Inadequate compliance from aid workers

One of the factors affecting the community's compliance to the prevention measures for COVID-19 is that humanitarian workers working in the camp are not also complying with the prevention measures, expressed by the community representatives. As expressed by a Majhi "*Why should we wear masks? Most of the law enforcement persons are not wearing, NGO workers keep gossiping closely with each other, they do not do it how can we!"*

Recommendations from the participants

The participants opined that the community volunteers, specially CHWs should be more engaged with the community and provide them with adequate time, attention and respect.

Also, they suggested that to develop trust and address rumors the humanitarian workers must be transparent in all their actions and communicate clearly and pro-actively with Rohingya. They should be compliant with COVID-19 preventive measures so that the community can follow them and have trust over them.

Communication

Limited access to reliable information

According to some participants, the displaced community in the camps have limited access to reliable information which made it difficult to gain knowledge and respond to the crisis. There were many rumors and misinformation, but the community had limited access to trustworthy information. Due to the initial strict lockdown during the pandemic, there was limited engagement with the community by the community volunteers or outreach teams. Household visits and community engagement events were irregular and less frequent than the normal period. There were no good alternative sources that could effectively engage the community with preventive measures.

Inconsistency, unclarity, and complexity of messages

There are some inconsistencies in messages due to some changes of conception on prevention. At the beginning of the COVID-19 outbreak, it was communicated that healthy people were not required to wear masks. However, later it was communicated that everyone needs to wear masks in public places to prevent COVID-19 infection. Such inconsistency of messages without explaining the reason behind the change caused confusion and affected trust.

The unclarity of messages or inadequate presentation of the content in a form that is understandable to the community was another factor of incompliance. As explained by one of the respondents, "*many of us have doubts about the rules of hand washing. Many people have misinterpreted the instructions and duration of hand washing for 20 seconds in various ways. Some have said that the government has asked them to wash their hands for 20 seconds, while others have said that they have been asked to wash their hands 20 times a day.*"

While most of the population is illiterate it is often difficult for them to interpret and understand the contents of the preventive messages. As expressed by one of the participants, *"we are the general people, we did not go to school, we cannot get complex words."* Many of the materials and contents are not comprehendible by the general population in the camps. As expressed, *"Baba, all the things' you people share are through mike or writing, poster with pictures are few, how much you expect us to understand through these?"*

Improper method of message dissemination

Although participants were familiar with different methods of message dissemination, miking through Tomtom (rickshaw-like motor vehicle) was reported as most frequently accessed source of information. However, they mentioned that such messages they cannot understand properly due to distance, interruption and unclarity. Although CHWs make frequent household visits and disseminate messages on COVID-19 prevention and control, participants mentioned that they do not stay in the house day long, so, they are not engaged with the CHWs and their messaging.

Recommendations from the participants

The participants suggested that folk songs or theaters can be used as one of the modes for reaching the community with risk communication messages and addressing the misinterpretation, rumors and local concerns. Facilitation, capacity building and mobilization of self-organized Rohingya groups can be considered for RCCE. They thanked the innovative approach of "go and see" visits by the community representatives to treatment and quarantine sites, which can be replicated more to enhance community trust and eradicate rumors and misconceptions. It was also suggested for more use of Burmese and Rohingya languages in promotional materials for better understanding by the community. Considering the high literacy rate, it was recommended to use meaningful culture appropriate pictures instead of text.

Gender and social inclusion

Gender

Gender inequalities impacted heavily in COVID-19 preventive measures. Women are threatened by their husbands that they will be divorced if they stay in the isolation and treatment center for treatment of themselves or their children. *"I was threatened to be sentenced 'Talak' by my husband if I stay in COVID center, my husband shouted at me and said there are male persons in the center who will rape me, they are bandit, characterless people."* There is a belief that women should continue to use their "Burkah" and they do not need to wear a mask. Women are more vulnerable to stigma and social isolation if they get infected. Women are already overburdened with the pressure of taking care of family members, especially children and older adult and doing household chores. Therefore, superimposition of stigma surrounding COVID-19 causes them to hide their symptoms. One of participants opined, *"Women are not subjected to quarantine because they are responsible for their families. They (women) would be afraid of rejection from their families, particularly from their husbands."*

Materials are not responsive to the special needs of vulnerable groups

The messages and the materials are not designed in a such that can be responsive to the special needs of vulnerable groups, e.g., older adult, persons with disability. One of the older adult persons stated, *"we are old folks, so we do not hear anything correctly. Most of the miking takes place on the road, far away from our house. Yeah, there are*

some posters here and there, but we find it tough because our vision is not great."

Stigma

There persists stigma surrounding COVID-19 in the camps. People infected with COVID-19 are stigmatized and socially isolated. Even, there is a negative perception of people toward those considered to be at higher risk of infection, such as the older adult and people with disabilities. There is a reluctance to aid or engage with those who were potentially infected or those who are considered at higher risk of infection. Also, there is fear among men that they will lose their jobs if they get infected with COVID-19. Due to fear of such stigma, people often hide their symptoms and do not present to the health facilities for isolation and treatment.

Recommendations from the participants

Representatives from different vulnerable groups, e.g., persons with disability and older persons requested to consider their special needs in the communication channels and messages. Some participants, specially, women also urged to address the concerns of inequalities and stigma.

Discussion

By observing the RCCE activities, the study identified a number of effective practices, such as choosing CHWs from the local community, establishing a long-standing relationship between the community and CHWs, involving female CHWs, using the local dialect when communicating, and collaborating with community and religious leaders. We identified some effective strategies that require further expansion, including the use of multiple communication channels and instruments as well as interpersonal communication abilities. We also identified certain gaps that need to be addressed, such as CHWs being overworked with numerous tasks and household visits, less effort being put into active listening, lack of formal guidance on how to deliver key messages, reiterating the same messages without properly tying them to daily struggles, and insufficient tying of messages to culture, context, and resources. Engaging the community, including people from diverse levels and vulnerabilities, our study revealed five critical themes connected to poor COVID-19 preventive practices: (a) culture, religion, and language; (b) local context and resources; (c) community trust and interaction with aid workers; (d) communication methods; and (e) gender and social inclusion. Religious misinterpretation, cultural barriers, language diversity, misinterpretation and poor understanding, physical barriers and lack of resources required to comply with preventive measures, breach of trust between the community and aid workers, inconsistent/complex messages, lack of gender, age and social inclusion, and stigmatization are some key factors. The community recommended some measures to consider to further improve the risk communication and communication strategy. This includes addressing issues with local religious beliefs, customs, and culture, utilizing Rohingya (or, in some cases, Burmese) in communication, enhancing social support for families of isolated or quarantined patients, providing financial aid to make up for lost wages while a patient is in the hospital, fostering better family cohesion or communication during facility-based quarantine or hospital stays, ensuring a regular supply of items for maintaining COVID-19 preventive measures, creating entertainment options for the home environment to reduce outdoor gatherings, enhance the engagement of community volunteers and CHWs in the neighborhood, making an effort to increase community and humanitarian workers' trust, exploring use of folk songs and theater for risk communication, building the capacity of Rohingya community groups, replicating the "go and see" visit strategies for treatment and quarantine sites, using culturally relevant meaningful images instead of text in communication materials, and addressing the special needs of vulnerable groups (e.g., women, persons with disability and older persons) in communication channels and messages.

A quantitative KAP study conducted by the same authors prior to this qualitative study found that the majority of the community had a good level of knowledge or awareness on COVID-19 and an average to good level of attitude, however, a significantly low level of practice toward the preventive measures (15). There was a significant improvement in knowledge and attitude among Rohingya refugees compared to the results of previous research (22), however, the practice was not improved as much as the level of knowledge and attitude (15). This improvement in the level of knowledge and attitude can be potentially linked with the extensive community outreach activities and best practices of community health interventions as identified by our study, which include the selection of CHWs from local community, deployment of female CHWs, longstanding relationship of the CHWs with the community, speaking in the local dialect, engaging with the community and religious leaders, and effort to build community trust, reliability and respect. The crucial role played by CHWs in COVID-19 RCCE were also recognized in several other studies in different settings of the world (23, 24).

The study found that overburdening the CHWs with too many tasks and a high target of coverage negatively affects their time spent per household and active listening and engagement. In Cox's Bazar, CHWs are already assigned with the tasks of health and hygiene promotion, promotion of vector control, routine immunization, SRH awareness-raising, health referrals, defaulter tracing, community-based birth and mortality surveillance, notification of unusual events, maintaining key health and demographic data of each household and recording rumor, community complaints and providing basic first aid in the event of an emergency (25). COVID-19 RCCE and enhanced surveillance is an added responsibility to them. High workload and unrealistic expectations of work from CHWs can interfere quality of social and behavioral change communication (26). Our finding is complementary to the study of Musoke et al. (27), which highlighted that overburdening of CHWs results in stress and anxiety leading to lost working hours. It is recognized in some COVID-19 public health guidance that older people, persons with disabilities and/or chronic illnesses face higher risk of COVID-19 and face inequality and barriers to access information, education and services (28). The earlier KAP study found the association between knowledge and practice level and age group, specifically, the older adult age group (≥61 years) had less level of knowledge (AOR 0.42, $p = 0.05$) (15). This could be explained by the findings of study that the RCCE strategy, messages and the materials were not responsive to the special needs of vulnerable groups, specially, older adult and persons with disability. Our study also found the negative perception of people toward those considered to be at higher risk of infection, such as the older adult and people with disabilities. This finding is similar to Lebrasseur et al. (29) who found that the COVID-19 pandemic had a major impact on

vulnerable populations, notably older people, who often experience loneliness, age discrimination, and anxiety. Therefore, the study recommends that RCCE strategies and contents should address the concerns of vulnerable groups, especially older persons and persons with disability. This is in line with the recommendation of ADCAP, which suggests identifying the barriers of older people and persons with disability, providing them access to information using a range of communication channels and different formats, using simplified languages, improving outreach strategies, and monitoring their access to ensure their effective inclusion (28). The study also discovered that gender inequality contributes to a lack of compliance with preventive measures, particularly when it comes to women who are restricted from accessing isolation and treatment services by their partners and who are more vulnerable to social isolation and stigma. However, it was also found that the RCCE strategy and contents are not adequately addressing the gender needs. Therefore, we recommend incorporating gender inclusive approaches in the RCCE strategy addressing the needs of women, men, girls and boys. This is aligned with the recommendation from a study in Pakistan, which concluded to incorporate gender aspect in designing effective communication and risk reduction strategies (30).

It is mentioned in the RCCE strategy for COVID-19 to ensure that the community engagement is culturally appropriate and empathetic (31). WHO community engagement guideline also emphasized local understanding and engagement consistent with the language, culture and context (11). Our study found that culture and religious beliefs were not adequately taken into consideration in the risk communication contents and strategies. Hence, there were religious misinterpretations and a tendency to improperly replace preventive measures with religious practices. Similarly, several cultural norms and perceptions were documented that hindered practice of preventive measures. Our study also found a lack of efforts at community health interventions in engaging the families and community in active decision making and action planning. There were initiatives to produce IEC materials, e.g., posters, key messages, in Burmese language. Some IEC materials (e.g., videos) are also produced in Rohingya language which achieved popularity (31). Since, Burmese is not the mother tongue of Rohingya and only people with some literacy can understand the language, Rohingya language should be preferred over Burmese in developing and disseminating IEC materials. Although the promotional materials well visualized local customs, the study found that there are many culture-friendly media, e.g., folk songs, traditional theater that have not yet been explored or included into the RCCE strategy. Therefore, we recommend that targeted strategies and contents should be designed to address the cultural and religious beliefs and local practices associated with COVID-19 preventive practices; and the families and community should be enabled for making informed decision and taking action to comply the preventive measure in the frame of local context. This recommendation is similar to the cultural model proposed by Airhihenbuwa et al. (32) who drew lessons from the Ebola response and HIV intervention and concluded that the COVID-19 communication strategy should be reframed to promote positive aspects of lived experience and overcome the negative practices within the context and culture of the communities. Similarly, Allgaier and Svalastog (33) also concluded that local knowledge, beliefs, and communities must be considered for effective control of Ebola outbreak with meaningful participation of local community. In many settings role of traditional and religious leaders has been well recognized including in Bangladesh, Sri Lanka and South Africa (34–36). This study also found the strong role of traditional and religious leaders, e.g., Majhis and Imams, in COVID-19 risk communication. However, this effort should be considered for further scaling up with trainings and engaging the Majhis and Imams in addressing stigma and discrimination, motivating people in testing procedures, isolation and quarantine and building community resilience.

The study also identified that insufficient resources often contribute to poor compliance. Poverty changes the community's priorities from COVID-19 preventive efforts. Some people raised concerns on social security of the family if they remain isolated or quarantined. Security of home and resources was cited as a reason for avoiding facility-based isolation and treatment. Although preventive messages urge the use of masks, disposal into a closed waste container, and frequent handwashing, participants reported insufficiency or unavailability of some preventive tools, e.g., mask, handwashing point and closed bins. The study also identified some physical factors that limit COVID-19 preventive practices, e.g., mask causes interruption of communication and spectacle fogging. Congested living arrangements at the shelters and crowding to access common toilets and water points are also some factors that limit maintaining social distance. Our findings are contributory to Patel et al. (37), who demonstrated how people with low socio-economic status get more exposed to COVID-19 due to their poverty and several socio-economic factors, including overcrowding and unstable employment.

The study identified a breach of trust between healthcare workers and the community due to rumors, additional precautions taken by the healthcare workers, and fear regarding the isolation treatment center. Some participants also questioned the protective behavior of the healthcare workers which affected their access to the health facilities. Inadequate compliance with preventive measures by aid workers was also found as a discouraging factor for the beneficiaries to comply with the COVID-19 preventive measures. Therefore, the study recommended to take actions to strengthen trust among the community, health workers and humanitarian actors during outbreak.

Recommendations from the study

Based on the observation of RCCE interventions, having feedback from the communities in the focus group discussions and relevant literatures and studies presented in the discussion section, the following recommendations were generated to strengthen the RCCE strategy.

General recommendation

- Adjustments need to be made to the frequency schedule that CHWs follow when going from one household to another. This will allow the CHWs to devote sufficient time to each household, allowing them to engage in attentive listening, provide sufficient explanation, solicit feedback, and address the concerns of the residents.
- CHWs should be part of empowering families and communities to execute strategies at the family and community level to put

COVID-19 preventive measures into action and monitor their effectiveness. This should be linked to the stakeholders responsible for supplying essential resources to the community, such as masks, soaps, hand washing devices, and so on.

- Consideration should be given to incorporating a variety of forms of entertainment into one's home setting in order to reduce the need for socializing in public spaces.
- It is important to provide enhanced social support to the families of those who are hospitalized or placed in quarantine. In lieu of the absence of one or more family members as a result of their stay at an isolation or quarantine facility, it should be ensured that the families are supplied with regular foods and commodities and that their children are getting proper attention.
- People who are quarantined or hospitalized should have the option of applying for incentives to make up for lost wages while they are away from work. Efforts should be made to strengthen family cohesion and communication during hospitalization or quarantine in a facility.

Culture, religion, and language

- Culture and context friendly methods and contents: Culture and context-friendly communication contents, methods and strategies should be designed. Different traditional methods, e.g., folk songs, theaters can be considered as further interventions.
- Extensive involvement of Majhi and Imam: Although some activities were noted regarding involvement of Majhis and Imams, these key stakeholders can be more extensively capacitated and mobilized for risk communication and behavioral change among the community.
- Use of Rohingya language: While designing information, education and communication materials attention should be given for more use of Rohingya language for better understanding by the community.
- Basic literacy program: This is one of the suggestions from the community to consider basic literacy program for improving understanding level of the community.
- Redesign risk communication contents, approaches and strategy: The risk communication messages, guidance, contents and approaches should address the following concerns as mentioned in the above section.

(1) Religious belief, traditions and culture of the community should be well reflected and addressed. Any misinterpretation and misperception should be properly addressed.
(2) Messages should be regularly updated based on updated scientific findings. Any confusions and unclarity of messages and guidelines should be properly explained.
(3) Messages, contents, approaches and methods should address the special needs of vulnerable groups, e.g., persons with disability and older adult people.
(4) Inequalities and stigma associated with COVID-19 should be well addressed in risk communication strategy.
(5) Shift tone of COVID-19 messaging to a more positive message on how to support community and family members during stressful times.

The RCCE strategy should focus on bottom-up communication to reduce suspicion and improve community awareness and perception of COVID-19.

Local context and resources

- Ensure supplies: Ensure regular supplies necessary for maintaining COVID-19 preventive measures should be ensured coupled with culture and context appropriate messages. This includes, masks, soaps, handwashing devices, proper waste management system.
- Marking and signages: Placement of physical barriers and ground markings coupled with culture/language appropriate signages to maintain physical distances at crowded places, e.g., market place, distribution points etc.
- Adequate space for shelter: Consider adequate space at each shelter enabling home isolation/quarantine when necessary.

Trust and interaction

- Addressing people's concerns: Concerns shared day to day by the community to CHWs should be documented and shared to relevant agencies and sectors for adequately address the same.
- Interpersonal communication skill: Although the majority of CHWs receive interpersonal communication training from team trainers, the use of these skills should be effectively monitored and followed up on. Consideration can be given to providing CHWs with follow-up or refresher training. Different IPC skills, such as gentle speaking, smiling, caring, positive body language, engaging community in problem solving and decision making, active listening with attention to people's opinion and reaction, using video or pictorial aids, analyzing the situation, taking the time to engage people, being respectful, realizing how to support and care, and creating a comfortable environment, should be well integrated into the role and approaches of CHWs.
- More engagement of the CHWs with the community: Community volunteers, specially CHWs should be more engaged with the community establishing good interpersonal communication and providing them with adequate time, attention and respect.
- Utilization of multiple channels: In order to assess the efficacy of various communication channels in Rohingya refugee camps, additional research must be conducted. Adaptation and adoption of channels should be planned as required. Instead of repeatedly using the same message and channel, inventive content and channels should be explored.
- Better compliance, cooperation and support from humanitarian workers: To develop trust and address rumors, humanitarian workers must be transparent in all their actions and communicate clearly and pro-actively with Rohingya. They should be compliant with COVID-19 preventive measures so that the community can follow them and have trust over them.

- Feedback: A well-established system should be there for getting feedback from the community should in every RCCE intervention.

Communication

- Identify and innovate more engaging channels of communication: Proper channel of communication should be identified or innovated. For example, folk songs or theaters can be used as one of the modes for reaching the community with risk communication messages and addressing misinterpretation, rumors and local concerns. Facilitation, capacity building and mobilization of self-organized Rohingya groups can be considered for RCCE. Community representatives can be engaged in "go and see" visits to treatment and quarantine sites to enhance community trust and eradicate rumors and misconceptions.
- Addressing rumors and misinformation: A community-based surveillance system should be operated actively identify and record rumors and misinformation; based on which a response system should be established involving CHWs.
- Transparency and up-to-date information: A transparent communication system needs to be established. The CHWs should be capacitated to share their standing, updates and the possible risks or uncertainty in future. The CHWs should be provided with updated information (on situation, strategy, plans etc.) on COVID-19 by their agencies so they can share the same with the community.
- More use of pictorials: Considering high literacy rate, IEC materials should use meaningful culture appropriate pictures instead of text.
- Appropriate message: The messages should be updated addressing the existing rumors and concerns, community's culture and context. This should be linked to access to adequate resources for effectiveness of the messages. For example, if wearing a mask is a recommendation, it should be linked how people can get a mask.
- Active listening: Active listening skills of the CHWs to be further strengthened. CHWs should establish a comfortable zone during their conversation so that the peoples' thoughts, fears, and concerns are shared, respected and taken into account.

Gender and social inclusion

Specific concerns and requirements of vulnerable groups, specially girls, women, persons with disability and older persons should be considered when designing the risk communication strategy and contents. The gender-based inequality and stigma should be taken into account.

Conclusion

RCCE is the cornerstone of reducing COVID-19 transmission. The study explored the effectiveness of RCCE strategies in the Rohingya refugee camps and identified the challenges and community recommendations in relation to COVID-19 preventive measures We identified several best practices, such as recruiting CHWs from within the community, maintaining long-term relationships with CHWs, involving female CHWs, communicating in the local dialect, and establishing connections with religious leaders. We also found areas that need improvement, such as the fact that CHWs are often overworked and unable to devote sufficient time to each individual household they visit, that they often repeat the same messages without making the necessary connections to the difficulties their clients face on a daily basis, that they rarely make the effort to connect their clients' needs with the appropriate cultural, contextual, and material resources, and that they rarely work to empower their clients and link them to those resources. Based on extensive community participation, including members of varying socioeconomic statuses and degrees of vulnerability, we identified five central themes associated with ineffective COVID-19 prevention strategies: (a) culture, religion, and language; (b) local context and resources; (c) community trust and interaction with aid workers; (d) communication methods; and (e) gender and social inclusion. Cultural barriers, limited availability of resources, distrust between the community and aid workers, inconsistent or complex messages, improper mode of message dissemination, a lack of gender and social inclusion, and stigmatization are just a few of the factors that limit to prevent the spread of disease. We encourage organization partners to use this study's findings and recommendations to create a comprehensive risk communication and communication engagement strategy for future outbreaks that takes into account people's culture and context, local concerns and needs, gender and social vulnerabilities.

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

All respondents choose to participate voluntarily. Written informed consent was obtained from the participants for the publication of any potentially identifiable images or data included in this article. Ethical issues in the study were reviewed and approved by the Institutional Review Board of North South University (2021/OR-NSU/IRB/0401). The study adhered to the "no-harm" principle, and no intervention in the project caused significant harm to the subject population or endangered their health or lives. There was no legal risk associated with the participation of the beneficiaries in this study. Local rules/regulations were respected during interaction with the beneficiaries.

Author contributions

CH was responsible for the conception and design of the study as well as drafting the manuscript, managed the project, and coordinated the team of researchers. All authors provided significant contributions to the project, including data collection, analysis, interpretation and

revision, read and approved the final manuscript, and agreed to be accountable for all aspects of the work.

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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.2023.1169050/full#supplementary-material

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Impact of wars and natural disasters on emerging and re-emerging infectious diseases

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Emerging Infectious Diseases (EIDs) and Re-Emerging Infectious Diseases (REIDs) constitute significant health problems and are becoming of major importance. Up to 75% of EIDs and REIDs have zoonotic origin. Several factors such as the destruction of natural habitats leading humans and animals to live in close proximity, ecological changes due to natural disasters, population migration resulting from war or conflict, interruption or decrease in disease prevention programs, and insufficient vector control applications and sanitation are involved in disease emergence and distribution. War and natural disasters have a great impact on the emergence/re-emergence of diseases in the population. According to a World Bank estimation, two billion people are living in poverty and fragility situations. Wars destroy health systems and infrastructure, curtail existing disease control programs, and cause population movement leading to an increase in exposure to health risks and favor the emergence of infectious diseases. A total of 432 catastrophic cases associated with natural disasters were recorded globally in 2021. Natural disasters increase the risk of EID and REID outbreaks by damaging infrastructure and leading to displacement of populations. A Generic National Action Plan covering risk assessment, mechanism for action, determination of roles and responsibilities of each sector, the establishment of a coordination mechanism, etc. should be developed.

KEYWORDS

emerging infectious diseases, re-emerging infectious diseases, wars, natural disasters, public health

1. Introduction

Emerging Infectious Diseases (EIDs) are illnesses that are newly defined or have existed but are increasing in incidence or geographic range which poses a threat to the population, either in a particular area or globally (1–3). Conversely, Re-Emerging Infectious Diseases (REIDs) are illnesses that existed in the past but reappear after they have been on a significant decline, and rapidly spread either in terms of incidence or to new geographical areas (2–5).

Many factors such as the destruction of natural habitats, which leads humans and animals to live in close proximity, changing climate and ecosystems, replacing reservoir hosts or intermediate insect vectors, and microbial genetic mutations are involved in the emergence of new diseases (6). Ecological changes due to natural disasters such as flood/drought and earthquakes, population migration resulting from war or conflict, interruption or decrease in disease prevention programs, and insufficient vector control applications and sanitation have great importance in disease emergence (1). Environmental and social determinants resulting

from natural disasters, wars, etc. are also of great importance in disease re-emergences as well as viral and microbial factors (4). Major contributing factors to the prevalence of EIDs and REIDs are summarized in Table 1.

Re-emergence of diseases that were considered to have ended at the beginning of the 20th century has begun in recent years (8). Wars and natural disasters have a great impact on the emergence/re-emergence of diseases in the population.

2. Impact of wars on human health

According to a World Bank estimation, two billion people are living in poverty and fragility situations (9). Wars destroy the health systems and infrastructure, curtail the existing disease control programs and cause population movement. Large population displacement into overcrowded camps and temporary shelters leads to an increase in exposure to health risks such as disease vectors favoring the emergence/re-emergence of infectious diseases (10, 11). Outbreaks of typhoid fever, cholera, dysentery, malaria, small-pox, typhus fever and influenza caused a significant number of deaths among soldiers and civilians in war zones during or after World War I and World War II (12, 13).

TABLE 1 Selected major and contributing factors to the prevalence of emerging and re-emerging infectious diseases [Adopted from Church (7)].

Major factors	Contributing factors
Global changes in population demographics and distribution	Population growth and density, migration to urban areas, increased widespread travel, immigration, and housing practices
Change in human behaviors	Food distribution and transportation services, liberation of sexual practices, increased need for childcare outside the home, alcohol and drug abuse, change in immunization practices
Changes in environment and land usage	Global climate changes such as increase in average temperature, deforestation, change in land usage, and natural disasters (Floods, intense droughts, catastrophic storms)
Chronic manifestations of infectious diseases	Modern medical technology in developed countries prolongs the life of people with life-threatening chronic diseases
Advanced pathogen detection	Advanced molecular methods have been developed for the detection of fastidious, uncultivable organisms
Microbial evolution	Adaptation of microorganisms to the environment in order to survive
Collapse of public health systems and bioterrorism	Decreased funding of the public health systems, insufficient public health infrastructure, mobility of population, increased international travel, immigration and refugees, wars, conflicts, bioterrorism

After a dramatic increase in morbidity/mortality rates in war-involved countries during and after World War I, tuberculosis was recognized as a war disease and became one of the main concerns associated with wars and conflicts (12, 14, 15). The disease re-emerged in Europe as a result of World War I where the incidence was declining (14). As most of current conflicts are prevalent in tuberculosis-endemic countries, tuberculosis continues to be an important threat not only to the people living in conflict areas, but also to the population living in unaffected areas and neighboring countries since huge numbers of people move to safer places internally or internationally (14–17).

One of the examples is the Syrian crisis which led millions of people to migrate to neighboring countries such as Türkiye. The proportion of foreign-country-born tuberculosis cases among total reported cases in Türkiye increased progressively from 1.1% in 2010 to 6.0% in 2014, 7.3% in 2017, and 10.84% in 2018. 587 Syrian cases constituted 53.0% of foreign-country-born cases and 4.87% of the total cases diagnosed in Türkiye in 2017 (18, 19).

The possibility of death in tuberculosis patients who received irregular or no treatment during the conflict was up to three times higher than the ones who received a full course of treatment during peacetime in Guinea-Bissau, West Africa (20, 21).

In a study assessing the impact of war on Ebola transmission and control in the Democratic Republic of the Congo (DRC), it was found that an increase in the number of cases had been observed over and over due to conflict conditions. Violence against healthcare workers and Ebola treatment centers inhibited the rapidity of case isolation, treatment, following up the contacts of cases, and vaccination programs due to continuous conflict events (22). War in the DRC contributed also to an increase in the transmission of sleeping sickness (23), river blindness (22, 24), and pneumonic plague (25–27) in addition to the spread of Ebola.

Destruction of healthcare infrastructure during wars resulting in weakened prevention and treatment programs can cause new strains of infectious diseases to emerge (28). Eradication of Guinea worm, river blindness, and polio programs have been disrupted resulting in challenges to delivering vaccines due to insecurity and conflict in countries experiencing war (28, 29).

As of 20 March 2023, around 8.1 million Ukrainians have moved to other European countries since 24 February 2022 after the attack by Russia on Ukraine, 5 million of whom were recruited into protection programs (30). Owing to the living conditions in the temporary shelters and the conditions they had faced during their movement, these displaced people are likely to acquire certain infectious diseases including EIDs or REIDs, and cases of EIDs or REIDs among this population are not unexpected (31).

There have been numerous reported outbreaks of conflict/war-associated EIDs/REIDs from a wide range of countries. Selected outbreak reports are presented in Table 2.

3. Impact of natural disasters on human health

Natural disasters increase the risk of infectious disease outbreaks, including EIDs and REIDs, by damaging infrastructure and leading to displacement of populations (41). The population residing in natural disaster-prone areas has risen in most countries due to land

TABLE 2 Selected EIDs/REIDs outbreaks associated with wars.

Year	Continent/Region	EID/REID	Summary
Africa			
1998	DRC	African trypanosomiasis	Because of the weakness of many general health services which seriously hamper the integration of control activities, the annual number of reported sleeping sickness cases rose from 5,825 in 1991 to 26,318 in 1998 (23).
2005	Angola	Marburg	Nine Marburg hemorrhagic fever cases were diagnosed on 21 March, 2005 by the Centers for Disease Control and Prevention (CDC) in the samples sent from Angola. Being the first natural outbreak of the disease to take place in an urban setting, 270 cases with a case fatality rate of 92 percent were reported within the outbreak. Almost three decades of civil unrest have created additional challenges in the effort to contain the outbreak (32).
2006	DRC	Plague	Several plague outbreaks have been reported in DRC. 100 cases of suspected pneumonic plague, including 19 deaths were reported on June 2006 (27).
2020–2021	DRC	Plague	Implementation of control measures had been difficult due to security concerns in the area. More than 400 cases have been reported during outbreaks in 2020 and 2021 which were aggravated by conflict, poverty, increased population movement and displacement, and instability in some areas (25, 26).
2020	Guinea	Yellow Fever	A yellow fever outbreak with 50 suspected cases was reported in Guinea in 2020. The yellow fever vaccination coverage was found to be 16% in the Koundara district which was one of the affected areas while the estimated vaccination coverage against yellow fever for the whole country had been 40% for the years 2016 to 2019 (33).
2022	DRC	Ebola	EVD re-emerged in the Beni health zone, which was affected by insecurity from armed groups, DRC, 21 August 2022. Frequent protests against the security measures put in place by the authorities and against international organizations increased the risk of refusal of outbreak control measures (34).
2022	Malawi	Polio	Malawi reported a wild poliovirus type 1 (WPV1), which was later shown to be genetically linked to a Pakistan sequence detected in 2020 in Sindh province, in February 2022. The detection of WPV1 outside Pakistan, where the disease is endemic demonstrated the continuous risk of international spread of the disease (35).
Asia			
1994	Tajikistan	Malaria	The number of laboratory-confirmed malaria cases increased from 175 in 1990 to 2,400 in 1994 in Tajikistan during the civil strife in 1992–1993 which caused massive internal displacement of people combined with deteriorating living conditions, and paralysis of the health system, especially public health programs. Additional to the major malaria outbreak, falciparum malaria transmission in some areas of the country was re-established for the first time in 35 years (36).
1997–1998	Pakistan	Cutaneous Leishmaniasis	A major cutaneous leishmaniasis outbreak was seen in an Afghan refugee camp in a non-endemic area in north-western Pakistan in 1997–1998. Before the outbreak, there were only a few cases in the camp, which was hosting more than 9,000 residents, mostly from eastern, central, and northern Afghanistan. Additionally, this outbreak caused disease establishment in the neighboring Pakistani villages (37).
2002	Afghanistan	Malaria	There had been a reemergence of malaria in Afghanistan with an estimation of 3 million cases in 2002 of which most of them in Kundoz Province. The annual incidence of *Plasmodium falciparum* and *Plasmodium vivax* malaria fluctuated from 0.0088 to 4.39 and from 3.58 to 13.37 episodes per 1,000 person-years, respectively. The introduction of *P. falciparum* and *P. vivax* malaria by returning refugees was one of the contributing factors (38).
2012	Lebanon	Leishmaniasis	The annual number of leishmaniasis cases reported in Lebanon sharply increased to 1,275 in 2012 due to mass population migration from Syria. All the cases were Syrian refugees from Aleppo, where they had been infected, causing re-emergence of the disease in the country. Lebanon reported ranged between 0 and 6 cases with no local transmission before 2012 (39).
Europe			
2000	Kosovo	Tularemia	A tularemia outbreak with 327 cases in 2000 was seen in Kosovo as a result of environmental disruption, mass population displacements, the collapse of public health functions, such as disease surveillance and outbreak response, and a breakdown of sanitation and hygiene due to more than 10 years of political crisis and warfare (40).

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shortage, urbanization, poverty, and population growth, and led to an increase in the public health impacts of natural disasters (42, 43).

A total of 432 catastrophic cases associated with natural disasters were recorded globally in 2021 though the average was 357 between 2001 and 2020 (44). The annual number of floods, which is the most common event among natural disasters, has increased from an average of 163 in the 2001–2020 period to 223 in 2021 (44). Additionally, globalization, climate change, and population movement cause natural disasters and their effects to become more complex (41).

Natural disasters are stratified into several categories by experts. Even though one classification divides natural disasters broadly into three general types (hydrometeorological, geophysical, and geo-meteorological disasters), any disaster in one category can have elements of another, for example earthquakes can cause avalanches and tsunamis, which can, in turn, cause flooding (45).

Hydrometeorological disasters such as floods from rains, hurricanes, cyclones, typhoons, and tsunamis are the most common natural disasters followed by geophysical disasters (Earthquakes, volcanic eruptions) while geo-meteorological ones are rarer than the others (45). Sometimes an emergence or re-emergence of an infectious disease agent can be, by itself, a natural disaster. Centre for Research on the Epidemiology of Disasters (CRED) classify these epidemics/pandemics as a biological sub-group of natural disasters (46). Plague outbreaks, especially the 14th century Black Death period and more recently the emergence of SARS-CoV-2 are the best examples. Even though these diseases cannot be attributed to any specific war or natural disaster they did have serious consequences on the control and prevention of other infectious diseases.

There have been numerous reported outbreaks of natural disaster associated EIDs/REIDs from a wide range of countries. Selected outbreak reports are presented in Table 3.

4. Drivers of emerging and re-emerging infectious disease outbreaks in wars and natural disasters

There are several causal factors in emerging and re-emerging infectious disease outbreaks in wars and natural disasters. Population movement and migration, environmental health disruption and ecological changes, displacement of domestic and wild animals, the collapse of health systems and disruption of disease control programs, inadequate surveillance and early warning and response systems, impaired laboratory services and diagnosis, and breakdown in infection control and treatment in effectiveness and development of drug resistance are the most accepted drivers of emerging and re-emerging infectious disease outbreaks in wars and natural disasters.

The impact of wars and natural disasters on emerging and re-emerging infectious disease outbreaks can be direct or indirect.

5. Direct impact with increasing conditions for vector/reservoir proliferation or environmental conditions

Waterborne, rodent-borne, and vector-borne diseases are the main groups of diseases associated with natural disasters. Emergence and increase in the incidence of waterborne diseases due to *Campylobacter*, *Cryptosporidium*, *Escherichia coli*, *Giardia*, Hepatitis A virus, Norovirus, *Shigella,* and *Salmonella* are generally reported from the affected areas (83).

It is widely recognized that natural disasters, especially hydrometeorological ones like floods, hurricanes, and cyclones, have the potential to lead to an increase in vector-borne diseases due to environmental factors (enlargement of vector habitats in number and area) enhancing vector population densities (84, 85). Existing mosquito breeding sites may be washed away at the beginning of flooding, but later on, new breeding sites for mosquitoes can occur due to overflows of rivers or rainfall and therefore enhance the risk of re-emergence of mosquito-borne diseases such as malaria, Chikungunya, and dengue (57, 85–87). There have been also emergence/re-emergence and outbreak reports of Chikungunya virus (CHIKV), Tahyna virus (TAHV), West Nile virus (WNV), and Japanese encephalitis diseases linked to flooding events (83, 88). Flooding can also cause a proliferation of disease vectors and microorganisms by changing the balance in ecosystems and the environment (43, 86). Mosquito-borne diseases, related to ecological changes which are suitable for mosquito breeding, also increase after earthquakes (49). Following the devastating earthquake in Ecuador in 2016, an important increase in the incidence of Zika Virus (ZIKV) was observed in the affected regions (89, 90). After the earthquakes in Iran in 2003, Cutaneous Leishmaniasis (CL) cases fluctuated, new foci emerged and the epidemiology of the disease changed (65, 66, 71, 91).

Mosquito-borne diseases were also reported during drought conditions. Drought causes reduce in the size of water bodies making them stagnant which leads to additional breeding places for mosquitoes (92). Also, the collection of rainwater due to inadequate water supply can increase the stagnant water sources (92). Drought-induced amplifications of the West Nile virus have been reported in the USA (55, 56). Malaria cases and deaths increased excessively due to the drought following an El Niño Southern Oscillation that occurred in Indonesia (68). Drought conditions caused numerous stagnant water puddles in rivers acting as mosquito breeding places (68, 93).

The risk of diseases transmitted by rodents such as leptospirosis and Hantavirus Pulmonary Syndrome increases as the probability of contact with bacteria and their animal hosts rise during heavy rainfall and flooding (94, 95). Flooding catalyzes the transmission of leptospirosis as a result of an increase in the number of infected rodents sheading leptopiras (57, 95). Insufficient garbage collection and management causing the spill of rubbish into streets after natural disasters can lead to an increase in rodent population (96, 97).

Rarely, unusual disease outbreaks such as coccidioidomycosis and scrub typhus have been observed following earthquakes. For example, an outbreak of acute pulmonary coccidioidomycosis related to exposure to high-level airborne dust following landslides after the 1994 earthquake occurred in southern California (52, 98). This outbreak illustrates the relationship between specific environmental conditions and the emergence of infectious diseases (52). After the massive earthquake in Nepal in 2015, scrub typhus emerged and outbreaks were reported from various parts of the country due to rodent infestation of the environment around temporary shelters which led people and rodents to be in close proximity (76, 77, 99).

Also, the destruction of infrastructure such as electricity, water supply, sewage disposal, and gas supply increases the risk of food poisoning and water-borne diseases after natural disasters (100, 101).

TABLE 3 Selected EIDs/REIDs outbreaks associated with natural disasters.

Year	Continent/ Region	Natural Disaster	EID/REID	Summary
Africa				
1987–1988	South Africa	Flood	Poliomyelitis	An outbreak of type 1 poliomyelitis with 23 cases reported triggered by the massive floods, experienced in the area 2 months earlier in the eastern part of South Africa between December 1987 and November 1988. The cases were most probably due to temporary breakdown in vaccination services and considerable surface pollution, including 'wild' poliovirus as a result of flooding (47).
2000	Mozambique	Flood	Malaria	After the heavy rain in Mozambique in 2000 the incidence of malaria increased by four to five times compared to the same period in other years (48).
America				
1991	Costa Rica	22 April 1991 Earthquake	Malaria	After the earthquake on 22 April 1991, measuring 7.4 on the Richter scale, statistically significant increases in the incidence of malaria were reported during the months immediately after the earthquake in Limon Province. The number of registered malaria cases for 1 June through 31 May period dramatically increased from 681 in the 1990–1991 period to 3,597 on 1 June 1991 through 31 May 1992 in the same region (49).
1991	USA	Drought	Leptospirosis	Five leptospirosis cases associated with swimming in a rural swimming pond were reported from a small town in rural Illinois between July 7 and 18 in 1991. The outbreak was attributed to the drought conditions creating an environment in the pond facilitating transmission of the organism from area animals to humans (50).
1993	USA	Flood	Leptospirosis	Following the floods in 1993, leptospirosis cases exposed to extensive flood water were reported in Iowa (51).
1994	USA	1994 Northridge, California Earthquake	Coccidioidomycosis	A coccidioidomycosis outbreak between 24 January and 15 March was reported in Ventura County following the January 1994 earthquake, centered in Northridge, California. It is suggested that the outbreak was caused by arthrospores spread in dust clouds generated by the earthquake (52).
1996	Brazil	Flood	Leptospirosis	An increase in the number of leptospirosis cases was reported during the subsequent weeks, after the heavy rainfall caused persistent flooding in several areas of Rio de Janeiro accounting for the largest epidemics in the city's history. The incidence of leptospirosis was around 1 case per 100,000 inhabitants in the city of Rio de Janeiro yearly before the outbreak. The disease incidence fluctuated to 42.05 per 100,000 inside the flood-risk area (53).
1998	Nicaragua and Guatemala	Hurricane Mitch	Cholera, Leptospirosis and Malaria	Hurricane Mitch in 1998 affected several countries in Central America which damaged health services, wate,r and sanitation networks, and caused population movements between neighboring countries. Thirty-eight cholera outbreaks with 33 deaths in Guatemala, and a leptospirosis outbreak in Nicaragua with 7 seven deaths were seen. Also, the number of reported malaria cases was much higher than the weekly average reported during the pre-Mitch period in Nicaragua and Guatemala (during the second and third weeks) (54).
2001–2003	USA	Drought	West Nile virus	Sporadic and focal transmission of West Nile Virus (WNV) in humans and sentinel chickens has been reported from Florida between 2001–2003. Transmission of WNV was associated with drought 2–6 months prior and land surface wetting 0.5–1.5 months prior probably due to drought that brought avian hosts and vector mosquitoes into close contact facilitating the epizootic cycling and amplification of the arboviruses within these populations (55).
2003–2011	USA	Drought	West Nile virus	Analysis of the field surveys of local mosquito communities and the prevalence of WNV within *Culex* spp. populations for transmission seasons of 2012 and 2011, and the WNV infection rates and climate data from nine transmission seasons (2003–2011) in New Jersey showed drought conditions (i.e., increased temperatures and decreased precipitation totals), were associated with increases in the prevalence of WNV and confirmed that climatic conditions have a strong impact on the prevalence of vector-borne diseases (56).
2004	Dominican Republic	Flood	Malaria	After the Hurricane struck the Dominican Republic, the east coast of the country received heavy rains and flooding resulting in increased mosquito breeding sites. The number of malaria cases increased sharply, and 2,012 cases had been reported within one month in 2004, which was approximately 1,500–2,500 cases annually in the country (57, 58).
2004	USA	Flood	Leptospirosis	After a flood in a university campus, two leptospirosis cases, of which one was a professor cleaning his flooded laboratory in sandals, were diagnosed (59).

(Continued)

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TABLE 3 (Continued)

Year	Continent/ Region	Natural Disaster	EID/REID	Summary
2005	Guyana	Flood	Leptospirosis	The widespread flooding following the unusually high rainfall in January along the Atlantic coast led to conditions favorable for epidemic leptospirosis. An outbreak of leptospirosis associated with flooding was confirmed *via* laboratory testing (60, 61).
2010	Haiti	Earthquake on January 12, 2010	Malaria	After the great earthquake, 11 laboratory-confirmed cases of whom seven of them were emergency responder U.S. residents, of *P. falciparum* malaria acquired in Haiti reported (62). By enhanced surveillance conducted between 4 March and 9 April, 2010, 317 more malaria cases were diagnosed most probably due to displaced persons living outdoors or in temporary shelters, putting them at increased risk for acquiring malaria (63).
2011	USA	Tornado	Mucormycosis	After the tornado on 22 May, 2011, a large cluster of cases of mucormycosis, with 13 *A. trapeziformis* infections in persons injured during a tornado reported, suggesting environmental fungi be considered as potential agents of soft-tissue infections in injured patients after disasters (64).
2016	Ecuador	Earthquake on April 16, 2016	ZIKV	A significant increase in the number of Zika Virus (ZIKV) cases was seen in the affected areas after the 7.8 magnitude earthquake in Ecuador on 16 April, 2016 (65, 66). The total number of ZIKV cases in the country rapidly escalated from 92 to 1,106 in just 3 months. Eighty percent of the cases were reported from the region most affected by the earthquake (67).
Asia				
1997–1998	Indonesia	Drought	Malaria	A dramatic increase in malaria and an associated 550 deaths were reported beginning in late August 1997 due to the prolonged and severe drought created by the prevailing 1997–98 El Niño Southern Oscillation (ENSO) in Irian Jaya, Indonesia. Drought conditions resulting in numerous, transient pools of standing water permitting a rapid increase in vector populations and movement and exposure of the population to high-risk malaria endemic lowlands were the main drivers of the outbreaks (68).
1999	Japan	Heavy rainfall	Leptospirosis	After the heavy rainfall in 1999, an outbreak of leptospirosis in the Yaeyama Islands was reported. Fourteen people who were exposed to contaminated soil or water were diagnosed with leptospirosis and required hospitalization (69).
2000	India	Flood	Leptospirosis	Two weeks after heavy rains led to floods in Mumbai in July 2000, an outbreak of leptospirosis was reported in adults admitted to public hospitals. Additionally, leptospirosis was diagnosed in 18 children who were admitted to one hospital between 24 July and 14 September 2000. All of them had contact with flood water (70).
2003	Iran	December 2003 Bam city Earthquake	Cutaneous leishmaniasis	After an earthquake created 10 million tons of rubble, creating suitable conditions for the propagation of sand fly vectors in Bam city of Kerman province in Iran, 2003, a new Anthroponotic Cutaneous Leishmaniasis (ACL) focus in the villages of Dehbakry county, of which there had not previously been any record of CL, was established and an outbreak reported in November 2004 (71).
2003	Iran	July 2003 Fars Earthquake	Cutaneous leishmaniasis	The annual incidence of CL in Fars, Zarindasht, increased from 58.6 cases/ 100,000 in 2002 to 864/100,000 in 2004 after the two earthquakes on 10 July 2003. The cases were predominantly Zoonotic CL (65).
2003	Iran	December 2003 Bam city Earthquake	Cutaneous leishmaniasis	Creating various risk factors; the earthquake caused a sharp increase in the incidence of anthroponotic cutaneous leishmaniasis (ACL) cases in Bam. The mean annual incidence of ACL increased from 1.9 per 1,000 for the period of 1999–2003 to 7.6 per 1,000 for the period of 2004–2008 (66).
2004	Indonesia	Tsunami	Melioidosis	Ten patients with pneumonia of whom four were with culture-confirmed melioidosis, were diagnosed among tsunami survivors as a result of immersion in contaminated saltwater during the tsunami (72).
2007	China	Flood	Malaria	Several floods occurred following the persistent and heavy rain in June and July in the Huaihe River Basin in 2007. The monthly mean monthly malaria incidence fluctuated from 13.76/10 to 95.78/10 in the area. Increased risk of malaria was significantly associated with flooding (73).

(Continued)

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TABLE 3 (Continued)

Year	Continent/ Region	Natural Disaster	EID/REID	Summary
2009	Taiwan	Typhoon	Leptospirosis, melioidosis	After Typhoon Morakot in 2009, unusual epidemics of leptospirosis and melioidosis cases were reported. Incidences of leptospirosis and melioidosis cases after the typhoon were significantly higher than those before the typhoon (74).
2011	Sri Lanka	Flood	Leptospirosis	In 2011, a large outbreak of leptospirosis with 32 cases was observed in Anuradhapura district, which was not previously classified as a leptospirosis endemic area, after two weeks of massive flooding (75).
2015	Nepal	Earthquake	Scrub typhus	Three months after the devastating earthquake in April 2015 in Nepal; the first and most significant fatal scrub typhus outbreak was reported in the country with 141 cases in 2015, and lasted for three years (76–78).
Europe				
1997 and 2002	Czech Republic	Flood	Leptospirosis	Leptospirosis has been reported rather sporadically, with an incidence rate of about 0.3% per 100,000 population in the Czech Republic. After vast floods in 1997 and 2002, the incidence of leptospirosis increased to 0.9 per 100,000 population (79).
1999	Türkiye	17 August 1999 Earthquake	Tularemia	After the 1999 earthquake, an oropharyngeal tularemia outbreak was reported from the Golcuk-Kocaeli region beginning with 5 cases followed by 129 more cases. This was the first outbreak in the region and most of the cases were from the houses located around natural springs in the new settlement constructed after the earthquake. The outbreak was probably due to pollution of natural springs infected by wild rodents or other infected animals (80).
2004	Finland	Tsunami	Melioidosis	Melioidosis cases were reported in survivors of tsunami that occurred in coastal areas of the Indian Ocean rim in December 2004. *Burkholderia pseudomallei* was isolated from three Finnish patients in January 2005 whom were visiting Thailand when the tsunami struck in December 2004 (81).
2010	Austria	Flood	Leptospirosis	Four leptospirosis cases were reported in athletes after a triathlon held in Langau, a village in Austria, due to intense rain on the eve of the triathlon that had caused flooding and "unusual turbidity" of the lake (82).

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Outbreaks of leptospirosis and malaria were reported in Guatemala and Nicaragua after Hurricane Mitch in 1998. The number of diagnosed malaria cases was considerably higher than the number of cases diagnosed before the hurricane (54).

Melioidosis cases were reported in survivors of the tsunami which occurred in coastal areas of the Indian Ocean in December 2004 (72, 81). The risk of waterborne and vector-borne diseases increases due to physical changes in the environment in tropical cyclones, followed by floods and sea surges (102).

Findings from previous El Nino episodes show that rains due to El Nino that result in increased rodent density heightens the risk of human contraction of Hantavirus pulmonary syndrome (103). A previously undescribed disease, subsequently identified as a previously unknown hantavirus (*Bunyaviridae*), emerged in the Southwest USA in 1993 after El Nino Southern Oscillation events the year before, which had allowed excess precipitation, warmer seasons, and plenty of food for rodents (21, 103). Also, a statistically significant association was demonstrated between malaria epidemics and El Niño most probably due to widespread flooding during El Niño (104).

6. Indirect impact through changes in human behavior

6.1. Population movement and migration

Population displacement is generally observed after natural disasters and wars (105, 106). Population displacement can lead to contact with infectious agents both for the migrating population and for the population at their point of arrival. The migrating population can introduce new infectious agents to the population living in their destination. Conversely, the migrating population can get in touch with new local pathogens. Additionally, the importation of the diseases from endemic regions to non-endemic regions by the migrants (107, 108).

According to UN Refugee Agency, 89.3 million people—of which 27.1 million were refugees—had to leave their homes in 2021 because of conflicts/war, assault, human rights violations, etc. Around 69 percent of people displaced across borders originated from Afghanistan, Myanmar, South Sudan, the Syrian Arab Republic, and Venezuela. The Syrian refugee population constituted 27 percent of the global refugee population with around 7 million and spread to 129 countries. Most of them are hosted in Türkiye (3.7 million), Lebanon (840,900), and Jordan (673,000) (109).

The displaced population is generally housed in temporary settlements or camps with overcrowded shelters, which increases infectious disease transmission and the number of endemic and epidemic-prone disease cases (11, 85). There have been numerous reports of EIDs/REIDs related to population movement associated with the destruction of health services and disease control programs.

Being the largest refugee crisis since World War II, the Syrian crisis is one of the examples of how conflict and refugees change epidemiological patterns of diseases not only in the source country but also in neighboring countries (85, 110). Cutaneous leishmaniasis (CL) has always been reported in Syria, particularly in the western part of the country. CL cases had increased from 17.709 in 2007 to 78.231 (suspected cases 111.144) in 2021 due to decreased public health services, reduced diagnosis and treatment access, population displacements, demolition of infrastructure, and worse living conditions as a result of war. Additionally, the epidemiology of the disease in Syria also changed during wartime; mass population movements from highly endemic areas raised the number of cases in some governorates, which used to report few cases before (111–113). Also, the CL situation in countries that hosted huge numbers of Syrian refugees altered. The disease re-emerged in Lebanon and the number of cases rose to 1.383 in 2014 (39, 114). Even though CL is endemic in Türkiye, the number of CL cases increased from 1.133 in 2008 to 5.362 in 2013 (115).

The risk of outbreaks of norovirus, *Salmonella enterica*, and chickenpox resulting from post-disaster living conditions increases after earthquakes (41, 100). Diarrhea and respiratory tract infections are the most common diseases reported after earthquakes (116, 117).

Movement of workers from the CL endemic regions to Bam city in order to work on construction projects, provision and creation of suitable conditions for propagation of sand fly vectors and many new resting and breeding sites, changes in the behavior of people following earthquake increasing their exposure to the bites of the vectors, and displacement of the wild mammals acting as the 'reservoir' hosts of the parasite (65, 66, 71, 91) were likely to be the main drivers.

In drought conditions both human and animal behavior changes, such as movement to places where water supplies can be found. This increases the likelihood of contact with one another (92, 93). Current theories suggest even the bubonic plague outbreaks in Europe in the 14th century were caused by the drought in Asia which led the introduction of infected rodents to Europe (118, 119).

6.2. Displacement of domestic and wild animals

During natural disasters, war, or conflicts animals can be lost, abandoned, or left unsupervised leading to a high number of stray or owned free-roaming dogs (120). Free-roaming dogs and cats are source of a variety of bacterial, viral, and parasitic infections which pose risks to humans (121). Free-roaming dogs change or increase the risk for emergence and transmission of zoonotic diseases in dogs and people such as rabies, echinococcosis, toxocariasis and even leptospirosis, Chagas disease, and leishmaniasis (122, 123).

Colonization of rodents can be increased by dog foraging activities in accumulated waste in camps (122, 124). Loss of rodent control resulted in the Lassa outbreak in refugee camps in Sierra Leone (106).

The risk of rabies can be increased due to a higher roaming dog population, increased aggression in dogs and dog bites, reduced/low vaccine rates in dogs, and post-exposure prophylaxis in humans (122) in natural disasters and wars. There have been some studies suggesting an increase in the incidence of dog bites following natural disasters (125–128). News stated that cases of exposure to dog bites have increased in the areas of northwestern parts of Syria and rabies cases were reported in 2022 (129).

An increase in contact with wildlife due to population movement is another risk factor for EIDs/REIDs in natural disasters and especially in conflicts (130). Disruption of livelihoods that is likely to increase the consumption of bush meat (131, 132) and increased exposure to animal hosts such as bats, increase the likelihood of zoonotic diseases (106, 130). Evidence suggested that Ebola may have been spread by these population movements in conflict areas (130).

European Centre for Disease Prevention and Control (ECDC) warns the countries accepting Ukrainians about rabies risk as it is still endemic in sylvatic animals and dogs and cats in Ukraine due to the reports describing displaced Ukrainians as fleeing with their pets (31).

7. Indirect impacts through the collapse of health systems and sanitation services

7.1. Collapse of health systems and disruption of disease control programs

The deterioration of the public health systems leads to an interruption in health measures such as immunization, surveillance, prevention and control programs, sanitation, and hygiene services and results in an increase in communicable diseases (105, 133). Disruption of public and animal health care systems has a major impact on the increase in vulnerability of displaced populations to different kinds of infections (85). Beyond the destruction of pre-existing local health facilities such as buildings, medical stores, and laboratories, access to functioning facilities can be blocked due to risks involved in traveling through the conflict zones (11, 134). Moreover, local medical staff are often also affected by the conflict or natural disaster, making them incapacitated (133).

The incidence of vector-borne diseases such as malaria can increase due to the disruption of control activities (135). Re-emerge of malaria in former Soviet Union republics is an excellent example of how the disruption of disease programs due to conflict can alter the disease epidemiology.

Malaria had been eradicated in all the republics of the former Soviet Union with a campaign launched at the end of the 1950s (136). Re-emergence of malaria started in the former Soviet Union republics due to internally displaced people and mass population movement from malaria-endemic neighboring countries, mainly Afghanistan, experiencing armed conflicts and war in the 1990s (137). Beyond this; the deterioration of preventive measures including malaria services due to economic collapse as a result of the disintegration of the former Soviet Union contributed to the situation (137). Malaria re-emerged in Armenia, Azerbaijan, Georgia, Kazakhstan, Kyrgyzstan, the Russian Federation, Tajikistan, Turkmenistan, and Uzbekistan and several outbreaks have been reported afterwards (137). During the civil war in 1992–1993, with the influx of massive population displacement united with deterioration in living conditions and breakdown of the health system, particularly public health programs, and refugees from Afghanistan, a major malaria outbreak reached 29.794 cases in 1997 in Tajikistan, where malaria had been eradicated in 1960. Moreover, *P. falciparum* malaria was re-established after 35 years in Tajikistan due to mass population movement to and from Afghanistan (36, 137).

Curtailment of vaccination programs is especially crucial. A decline in or suspensions of vaccination programs have serious effects not only on the local disease programs but also globally implemented ones. Conflicts are now causing re-emergences of diseases that were in the eradication phase such as polio (138). The World Health Assembly put in force a resolution for polio eradication globally in 1988 (139). Due to conflict the three doses of polio vaccine coverage dropped to 35% and led to a large polio outbreak in Somalia in 2005 (10, 140).

The last case of wild poliovirus in Syria had been reported in 1999 (141). But in 2013 the disease re-emerged and 36 children of whom most had not been vaccinated against polio were paralyzed (141). In addition, another poliovirus outbreak in 2017 left 74 children paralyzed in Syria (142). The emergence of poliovirus in Syria manifests the public health results of the war (143).

The last case of autochthonous wild polio was reported in 2020 in Africa, and in Malawi the last clinically confirmed wild polio case was reported in 1992 (35). Wild poliovirus type 1 (WPV1) cases were diagnosed in Malawi and in Mozambique on 17 February 2022 and 15 May 2022, respectively, which the WHO considered at high risk of spreading, particularly to the countries of Southeast Africa, owing to their low immunity and surveillance, huge population movements, and decreased immunization rate (35, 144).

Yellow fever outbreaks were reported in West Africa as a result of the curtailment of immunization programs and spontaneous or forced migrations of thousands of people due to conflict. The circulation of the yellow fever virus in Africa has increased significantly between 2000 and 2004 (10, 145). Yellow fever re-emerged in 2020 with two outbreaks in West African countries (Guinea and Senegal) and enlarged to 12 African countries of which most have been facing political instability and insecurity (146). The outbreak continued in nine countries of the WHO African Region (Central African Republic (CAR), Cameroon, Chad, Côte d'Ivoire, DRC, Ghana, Niger, Nigeria, and the Republic of Congo) in 2021 with aggregation of decreased routine immunization and increased population movement (147). A resurgence of yellow fever began additionally in four countries (Kenya, Niger, Sierra Leone, and Uganda) in 2022 (148).

Security problems are another concern in wars, and can hinder the implementation of control programs and also can cause delays in response in cases of disease outbreaks (10).

There have been various reports of outbreaks of plague in DRC. A total of 8,379 cases have been reported between 2004 and 2014 in the country (149). The disease re-emerged in 2020 (25, 26) and the outbreak continued in 2021 (26) and 2022 (150). Worryingly, cases are also reported from areas that have not seen cases for several decades (26). The collapse of control programs impeded the access of the population to health facilities, the insecurity of the area, and population displacement due to a prolonged and escalating conflict situation were the main drivers of the outbreaks (26, 149–151).

7.2. Insufficient surveillance and impaired early warning and response systems

An effective disease surveillance system is essential in order for timely detection of infectious disease outbreaks both in human and animal populations (85, 152). Surveillance is needed to assess the situation of diseases, follow up the alterations in epidemiology, characterize health risks, trace the populations' health, guide immediate and long-term measures, prioritize of financial resources for health, and complement more targeted epidemiological and laboratory investigations (153). However, surveillance systems are often weak and adversely affected in conflict situations and disasters or are not designed to serve emergency preparedness and response activities, give early warning of diseases, or gather information necessary to assess needs (10, 152, 153).

Existing surveillance systems may not be functioning or reachable due to the conditions (154). For example, in the earthquake in Haiti in 2010, the identification of the emergence of cholera took several days. The surveillance system and infectious diseases control programs took 2 weeks to be set up following the earthquake (154, 155).

7.3. Impaired laboratory services and diagnosis

Laboratory services and facilities face many constraints in disasters and conflict areas including getting the affected region, deficiency in reagents and equipment, limited electrical power and utensil supplies, inadequate personnel, and ineffective supervision. In some situations, laboratories themselves may be in a state of emergency (156, 157). Security risks, damaged infrastructure, and discontinuity in supply chains due to blockades are additional burdens in laboratory systems in conflict-affected areas (158).

Disease surveillance is one of the important components of disaster assessment and monitoring the effectiveness of interventions (156). Diseases that have typical clinical presentations, such as measles, do not require laboratory investigations for diagnosis however most infectious diseases need laboratory facilities to make or confirm the diagnosis (156). Timely and accurate diagnosis is essential in conflict situations and disasters in order to realize disease clusters or even outbreaks, as well as generate data to manage public health interventions (158).

The role of laboratory services in disasters is the prevention or control of infectious diseases, by identification of the causative agent(s) of outbreaks which is especially important in effective disease/outbreak control and the management of conditions that occur secondary to the prime cause of the outbreak/disaster (21, 156). The decrease in etiologic diagnosis can cause an increase in broad-spectrum antibiotics usage which can contribute to the emergence of antimicrobial resistance (21).

Displaced persons or refugees are generally at great distance from access to laboratory facilities (156) which makes them more vulnerable to disease outbreaks. Testing for different pathogens, particularly water-borne ones is especially important in these groups with poor hygiene and overcrowding in order to manage disease outbreaks (158).

The debilitating earthquake in 2005 in Pakistan posed a unique problem with the supplies for the maintenance of laboratory services. The health management in affected areas was almost paralyzed and the existing healthcare system was completely demolished leading to total disruption of the primary and secondary healthcare system. Additionally, health staff became vulnerable due to landslides and weather conditions. The establishment of diagnostic laboratory services was a unique and special challenge. Unavailable appropriate shelters specified for laboratory equipment caused to work in uncontrolled conditions. The efficiency of sensitive equipment was affected due to drastic changes in temperature and electricity supply as the generators were limited. There was a great problem with effective logistic and technical support, and the supply of reagents and diagnostic kits (159). The weak health system including insufficient laboratory diagnostic capacity and a lack of experience in patient treatment and community awareness resulted in the further spread of Ebola in Sierra Leone in 2014 (160). Patients died due to the late laboratory confirmation, leading them to not being transported to treatment centers. Additional problems regarding inappropriate specimen delivery systems, a lack of consistent electricity which resulted in broken laboratory equipment and water supply, and the disruption of instruments owing to high temperature and humidity had been also experienced during the outbreak (160).

7.4. Breakdown in infection control

Breakdown in infection control in natural disasters and wars is generally observed. The principles of infection prevention and control (IPC) remain especially important given the disrupted health services, insufficient trained staff and personal protective equipment, and the unhygienic circumstances after disasters, which can enable the amplification of infectious diseases. Infection control is especially important in areas where there is a transmission risk of infectious diseases such as Ebola, Middle East Respiratory Syndrome (MERS) (10, 138).

Staff of healthcare centers are at high risk of contracting and transmitting the diseases to their family and the community (161, 162). Prior to infection control procedures, the relative risk of healthcare workers contracting Ebola during the outbreak in West Africa was approximately 100 times higher than in the general population (162, 163). Nosocomial transmission played a major role in two Ebola outbreaks in conflict-affected countries, the DRC in 1995 and Uganda in 2000 (10), most probably from a breakdown in infection control. Another nosocomial outbreak of Lassa fever occurred in Sierra Leone in 2004 due to a breakdown of infection control measures due to a weakened health system during war years (10, 164).

A Marburg hemorrhagic fever outbreak was recorded in long-term war affected Angola in 2004–2005. The outbreak was amplified by multiple usage of injectors and multi-dose ampoules in health facilities (10, 32, 165).

7.5. Treatment ineffectiveness and development of drug resistance

The collapse of health facilities, impeded access of the population to surviving health facilities, and insufficient quantities of treatment drugs can cause the cessation of continuing treatment regimens and usage of un-prescribed drugs or inappropriate drug regimens and outdated drugs resulting in pathogens' increase in infectious diseases transmission and resistance to drugs in conflict situations and natural disasters (43, 85, 166).

The emergence of Antimicrobial Resistance (AMR) in microorganisms is a major health problem. It is linked to insufficient health facilities, inappropriate sanitation measures, weak health care, overuse of antimicrobials, and lack of or outdated treatment guidelines (167, 168). Evidence also indicates that increased international travel and migration including forced migration, can contribute to the spread of drug resistance. Displaced populations and migrants may have a higher burden of AMR due to specific factors such as poor living conditions, limited access to good-quality health care or interruption of treatment during their journey, and poor nutrition (167–169).

Studies show that refugees have an elevated prevalence of AMR carriage and infection and significantly higher rates of methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Enterobacteriaceae* (MDRE) than the host country population (168, 170). A study which compared the AMR levels of refugees and Germans found that the refugee group carried five or more antibiotic resistance genes whereas most Germans carried three or fewer (171, 172). In a study conducted in Türkiye, it is found that MRSA prevalence and extended-spectrum β-lactams (ESBL) positivity rates of Syrian refugees were higher than that of Turkish citizens (172).

The rapid worldwide emergence and spread of *Acinetobacter baumannii* (*A. baumannii*) is one of the major causes of hospital-acquired infections in recent years (173, 174). *A. baumannii* strains are of an especially high concern in military field hospitals and the ones established after natural disasters (173, 175). Drug-resistant or even multi-drug-resistant (MDR) *A. baumannii* are a great issue of concern in patients with war injuries as war-associated wounds are prone to colonization of multidrug-resistant *A. baumannii* (176). Gaining attention during the 2003 and 2005 period military operations in Iraq, since then there have been several reports of warfare-associated MDR *A. baumannii* infections (174, 176–180).

A study indicated that drug resistance, and MDR-TB in particular, was significantly higher in patients from the refugee population than the local population in Kenya most probably due to a poorly functioning TB treatment program and an incomplete course of medication (166). Drug-resistant TB including MDR-TB has emerged in republics of the former Soviet Union after the fall of the Soviet Union due to civil war in most of the countries, leading to a large number of internally displaced persons, the collapse of public health systems and increased poverty and decline in socioeconomic status (181–184). TB remains a major public health problem in Ukraine having the second highest number of TB cases (31000), with an incidence of 71 cases per 100,000 in the World Health Organization (WHO) European Region. Additionally, the burden of multi-drug resistant tuberculosis (MDR-TB) in Ukraine is extremely high (185). Experts warn about the risk of suspension of TB and DR-TB treatment due to war could have significant results, like amplification of drug resistance, continuous transmission of infection, and death (186).

7.6. Environmental health services disruption and ecological changes

The impact of both natural disasters and wars creates several public health problems. The deterioration in environmental health services is one of the major problems (187). Alteration in ecology leads to an increase in vector proliferation and the movements of wild and domestic animals pose risks for disease outbreaks (188). Disasters alter the microbial population in the affected area which can cause new ecological interactions (118).

Vector-borne diseases represent a large group within emerging diseases and major causes of morbidity and mortality. Ecological changes and environmental conditions are very important in vector-borne agent transmission (21). The mosquito population is dramatically affected by environmental conditions such as rainfall and flooding (21).

A tularemia outbreak was reported in 1999–2000 during the civil war in Kosovo due to ecological changes, huge population movements, and deterioration of sanitation (40).

Natural disasters can cause disease outbreaks as a result of the contamination of water sources with feces and chemicals, and the living conditions of populations in overcrowded shelters (189).

8. Indirect impact through individual vulnerabilities

Malnutrition, which increases vulnerability to EIDs/REIDs, is a common situation observed both in wars and natural disasters, especially in long-term events such as droughts. Food security created by conflicts, civil strife, and extreme weather conditions—particularly repeated droughts—is the main reason for malnutrition in less developed countries (190, 191). According to World Food Programme around 258 million people were acutely food-insecure in 2022 in 58 countries (191). The Greater Horn of Africa including Djibouti, Ethiopia, Kenya, Somalia, South Sudan, Sudan, and Uganda is the worst food-insecure region for decades. A rising trend in food insecurity is being currently observed in the region due to extreme weather events such as drought and flooding, conflict and instability, high food prices, and the socioeconomic impacts of COVID-19 (192, 193).

Food insecurity can cause impairment in the immune system due to malnutrition making people more susceptible to infectious diseases and generally associated with population displacement, overcrowding, the collapse of health system measures such as vector control and vaccination programs, and poor sanitation which poses the risk of infectious diseases outbreaks (192, 194). Particularly women, children, and people living with chronic diseases like human immunodeficiency virus (HIV) and tuberculosis are vulnerable due to food insecurity (192). There are several well-established studies confirming the bidirectional link between HIV and food insecurity: Food insecurity may increase susceptibility to HIV, and HIV may lessen the HIV patient's ability to make and/or aquire food (190).

Food insecurity can also contribute indirectly to shape, re-shape, or enhance vulnerabilities to EIDs/REIDs, especially in less developed countries. For example, the risk of contracting HIV/AIDS is much higher in women than men now even though men were more likely to contract the disease when the disease emerged (190, 195). The reason for this inequality is a mixture of several biological, cultural, economic, and social factors, especially in less-developed countries. A study assessing the data of 91 less-developed countries indicated that drought-induced food insecurity was directly and indirectly associated with women's vulnerability to HIV. Women generally were more likely to get a lower amount of food than men, which causes nutrient deficiencies leading to increased susceptibility to infection. Additionally, food insecurity was also associated with social vulnerabilities such as a decrease in women's access to health facilities and forced engagement in risky sexual behaviors, etc. (190).

A systematic review analyzing 111 studies even suggested an indirect relationship between drought and HIV treatment in African countries. Changes in livelihood and economic conditions due to drought-induced water and food insecurity and population movement appeared to affect HIV treatment adherence according to the review

(196). Another review's findings identified a relationship between food insecurity and initiation of antiretroviral therapy (ART) and treatment adherence (197).

9. Conclusion

The impact of wars and natural disasters on EIDs/REIDs varies depending strongly on the level of underlying fragility or vulnerability in the affected country or society. Less developed countries are significantly more vulnerable to disasters due to having a large number of vulnerable populations, limited response capacity, weak health systems, etc. (198). The inadequate health infrastructure and insufficient human and financial resources before, during, and after wars and natural disasters in less developed countries may increase the vulnerability, and EIDs/REIDs incidences such as malaria and HIV reach epidemic proportions (198). In contrast, EIDs/REIDs outbreaks can be confined within a particular area in developed countries as a result of strong health systems and their coping capacity with disasters.

However, development is not a guarantee of 'invulnerability," different levels of vulnerabilities within societies or populations even in developed countries can also be observed (198). The older adults and poor black populations were a significantly high proportion among victims of Hurricane Katrina in New Orleans in 2005 (199, 200). Similarly, the young, the older adults, and women were affected more than others during the earthquake in Italy in 2009 (200).

Conflicts also can generate additional vulnerabilities during natural disasters owing to weakening the respond capacity of countries. Prevailing vulnerabilities in conflict situations are generally aggravated by disasters and conflicts may worsen the impact of disasters. There are some studies suggesting that conflict has been higher in drought situations (201).

In a systematic review analyzing 132 studies, population displacement was the most frequently reported risk factor in infectious disease outbreaks followed by water, sanitation, and hygiene (WASH), housing, and vector/animal after disasters (202). In another review crowded conditions, forced displacement, poor quality shelter, poor water, sanitation and hygiene, lack of healthcare facilities, and lack of adequate surveillance were found to be the key risk factors cascades for communicable disease outbreaks in complex humanitarian emergencies (203). All these factors exacerbate the pre-existing vulnerability and increase the probability of post-disaster infectious disease outbreaks (202).

The extent (geographically, number of people affected, duration, etc.) of the disaster is also important in the context of the coping capacity of the country. For example, the devastating earthquake on 6 February 2023 with a 7.8 magnitude followed by 5,700 aftershocks directly affected 11 provinces living 15 million people in Türkiye and 11 million families in the Syrian Arab Republic (204, 205).

A Generic National Action Plan covering risk assessment, a mechanism for action, determination of roles and responsibilities of each sector, establishment of a coordination mechanism, etc. should be developed. Each province/region should prepare its own plan of action considering local conditions such as epidemiology of EIDs/REIDs, vector and reservoir species and their distribution, response capacity and additional needs, etc.

Even though there have been common risks and drivers for EIDs/REIDs in wars and natural disasters, they have idiosyncratic risks. For example, security is a great problem in wars while the continuity of a natural disaster itself, such as in an earthquake poses, additional risks in implementing control measures.

In order to increase response capacity, planning and investments including awareness raising additional to finance are needed for disaster preparedness and early warning. The establishment or adaptation of the current EIDs/REIDs Surveillance and Early Warning System covering the probable effects of wars and natural disasters on EIDs/REIDs is a prerequisite for recognizing disease clusters/outbreaks, and monitoring and evaluating control interventions. Being an important part of a strong surveillance system, enhanced laboratory services should be planned to include mobile even field laboratories which are capable of sustaining their work under inappropriate conditions in order to provide rapid diagnosis of diseases. Maintenance of surveillance and control activities, waste management services, and food security are of great importance during and after wars and natural disasters in order to reduce their impact on EIDs/REIDs. Given the risk of EIDs/REIDs is much higher in overcrowding and unsanitary conditions, priority should be given to the displaced population and refugees living in temporary shelters and regular screenings can be performed especially for vector-borne diseases such as malaria and leishmaniasis.

Free roaming animals, whether domestic/wild or stray/owned, should definitely be considered, and special control interventions should be implemented as most of the EIDs/REIDs have zoonotic origin. Planning of vector and rodent control is of great importance too. The risk of occurrence of natural disasters and, EIDs/REIDs following natural disasters and during conflict conditions are increasing due to climate change which contributes to creating environmental changes especially favorable for vector-borne diseases.

Therefore, the impact of wars and natural disasters on EIDs/REIDs should be taken seriously. Recommended actions in order to prevent and control EIDs/REIDs in wars and natural disasters are summarized in Table 4.

Author contributions

ST, AT-O, and EA: conceptualization, writing—review and editing, and visualization. ST and AT-O: methodology. AT-O and EA: validation. ST: resources and writing—original draft preparation. EA: supervision. All authors contributed to the manuscript development and approved the final version to be published.

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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.

TABLE 4 Recommended actions in order to prevent and control EIDs/REIDs in wars and natural disasters.

Action	Recommendations
Development of a generic national action plan	• Risk assessment for different scenarios (conflict, earthquake, flood, etc.) • Mechanism for action • Determination of roles and responsibilities of each sector • Establishment of a coordination mechanism • Standard algorithms for EIDs/REIDs prevention and control • Mobile teams for active case detection such as malaria, cutaneous leishmaniasis • Coordination with Non-Governmental Organizations and international agencies (WHO, ECDC, etc.) • Defining supply management system (laboratory equipment and kits, treatment drugs, vaccine, vector and rodent control equipment and products, etc.), etc.
Preparation of provincial/regional action plans	• Consideration of local conditions such as epidemiology of EIDs/REIDs • Specification of vector and reservoir species and their distribution • Assessment of response capacity and additional needs
Establishment or adaptation of the current EIDs/REIDs surveillance and early warning system	• Flexible surveillance system (computerized, paper-based, etc.) • Determination of data sources • Syndromic surveillance • Enhanced laboratory services • Mobile laboratories • Field laboratories • Special arrangements to specimen transport
Environmental health measures	• Supply of safe drinking water • Basic sanitation facilities
Planning of vector and rodent control	• Regularly disposal of excreta, wastewater, and solid wastes • Vector control in the affected area • Rodent control in the affected area especially around the temporary shelters
Planning of free-roaming-animal control	• Planning of new settlements far from wildlife • Shelters for domestic animals • Vaccination, anti-parasite medication, etc. services to animals hosted in shelters
Arising awareness and capacity building	• Training of health staff • Public awareness and education programs

Publisher's note

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Risk of SARS-CoV-2 transmission in the close contacts in a small rural area in the Veneto Region (NE-Italy): past evidence for future scenarios

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Background: During the first pandemic phase of COVID-19, an epidemiological study, named First survey, was conducted on the population of a small rural area in northern Italy. In spring 2020, the results showed how a prolonged lockdown slowed down the spread of the virus.

Methods: After contacting positive First Survey subjects and their families, those who decided to join voluntarily underwent a blood test to assess the presence of qualitative IgG about 2 months after the previous one. This was to determine if IgG persisted in individuals who tested positive in the First Survey as well as to assess the antibody status of their close family members, to determine if they were unintentionally infected.

Results: Based on serological analysis, 35.1% of the samples contained blood IgG. In subjects who tested positive during the First Survey, 62.5% remained IgG positive more than 2 months later. Among family members who were exposed to a positive relative, 23.7% were infected. Linear regression analysis showed that the presence of an infected person within a household resulted in the infection spreading to the others, but not excessively. Induced isolation extinguished the infection regardless of the extent of the contagion (intra-family or extra-family). Micro-outbreaks of SARS-Cov-2 infection which arose in the same household from extra-familial infections played a decisive role on the statistical significance of IgG-positive subjects ($p < 0.001$).

Discussion: The study reveal 52.6% of the IgG-positive subjects in the Second Survey came from the First Survey and 47.4% were family members previously in contact with positive subjects. Data suggest that there have been undiagnosed patients feeding the spread of the virus since the beginning of the pandemic. In conclusion, for future pandemics, it will be necessary: i) to ensure the rapid isolation of symptomatic patients and the early identification of their close contacts, ii) to carry out the maximum number of tests in the shortest possible time, both on symptomatic and asymptomatic subjects, and iii) to implement

information campaigns to make people aware of their risks, and implement clear, non-conflicting communication.

KEYWORDS

SARS-CoV-2, COVID-19, immunity, risk for transmission, social contacts, non-pharmaceutical interventions

1. Introduction

1.1. Clinical, epidemiological and immunological features of SARS-CoV-2

Coronavirus-2 (SARS-CoV-2), the virus responsible for Coronavirus disease 2019 (COVID-19), emerged and spread worldwide and caused a health crisis that had never been seen before, as neither vaccines nor effective pharmaceutical treatments were available at the time (1). On March 11, 2020, the World Health Organization (WHO) declared the disease a pandemic after it was first described in China in Wuhan in December 2019 (2–4). A clear mechanism of infection transmission was discovered early on: aerosols, or microscopic respiratory particles suspended in the air, or droplets, larger respiratory particles falling within 2 meters of the source (5); this can also happen with asymptomatic people or before symptoms appear. Contact with fomites, inanimate objects, or surfaces infected with the virus, is another method of transmission (6–8). Due to these factors, contagion could occur both near and far, with the risk of transmission in the near field for a person close to an infected person far greater than the risk of transmission from afar (9), as in the case of cohabitants. SARS-CoV-2 was originally transmitted from animals to humans, which characterized it as a zoonosis. Since then, human-to-human transmission events have occurred (8), resulting in symptoms ranging from mild fevers, coughing, dyspnea, cytokine storms, respiratory failure, and death (10–15). An individual's immune system and the SARS-CoV-2 virus closely interact to create COVID-19 disease. A crucial role for the immune system is thought to play in determining the severity of COVID-19. Cells of the lower respiratory system are infected by the SARS-CoV-2 virus, causing a rapid immune response so powerful which damages them (16–20). Using immunoassays, you can determine both active viral infections and past exposures. To date, several companies and research institutions have developed serological tests for detecting antibodies against SARS-CoV-2 in serum or plasma samples. Coronavirus serological tests primarily target spike protein (S), the most commonly exposed protein, and nucleocapsid protein (N), an abundantly expressed protein during infections. Serologic data can complement the results of RT-qPCR and contribute to seroepidemiology characterization (21). Induction of neutralizing antibodies has been the focus of most correlative studies on immune protection against SARS-CoV-2 (22–25). Despite this, in patients with less severe cases of COVID-19, antibody responses are not always detectable (26–28). Additionally, SARS infections tend to induce short-lived B-cell memory responses (29, 30). As a contrast, T-cell memories can persist for several decades (30–32).

1.2. Public health response: the Italian experience

Global health systems have suffered immeasurable pressures and disastrous consequences due to rapid pandemic spread and unprepared public policies to counteract it (33, 34).

During the early stages of the pandemic, Italy was one of the most affected countries after China. On February 21, 2020, the Italian National Health Service reported two hot-spots of COVID-19 cases in northern Italy: Vo' Euganeo (Padua), Veneto region, and Codogno (Lodi), Lombardy region (35–38). Viral spread in the two regions was controlled using different strategies. Lombardy only investigated symptomatic cases, while Veneto tested both symptomatic and asymptomatic individuals. A different strategy resulted in a different impact of infection: in Lombardy, COVID-19 cases increased rapidly, and many patients developed severe forms (38).

Two red zones were set up by the Italian government on February 24 to contain the outbreak, with quarantined areas, severe mobility restrictions and temporary closings of schools and stores (36, 39, 40). Supermarkets and pharmacies with a distance of at least 1 meter between customers were the only businesses that could remain open (37). The epidemic spread rapidly throughout the country but mostly in northern Italy in the weeks that followed. On March 8, 2020, the above extraordinary measures were extended to the entire Lombardy region and neighboring provinces. After almost 100 percent of the total deaths from Covid-19 increased in the 48 h leading up to the decree on 11 March, Italy was put under lockdown. This makes Italy the second most infected country in the world after China (36, 37, 41). Since 31 March, the number of newly reported Covid-19 cases in Italy has stabilized after steadily increasing for almost 4 weeks (36). After expiration on 3 April, a restrictions decree was extended until 18 May (37, 41). Italian authorities initiated Phase 2 following 69 days of lockdown, known as "Coexistence with the virus" (42). Infections, which exceeded 6,000 at the end of March, began to decline at the end of May with daily increases of fewer than 500 (43, 44). As part of the previous epidemiological study, it was observed how much the virus circulated in a small rural Italian community during the spring of 2020, and how the infection developed after a prolonged lockdown, when the situation improved, and limitations had been reduced (45). Only 0.2% of the population tested positive for NAAT by nasopharyngeal swab during the first Phase of the epidemic, according with the studies carried out during the same phase in Vò Euganeo, where a reduction in infections from 2.6 to 0.3% was found following the application of restrictions (38). A random sampling of the general population was used to test for anti-SARS-CoV-2 immunoglobulin levels during phase 2, which showed 97.9% of respondents were negative, while 2.1% had mildly

symptomatic or asymptomatic infection resulting from distantly positive IgG (45).

1.3. Aim of the study

Two months later, almost every subject (16 out of 19) found to be IgG positive against SARS-CoV-2 in the previous study by Bassanello and colleagues (referred to as the First Survey) (45) was retested by the same method, as were their family members (referred to as the Second Survey).

This study seeks to evaluate the risks of infection in the close contacts based on the intervening familial relationship and to retrospectively analyze the group of people who surrounded positive subjects early in the epidemic. In addition, the seroprevalence of subjects who were found positive in the first study 2–3 months after asymptomatic or pauci-symptomatic infection was observed. Several studies have now shown that anti-SARS-CoV-2 antibodies persist in nonvaccinated subjects for months after infection (46–49). Ex-post considerations will also be made about the effectiveness of restrictions at the beginning of the pandemic when vaccines were not yet available and there were no proven standardized drug therapies.

2. Patients and methods

The study was conducted between May 2020, and August 2020, in the Municipality of Monastier di Treviso in the Veneto Region (Northeast Italy). Data were collected in collaboration with Giovanni XXIII Hospital, a private healthcare Center that is part of the National Health Service.

2.1. Study design

2.1.1. First Survey

During the first pandemic phase of COVID-19, characterized by the instant lockdown and a subsequent gradual release of personal and social constraints, a study on a complete voluntary basis was conducted about a population of 922 adult subjects (a quarter of the city's population of about 4,400 people) and representative of one subject for each family (about 1750 families). Recruitment for this investigation began on May 25th, 2020. For the sake of simplicity, we define this investigation as *First Survey*. In addition to venous blood sampling, a complete analysis of the patient was performed, examining symptomatology, exposure to infected individuals, and comorbidities. In 19 subjects (2.1% of the study cohort), IgG antibodies were positive and IgM antibodies tested negative. Serological positivity was significantly correlated with only fever (especially when accompanied by a decrease in taste and smell), quarantined subjects, and some COVID-19 contact subjects (45).

2.1.2. Second Survey

In the summer of 2020, about 60 to 75 days after the First Survey, a subsample of patients with serum IgG positivity was evaluated along with their family members. To simplify, we refer to this new subsequent investigation as the *Second Survey*. Overall, 54 subjects were recruited, including 16 out of 19 positive subjects in the First Survey and 38 from their family networks (Figure 1). In the First Survey, the latter subjects were not included. The aim of the Second Survey is to investigate the living environment of the person who tested positive during the First Survey: the risk of transmission of the infection to family members, based also on the intervening kinship relationship, and the persistence of seroprevalence to anti-SARS-CoV-2 antibodies.

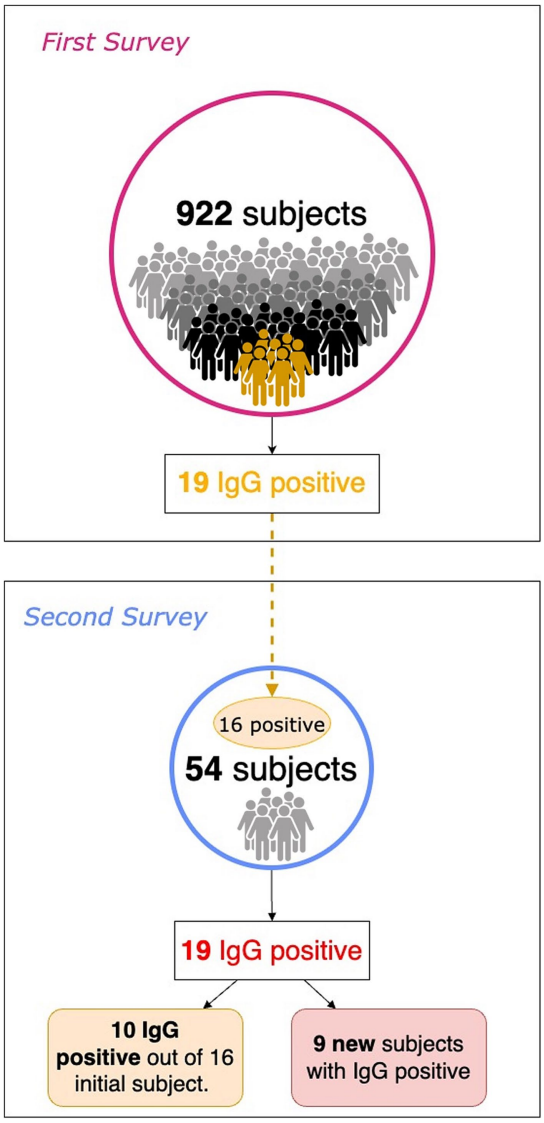

FIGURE 1
Population comparison between First and Second surveys.

2.2. Study protocol and data collection

Peripheral venous blood samples (5 mL) were collected in Serum Separator Tubes (BD Diagnostic Systems, Franklin Lakes, NJ, USA) and centrifuged at room temperature at 1600 rpm for 10 min. Specific qualitative determination of Anti-Nucleocapsid (N) and Anti-Spike (S) IgG and IgM antibodies directed against the SARS-CoV-2 plasma levels were measured by immunochromatographic method and a two-phase immuno-enzyme sandwich method with final fluorescence detection (ELFA).

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At the time of sampling, a complete medical history was obtained. Four closed-ended questions were asked by the health professional to the patient in the questionnaire:

1. *Presence of symptoms even in the previous weeks/months* (none, fever, coughing, general malaise, diarrhea, flu symptoms, sore throat, nasal discharge, altered taste or smell);
2. *Relationship in family context* (single, son/daughter, parent, grandfather/grandmother, husband, wife);
3. *Previous quarantine or buffer due to risk factors*;
4. *Contact with infected subjects through social and / or work activities*.

Symptomatology was classified according to priority (none, main symptom and up to 3 secondary symptoms).

Symptom numerosity (none, one, two, three, or four symptoms simultaneously) of each study participant was also collected. Scientific literature that existed during data collection guided the choice of grouping symptoms by number and type. The aim was to assess whether a diagnostic model and/or a main symptomatology could be hypothesized. In the current state of knowledge, this model does not exist. Five groups of symptoms were identified: *absence of symptoms (0)*, *primary symptom (1)*, *1° Secondary symptom (2)*, *2° Secondary symptom (3)* and *3° Secondary symptom (4)*.

Subjects who declared no symptoms were included in the group (0). Symptoms of fever, illness, diarrhea, flu symptoms, sore throat, nasal discharge, alteration of taste or smell, cough, headache, skin rash, dyspnoea, or not detected, could be included in each of groups (1) to (4) in order of importance in the presentation of each symptom.

The study was conducted in accordance with relevant guidelines and regulations, respecting the Privacy of patients as approved by the Ethics Committee of the Giovanni XXIII Hospital (study protocol # 12/2020 of 10 April, 2020). Informed written consent was obtained from all the participants included in the study. For minors, parental consent has been obtained.

2.3. Contact patterns and data analysis

For the statistical analysis, age, gender, family relationships, symptoms (additional or single), previous quarantine, contact with infected individuals, and molecular COVID-19 swab outcome were taken into account (Table 1). The same methods previously described were used to test all subjects for anti-SARS-CoV-2 antibodies. Regarding the 16 subjects already positive in the First Survey, the persistence of antibodies over time was considered. In the 38 subjects without previous analysis, the absence of previous exposure to the virus was assessed. Statistical analysis was performed using the Student-*t* test for paired data. Simple regression was used for correlation analysis. Groups were compared using the unpaired Student *t*-test (Tables 2, 3). Statistical significance was set at $p \leq 0.05$.

3. Results

Overall, 54 subjects participated in the study, with a mean age of 40.5 ± 19.2 years, 25 males and 29 females among them. Medical history information, shown in Table 1, was collected during the study

TABLE 1 Characteristics of the study group.

	All patients (n = 54)
Age (yr, mean ± SD)	40.5 ± 19.2
Gender (male/female)	25/29
Relationship	
Single	1 (1.9%)
Son/Daughter	25 (46.3%)
Parent	7 (13.0%)
Grandfather/Grandmother	1 (1.9%)
Husband	9 (16.7%)
Wife	11 (20.4%)
Absence of symptoms (0)	21 (38.9%)
None	21 (38.9%)
Primary symptom (1)	11 (20.4%)
Fever	23 (42.6%)
Illness	0
Diarrhea	1 (1.9%)
Flu symptoms	4 (7.4%)
Sore throat	0
Nasal discharge	0
Alteration of taste or smell	3 (5.6%)
Cough	0
Headache	1 (1.9%)
Skin rash	1 (1.9%)
Not detected	0
1° Secondary symptom (2)	11 (20.4)
Diarrhea	1 (1.9%)
Flu symptoms	2 (3.7%)
Alteration of taste or smell	4 (7.4%)
Cough	3 (5.6%)
Headache	11 (20.4%)
Dyspnea	1 (1.9%)
2° Secondary symptom (3)	10 (18.5%)
Illness	1 (1.9%)
Nasal discharge	2 (3.7%)
Alteration of taste or smell	8 (14.8%)
3° Secondary symptom (4)	1 (1.9%)
Alteration of taste or smell	1 (1.9%)
Quarantine	
Yes	8 (14.8%)
No	46 (85.2%)
Contact with infected subjects	52 (96.3%)
Previous IgG positive in First Survey	16 (29.6%)
Total IgG positive in Second Survey	19 (35.2%)
Covid-19 NAAT	
Not performed	47 (87.0%)
Performed	7 (13.0%)

TABLE 2 Characteristics of the subjects grouped by IgG positivity and IgG negativity, and *p*-values of statistical comparison between groups (bold values are significant).

	IgG negativity	IgG positivity	*p*
Age (yr, mean ± SE)	37.4 + 19.5	46.3 + 17.7	0.1
Gender (M/F)	**21/14**	**4/15**	**<0.005**
Relationship			0.1
Single	0	1	
Son/Daughter	19	6	
Parent	2	5	
Grandfather/Grandmother	1	0	
Husband	6	3	
Wife	7	4	
Absence of symptoms (0) and Primary symptom (1)			0.2
None	17	4	
Fever	12	11	
Illness	0	1	
Diarrhea	3	1	
Flu symptoms	2	1	
Sore throat	0	1	
Nasal Discharge	1	0	
1° Secondary symptom (2)			0.6
Diarrhea	0	1	
Flu Symptoms	1	1	
Alteration of taste or smell	1	3	
Cough	2	1	
Headache	6	5	
Dyspnea	1	0	
2° Secondary symptom (3)			0.2
Illness	0	1	
Nasal Discharge	2	0	
Alteration of taste or smell	6	2	
3° Secondary symptom (4)			n.d.
Alteration of taste or smell		1	
Symptoms by numerosity			**0.017**
0	**17**	**4**	
1	**7**	**4**	
2	**3**	**8**	
3	**8**	**2**	
4	**0**	**1**	
Quarantine			**<0.001**
No	**35**	**11**	
Yes	**0**	**8**	
Contact with infected subjects			0.3
No	1	0	
Yes	34	18	
Not detected	0	1	
Contact settings			
Family infection	**30**	**8**	**<0.001**
Extra-Family infection	**5**	**11**	**<0.002**

TABLE 3 Characteristics of the subjects grouped by IgG positivity and IgG negativity and *p*-values (bold values are significant) in Second Survey.

	IgG negativity	IgG positivity	*p*
Previous IgG-positive subjects in First Survey	**6**	**10**	**0.006**
Familial in contact with previously positive subjects in First Survey	**29**	**9**	
Covid-19 NAAT			**0.007**
Not performed	**34**	**13**	
Positive NAAT	0	4	
Negative NAAT	1	2	

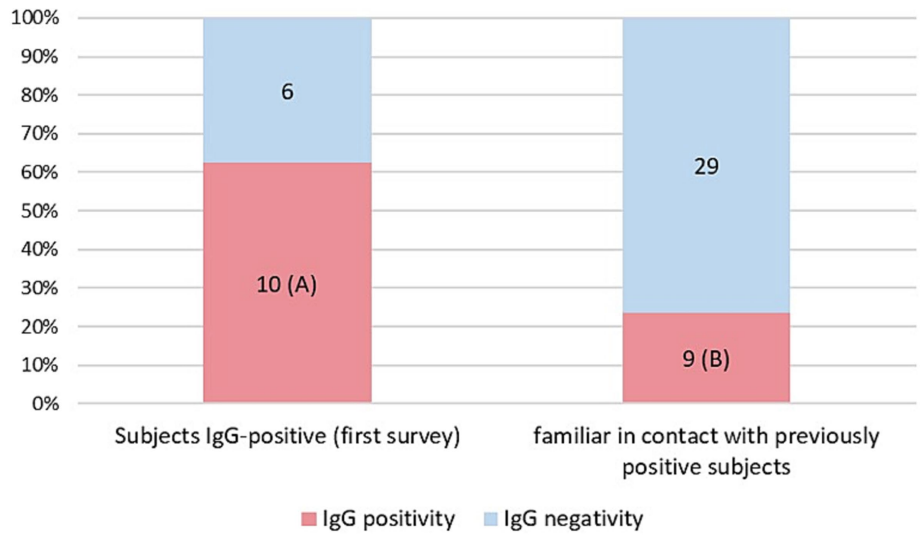

FIGURE 2
Percentage of IgG positive patients in the two different groups: IgG positive subjects in the first Survey still positive (A) and IgG positive subjects in family members previously in contact with positive subjects (B).

after patient consent was obtained. This data included: relationship within the family context (single, son/daughter, parent, grandparent/grandmother, uncle/aunt, husband/wife), the number and presence of symptoms, which occurred in the past few months (none, fever, illness, diarrhea, flu symptoms, sore throat, nasal discharge, altered taste or smell, cough, dyspnea), a previous period of quarantine resulting from positivity or cohabitation with positive individuals, and any previous contact with infected individuals.

Each participant's information was collected. No data missing is present. A descriptive analysis of the sample under study revealed that 46.3% of the subjects were children.

Considering the division of symptoms according to priority, 21 subjects (31.8%) had never experienced symptoms, while 33 had more than one symptom (whose 22 had two or more symptoms). In terms of primary symptom and first secondary symptom, out of the total sample, the most frequent were fever (42.6 percent) and headache (20.4 percent), respectively. The other symptoms occurred less (generally <5–8% each). A total of 52 subjects reported close contact with an infected person, but only 8 of them received a contumacies procedure, i.e., quarantine (14.8%). Only 7 subjects in the study population had a COVID-19 NAAT molecular swab. An overview of study group characteristics and outcomes can be found in Table 1.

As a result of processing the venous blood samples of the 54 subjects, 19 samples (35.1%) contained blood IgG (Table 3). Among the 16 subjects who were positive at the First Survey, 10 remained positive, i.e., 62.5% of the subjects had persistence of IgG positivity more than two months later. Of the 38 family members in contact with a positive relative had 9 newly infected relatives in the family context, i.e., only 23.7% became infected following exposure (Figure 2). Analyzing the total number of IgG-positive subjects in the Second Survey, it is observed that 52.6% were positive subjects from the First Survey and 47.4% from family members previously in contact with positive subjects (Figure 3).

In linear regression analysis (Table 2), it was found that stratification by symptom priority, age, and kinship was not statistically significant in predicting IgG positivity. Conversely, the association between IgG positivity and the number of symptoms was statistically significant. Similarly, IgG positivity is also related to gender, quarantine performance, close contact settings (i.e., family or extra-family exposure) and NAAT Covid-19 results.

4. Discussion

During Phase 2 of the first pandemic wave of SARS-CoV-2, which struck Italy in the spring of 2020 and affected 2.1% of the population, a cohort study was conducted in a small rural municipality in the Veneto region (north-eastern Italy) (45). Following contact with

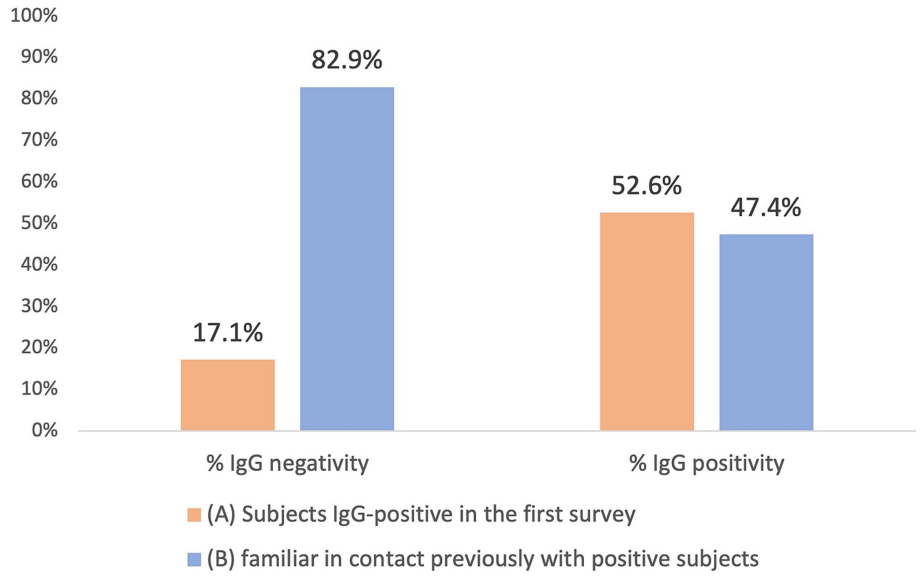

FIGURE 3
Comparison between IgG positive and IgG negative in the two different groups: IgG positive subjects in the first Survey group (A) and family members previously in contact with positive subject (B).

positive subjects from the First Survey and their families, those who voluntarily participate underwent a blood test to assess the presence of qualitative IgG approximately two months after the initial one. We conducted a study to determine if IgG persisted in individuals who tested positive in the First Survey as well as to assess the antibody status of their close family members, to determine if they were unintentionally infected, developing an undiagnosed asymptomatic or pauci-symptomatic condition.

4.1. Gender differences in infection risk and contagiousness

The analysis revealed a statistically significant difference between males and females, suggesting a higher infection risk in females. Initially, there were conflicting hypotheses in the scientific literature regarding gender-based contagiousness during the early stages of the pandemic. Some studies leaned towards male prevalence (50–53), while others toward female prevalence (54–56). However, current systematic reviews and larger population samples have shown that such hypotheses do not hold (57, 58).

4.2. Symptomatic patterns and antibody status

The study explored the correlation between IgG positivity and the number of symptoms exhibited by the subjects, revealing a statistically significant association. Fever, a common symptom, was often linked to secondary symptoms, such as headache and/or loss of taste or smell (51, 59, 60). These features were specific to the time under investigation, predating the emergence of less symptomatic viral variants and the development of vaccines that have mitigated symptomatology (60–62). This study also confirmed that the presence of multiple symptoms in the early stages of the pandemic was an important factor in suspecting the presence of the disease, as COVID-19 was the most common, symptomatic, and heterogeneous disease at the time of lockdown (63–66). However, stratification according to symptomatological priority was not significant, refuting both the hypothesis of predictive diagnostic patterns and the recognition of a primary symptomatology (67, 68). Although initially supported by the scientific literature, these hypotheses have now been disproved (59–62). Due to the heterogeneity of symptoms caused by COVID-19 and the emergence of variants constantly changing its characteristics, it was impossible to create a standardized symptom pattern (60–62, 69, 70).

4.3. Impact of quarantine and close contact settings

The significant correlation between IgG positivity and quarantine can be attributed to the meticulous and precise *contact tracing* efforts of health workers, especially during the early stages of pandemic. They accurately identified a high risk of infection by carefully examining the detailed descriptions provided by the respondents. Therefore, it was possible to speculated specific thresholds of effectiveness for contact tracing in decision-making (71–73).

When considering close contact settings, it is evident that micro-outbreaks of SARS-CoV-2 infection arising in the same household from extra-familial infections (i.e., no cohabiting relatives, friends, or co-workers) played a decisive role in the statistical significance of IgG-positive subjects ($p < 0.001$). This was most likely due to the mode of virus transmission and less attention paid during phase 2 of the pandemic when infection was believed to occur more easily within intra-family households due to the greater intimacy (74–77). Hence, it is possible that people took greater care within their own households, partly out of concerns over infecting a relative, than with extra-familial

contacts considered less at risk (76, 77). According to the current literature, infection risks are high even at several meters and/or when wearing unsuitable personal protective equipment (PPE), like fabric or surgical masks (78). Thus, the presence of an infected person in a household resulted in an inevitable spread of infection among household members, but not with an excessive rate of diffusion (75, 77). Regardless of the mode of infection (intra-family or extra-family), induced isolation still extinguished the infection (79, 80). It was already noted in the previous study and other investigations that quarantine of close contacts resulted in an increased risk of infection (compared to those who had not declared any contact), but at the same time allowed, along with lockdown, he extinguishment and prevention of further spreading of the family outbreak (77, 79, 81). Based on this current analysis, only 23.7% of family members became positive as a result of close contact with family members, which is similar to some studies (76), but nearly 10 percentage points higher than others (56).

4.4. Family transmission and persistence of seropositivity

Although contagion occurs within families, there is a greater likelihood of positive outcomes occurring between parents and children, presumably due to the difficulty of isolating children (75, 76, 82). In contrast, grandparents' negativity indicates that their presence led to greater attention to providing protection to older adult subjects by reason of their frailty (83). SARS-CoV-2 rates of familial secondary infections have also changed significantly as a consequence of vaccination and variants, making it impossible to generate a predictive model based on them (75). The persistence of seropositivity in positive subjects from the First Survey is noteworthy, with 10 subjects (62.5%) remaining positive in the Second Survey. This suggests the presence of anti-SARS-CoV-2 antibodies for at least 2 months after infection (46, 47). Several studies have demonstrated that patients with mild COVID-19 maintain hight antibody titers during the initial 1–2 months (84), but these levels decrease significantly thereafter, affecting 44% of cases (85, 86).

4.5. Factors affecting IgG positivity in COVID-19: impact of contact setting and quarantine

Linear regression (Table 2) shows that stratification by symptom priority, age, and kinship is not statistically significant in correlation with IgG positivity. Only two variables show significant associations: contact setting, with extra-familial contacts having a greater likelihood of being positive and having quarantined (87).

In this study, carrying out a quarantine greatly increases the likelihood of being positive. The reason for this is that living with a positive person significantly increases the chances of getting infect, particularly in small living environments and depending on the level of rigor of the quarantine (79).

Despite being statistically significant, the number of symptoms now has a much different clinical and scientific value compared to previous studies. It is important to consider the absence of symptoms, as the lack of symptoms often suggests negative IgG results (88). Although, some asymptomatic positive cases are also present, which, according to many studies, contributed to the pandemic's early outbreak and resulted in lockdown (70, 89). As the number of symptoms increases, the likelihood of having a positive IgG appears to increase linearly. With the advent of variants and mass vaccination, this linearity has been significantly altered. Currently, having more symptoms has no significant impact on predicting the likelihood of the general population being positive for COVID-19. The number and type of symptoms are not sufficient elements for determining whether COVID-19 is present.

4.6. Importance of testing and information campaigns

Data support the speculation that since beginning of pandemic, there has been an undiagnosed group of patients who contributed to the spread of the virus, as also revealed by some mathematical models (73, 81). Accordingly, nearly 60% of the infected individuals were undetected during the same period under analysis (81, 90). In this study, these individuals are mainly represented by family members of those who tested positive, who were typically asymptomatic or had mild symptoms, and therefore did not undergo a swab test. In fact, within this group, only a few people opted for COVID-19 swabs, and almost all of them were family members of the positive subjects in quarantine. Recent studies have evaluated periodic testing as an alternative to quarantine to mitigate the risk of COVID-19 transmission (91). As shown by Romagnani et al., the difference in the evolution of the epidemic in the early stages between the Veneto and Lombardy regions is strongly influenced by the number of subjects tested. Testing both symptomatic and asymptomatic individuals during epidemic peaks has been proven highly effective in curbing the spread of the virus (38). In the healthcare setting, a sequential approach with PCR testing involves adopting an organized and systematic method for conducting tests on both staff and patients. This approach entails administering the tests in a specific order or sequence, ensuring comprehensive and efficient screening. Thanks to this precise methodology, the Giovanni XXIII Hospital in Monastier di Treviso has been able to effectively manage and prevent the spread of Covid-19, maintaining its status as a Covid-free facility (92). Consequently, based on this evidence, several essential measures must be included in countering future pandemics:

- Promptly isolate and test symptomatic patients immediately upon the onset of symptoms using molecular testing.
- Conduct detailed and accurate *contact tracing* to identify close contacts, both within the family context and in extra-familial settings, as these individuals could unknowingly contribute to the virus spread, particularly if asymptomatic.
- Perform molecular testing regularly for close contacts and adjust the test frequency based on the epidemiological trend of the pandemic, which may be influenced by the development of virus variants. Utilize forecasting models to aid in determining appropriate testing intervals (93). Additionally, include serological testing to gain a comprehensive understanding of the actual extent of virus circulation in the target population. By combining molecular and serological testing, a more

comprehensive and informed approach to managing the pandemic can be achieved.
- Implement timely information campaigns to raise awareness about the risks associated with the spread of infection disease, ensuring clear and consistent messages. It is crucial to include extra-family contacts in these awareness campaigns, given their role in the spread of the virus, as observed in this study.

Consequently, it is crucial that communication is institutionalized, grounded in scientific data, and initiated from the initial stages of an emergency to ensure its effectiveness. Swift and widespread communication aids in disseminating prevention measures, testing and treatment guidelines, and updates on the evolving situation. Additionally, it serves to combat infodemic and misinformation that can cause confusion and panic among the public. Governments, health authorities, and media outlets play a pivotal role in rapidly and extensively communicating information during emergency situations, thereby keeping the public well-informed and engaged in the response efforts.

5. Limitations

The findings pertain to a specific context, namely that of a geographical area in a rural municipality. Some studies have demonstrated that effective strategies for addressing COVID-19 can vary depending on the geographic level of evaluation, such as city, county, or neighborhood (93). Each of these geographic units may have unique characteristics, as population densities, healthcare infrastructure, and socio-economic factors and population behaviors that influence the spread and impact of the virus presenting different challenges and resources in responding to the pandemic. Changing the context, or the geographic level of analysis, can indeed alter the endings. Different geographical units may have different infection rates, vaccination rates, and health outcomes. As a result, strategies and interventions that prove effective at one level might not necessarily be suitable or successful at another level.

In addition to the intrinsic geographic diversities of the analyzed area, the limitations of this study arise from the small sample size analyzed and the data collection taking place during the early and intricate stages of the SARS-CoV-2 pandemic. Nevertheless, early sampling also provides strength as it allows critical examination of the onset of the pandemic, shedding light on the challenges and complexities of managing an unforeseen pandemic. As a result, some actions taken have been one-sided, overlooking the broader context, and underestimating crucial factors, such as the significant role of extra-family contacts in the spread of the virus. On the other hand, efficient management of close contacts has proved to be essential to contain emerging outbreaks within households. Thus, this preliminary study aims to highlight the risk of disease transmission among close relatives and emphasize the possibility that undiagnosed cases could contribute to the spread of future pandemics.

6. Conclusion

This study was conducted in 2020, during a time when the pandemic was in its early stages and significantly different from the current phase. Vaccine development and emergence of SARS-CoV-2 variants have substantially altered the trajectory initially predicted. However, several aspects of SARS-CoV-2 infection remain relevant and may serve as warnings for future decisions in managing other air-spread infections, like the one causing COVID-19.

The study reveals that at the beginning of the pandemic, when the NAAT swab was available especially for symptomatic cases, after 2 months almost half of the IgG positive subjects (47.4%) were family members who had been in contact with a positive relative. Therefore, serological analysis of anti-N and anti-S antibodies becomes necessary to assess the actual impact of virus shedding in the general population. Monitoring the serological status of patients at an early stage of the pandemic is of paramount importance because it demonstrates that anti-N antibody-positive individuals remained so even after 2 months from the initial infection. It is equally essential to stress that while the NAAT swab is considered the gold standard for diagnosis, it alone cannot provide a comprehensive understanding of the epidemiological extent of the pandemic. The First Survey suggests implementing measures such as quarantine and, if necessary, lockdown in limited epidemic situations to effectively contain the pandemic. However, it is crucial not to underestimate the importance of close contacts which, if undetected, could fuel a pandemic, and evade public health control. This evidence must be considered to set possible alerts for future infectious diseases outbreaks. To conclude, we must recognize the pivotal role played by contacts in spreading pandemics and contemplate the most effective ways to contain outbreaks based on the specific stage of the epidemic when action is taken. Taking a forward-looking and inclusive approach is imperative, without underestimating anything that could contribute to the ongoing epidemic, whatever its nature.

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 the Giovanni XXIII Hospital (study protocol # 12/2020 of 10 April, 2020). 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

MB: conceptualization, investigation, and writing – original draft preparation. RG: original draft and writing – review & editing. EB: conceptualization and writing – review & editing. UC, MD'A, and AG: supervision and writing – review & editing. AF: data curation, writing – review & editing. AB and TB: conceptualization, supervision, writing, and validation – review & editing. All authors contributed to the article and approved the submitted version.

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.2023.1223109/full#supplementary-material

SUPPLEMENTARY DATA SHEET 1
The supplementary document describes the complete statistical analysis, including the source codes of the statistics.

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Characteristics of clinical trials of influenza and respiratory syncytial virus registered in ClinicalTrials.gov between 2014 and 2021

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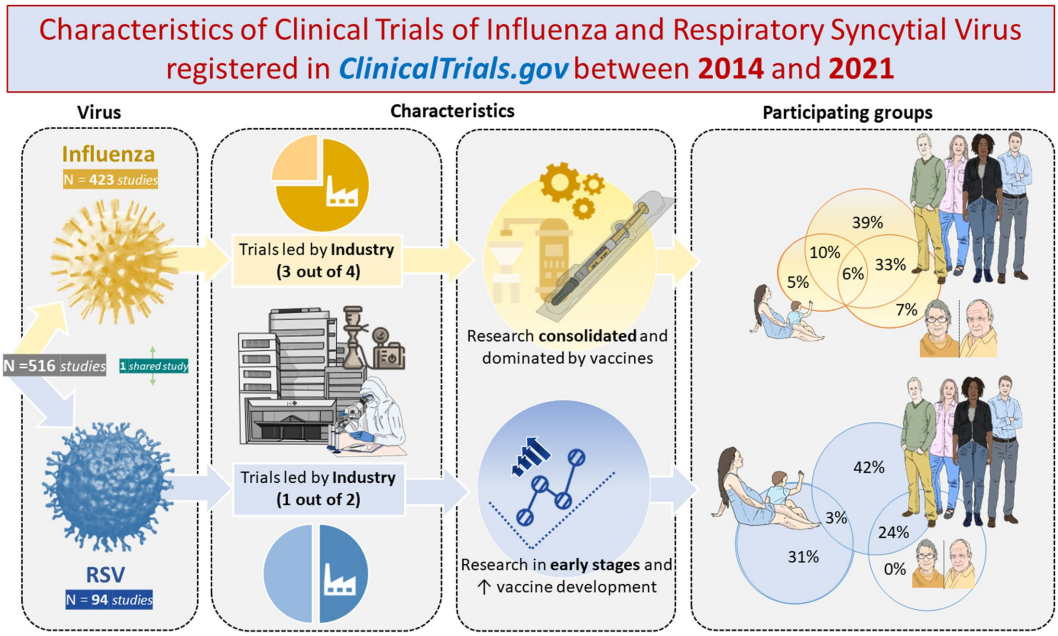
The randomized clinical trial (RCT) is the ideal and mandatory type of study to verify the effect and safety of a drug. Our aim is to examine the fundamental characteristics of interventional clinical trials on influenza and respiratory syncytial virus (RSV). This is a cross-sectional study of RCTs on influenza and RSV in humans between 2014 and 2021 registered in ClinicalTrials.gov. A total of 516 studies were identified: 94 for RSV, 423 for influenza, and 1 for both viruses. There were 51 RCTs of RSV vaccines (54.3%) and 344 (81.3%) for influenza virus vaccines (p <0.001). Twelve (12.8%) RCTs for RSV were conducted only with women, and 6 were conducted only with pregnant women; for RCTs for influenza, 4 (0.9%) and 3, respectively. For RSV, 29 (31%) of the RCTs were exclusive to people under 5years of age, and 21 (5%) for influenza virus (p<0.001). For RSV, there are no RCTs exclusively for people older than or equal to 65years and no phase 4 trials. RCTs on influenza virus and RSV has focused on vaccines. For the influenza virus, research has been consolidated, and for RSV, research is still in the development phase and directed at children and pregnant women.

KEYWORDS

influenza virus, respiratory syncytial virus (RSV), infectious diseases, clinical trials registry, randomized clinical trial (RCT)



GRAPHICAL ABSTRACT

Introduction

Respiratory syncytial virus (RSV) and influenza viruses are important causes of morbidity and mortality globally (1, 2). Both respiratory viral infections can result in severe disease and death in older adult individuals, children, pregnant women and people with underlying chronic conditions, even more so in low- and middle-income countries (1–4). There is an urgent need for better tools to prevent, detect, control and treat influenza and RSV, including more effective vaccines and antiviral drugs for influenza (4–6) and obtaining vaccines licensed for RSV (7–9).

Clinical trials with drugs are the ideal and mandatory type of study to verify the effect and safety of a drug. The registration of the clinical trial protocol is mandatory for the promoters, who must record the key elements and report the results of the clinical trial and adverse events (10) regardless of the direction or strength of the results (11). The transparency of information and public access to the results of clinical trials are essential for the protection and promotion of public health. In this way, scientific knowledge and its dissemination among health professionals and citizens are promoted, progress in clinical research is promoted, and value is given to the participation of patients in clinical trials for the benefit of all.

Different countries and organizations have specific regulations and their own registries for clinical trials. ClinicalTrials.gov is a Web-based resource that provides patients, their family members, health care professionals, researchers, and the public with easy access to information on publicly and privately supported clinical studies on a wide range of diseases and conditions conducted in the United States (12). There are different works that analyze the clinical trials registered in ClinicalTrials.gov in a general way (10), as well as for infectious diseases (9, 13) and the older adult population (14) or for SARS-CoV-2 (15–17). There is no specific one for the flu.

The present work aims to evaluate the clinical trials registered in ClinicalTrials.gov on influenza and RSV, the two most prevalent respiratory viral diseases before the emergence of COVID-19, between 2014 and 2021.

Materials and methods

Cross-sectional study of clinical trials on influenza or RSV in humans registered in ClinicalTrial.gov between 2014 and 2021. The data were obtained as of February 2023 through "the Database for Aggregate Analysis of ClinicalTrials.gov (AACT) (12, 18), as a cloud-hosted PostgreSQL database" using R's RPostgreSQL library (19). ClinicalTrials.gov studies that met the following conditions were included: (1) In the data table of "studies," those which were registered as "interventional" under the variable "study_type" and had the phase of the clinical trial defined under the variable "phase"; (2) Those that have a start date between 2014 and 2021, inclusive, under the "start_date" variable of the "studies" table; (3) Those that have the term Medical Subject Heading (MeSH) equal to "Influenza, Human" or "Respiratory Syncytial Virus Infections" under "browse_conditions" in the data table. Finally, the clinical trials obtained were manually reviewed, eliminating those that did not satisfy the previously established conditions or that were studies on vaccination policies.

The information extracted from the clinical trials was established under general characteristics and design, and methodological attributes. The following general characteristics were established: primary research proposal (treatment, prevention, diagnostic, and other), study registration established as the beginning of recruitment before or after registration in ClinicalTrials.gov, source of funding (Industry, National Institutes of Health [NIH] and Other), review by a DMC (Data Monitoring Committee), regions involved in the study

(Africa, Asia and Pacific, Central and South America, Europe, Middle East, North America and Missing), study status, population included in relation to gender and age, and type of molecule evaluated in clinical trials (antiviral drugs, antibodies, vaccines and others). In the category other than the type of molecule, treatments such as "traditional Chinese medicine," probiotics, antifungals, antiprotozoals, antibiotics and the like were included. The characteristics of the design and methodology collected were: documentation of the protocol and the statistical analysis plan (SAP), phase of clinical trial, allocation, interventional group, number of arms, number of recruited patients, and blinding. When possible, values of missing characteristics were inferred based on other available data. For example, for studies reporting an interventional model of a single group and number of groups as 1, the value of allocation and blinding was designated as nonrandomized and open, respectively (10).

All categorical variables were reported with absolute and relative frequencies. Data were stratified by influenza virus and RSV. Comparisons between groups were performed using the two-tailed chi-square test with an alpha error equal to 0.05. The graphic information was represented through Venn diagrams and ternary diagrams. The ternary diagram is a triangular graph that visualizes in a two-dimensional way the relationships between phase (represented by dots in the diagram) and the percentage of intervention/treatment (represented on each of the three axes). This graph was also used to represent the relationship between the years and the intervention/treatment. In this representation, the category other than the variable type of intervention molecule was eliminated. The Venn diagram presents the age groups to which clinical trials are directed. The age groups were established based on the scientific literature (3, 20): (1) less than 5 years old, (2) greater than and equal to 5 years old and less than 18 years old, (3) greater than or equal to 18 years old and less than 65 years old, and (4) greater than or equal to 65 years old. R was used for all statistical and graphical analyses (19) using the R libraries (packages) ggVennDiagram and Ternary.

Results

A total of 516 clinical trials that met the established criteria were selected from the total of 441,919 records included in ClinicalTrials.gov. A total of 423 clinical trials were of influenza virus, and 94 were of RSV. A study is shared in both groups of viruses.

Table 1 shows the characteristics of the clinical trials. The most frequent primary endpoint in clinical trials was prevention, with 56 (60.2%) for RSV and 282 (67.0%) for influenza. The industry conducted 78 (83.0%) clinical trials for RSV versus 237 (56.0%) for influenza, $p < 0.001$. Significant differences were found between the percentage of vaccines developed for RSV, 51 (54.3%), versus the percentage of vaccines developed for influenza virus 344 (81.3%), with a $p < 0.001$. The pharmaceutical industry participated in more than 50% of the RSV and influenza clinical trials. In 49.2% of the clinical trials registered in ClinicalTrials.gov, there was participation of North American centers. Highlight the interest in RSV in developing clinical trials only in women, 12 (12.8%), 6 directed at pregnant women, compared to 4 (0.9%) clinical trials for women in the influenza virus, 3 directed at pregnant women.

The Venn diagram (Figure 1) shows the age composition of the clinical trials included in the study. We found that a significant percentage of RSV studies included patients under 5 years of age compared to influenza studies, 29 (31%) for RSV versus 21 (5%) for influenza studies, $p < 0.001$. On the other hand, 23 (24%) of the RSV clinical trials included a population aged 65 years or over, compared to 184 (46%) of the influenza trials, $p < 0.001$. It should be noted that no RSV study exclusively included patients aged 65 or over compared to 29 (7%) influenza studies.

Phase designs less than or equal to 2 are the majority in RSV, above 80%. On the other hand, in the influenza virus, it was observed that the investigation is distributed homogeneously in each of the phases. The usual allocation for clinical trials in both viruses was in parallel arms, greater than 80%, and with two arms, greater than 40% (Table 2). The number of clinical trials that published the protocol and the SAP was similar between both infectious diseases, 122 (23.8%).

Regarding the type of molecule investigated in the different phases of RSV clinical trials (Figure 2A), in phase 1 clinical trials, approximately 60% were vaccines, 33% antibodies and 5% antivirals. All phases (Figure 2A) accounted for between 45 and 70% of vaccine research, with the exception of phase 2/phase 3, in which 100% were antiviral, although there were only 2 studies. Figure 2B shows the evolution of the trials over the years included, noting that the vaccine trials have gone from 30% in 2014 to 68.8% in 2021. The number of RSV studies has remained stable throughout the years, ranging from 10 (2015, 2019) to 16 annual trials (2021).

In the case of influenza (Figure 3A), more than 75% of the trials, regardless of the phase, are for vaccines, and in all years, more than 80%. In 2020 and 2021 (Figure 3B), the study of monoclonal antibodies against the influenza virus increased by up to 10%. The number of studies on the influenza virus decreased over the 8 years studied, from 67 clinical trials in 2014 to 46 by 2021 and 28 studies in 2020.

Discussion

The data from our study show research mostly conducted by the pharmaceutical industry, with approximately half of the studies reporting the presence of DMC. For influenza, research is consolidated and dominated by the development of vaccines, compared to research on RSV in which studies in early stages abound and with less predominance of research on vaccines, although increasing. Research on influenza has a higher participation of the older adult compared to RSV research, in which the participation of children and pregnant women was clearly higher.

Different authors have characterized the clinical trials that appear in ClinicalTrials.gov (9, 10, 13). Only 38.4% of clinical trials targeting infectious diseases registered on ClinicalTrials.gov between 2007 and 2010 (13) had a primary objective of prevention, compared to 65.5% of the clinical trials of RSV and influenza aimed at prevention existing in ClinicalTrials.gov between 2014 and 2021 in our study. The increase may be due to the exclusion criteria for clinical trials established in our study. On the other hand, it is reasonable to think that research efforts are aimed at prevention given the high burden of morbidity and mortality at the global level of infectious viral diseases, such as influenza and RSV, as opposed to bacterial diseases. This high morbidity and mortality due to RSV in the older adult population (20), and the research strategies promoted in 2015 by the World Health Organization (WHO) to provide guidance on clinical endpoints and development pathways for vaccine trials with a focus

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TABLE 1 Characteristics of clinical trials on influenza and RSV registered in ClinicalTrials.gov between 2014 and 2021.

	Level	Overall (*n* = 516)	RSV (*n* = 94)	Influenza virus (*n* = 423)
Primary purpose	Diagnostic	2 (0.4)	0 (0.0)	2 (0.5)
	Prevention	337 (65.7)	56 (60.2)	282 (67.0)
	Treatment	121 (23.6)	29 (31.2)	92 (21.9)
	Other	53 (10.6)	8 (8.6)	45 (10.7)
Intervention/Treatment	Anticuerpos	28 (5.4)	10 (10.6)	18 (4.3)
	Antivirals	68 (13.2)	31 (33.0)	37 (8.7)
	Vacuna	394 (76.4)*	51 (54.3)	344 (81.3)
	Otros	26 (5.0)	2 (2.1)	24 (5.7)
Study registration	Before first participant enrolled	199 (38.6)	32 (34.0)	168 (39.7)
	After first participant enrolled	317 (61.4)	62 (66.0)	255 (60.3)
Lead sponsor	Industry	314 (60.9)	78 (83.0)	237 (56.0)
	NIH	54 (10.5)	14 (14.9)	40 (9.5)
	Other	148 (28.7)	2 (2.1)	146 (34.5)
DMC	Study has DMC	250 (56.7)	37 (43.5)	214 (59.9)
	Study does not have DMC	191 (43.3)	48 (56.5)	143 (40.1)
Region**	Africa	36 (7.0)	17 (18.1)	19 (4.5)
	Asia And Pacific	165 (32.0)	41 (43.6)	125 (29.6)
	Central and South America	48 (9.3)	22 (23.4)	26 (6.1)
	Europe	154 (29.8)	49 (52.1)	106 (25.1)
	Middle East	31 (6.0)	13 (13.8)	18 (4.3)
	North America	254 (49.2)	61 (64.9)	194 (45.9)
	Missing	35 (6.8)	2 (2.1)	33 (7.8)
Overall status	Active, not recruiting	29 (5.6)	9 (9.6)	20 (4.7)
	Completed	388 (75.2)	69 (73.4)	320 (75.7)
	Enrolling by invitation	2 (0.4)	0 (0.0)	2 (0.5)
	Not yet recruiting	1 (0.2)	0 (0.0)	1 (0.2)
	Recruiting	30 (5.8)	4 (4.3)	26 (6.1)
	Suspended	2 (0.4)	1 (1.1)	1 (0.2)
	Terminated	21 (4.1)	7 (7.4)	14 (3.3)
	Unknown status	35 (6.8)	1 (1.1)	34 (8.0)
	Withdrawn	8 (1.6)	3 (3.2)	5 (1.2)
Gender	Both	496 (96.1)	80 (85.1)	416 (98.3)
	Female only	15 (2.9)	12 (12.8)	4 (0.9)
	Male only	5 (1.0)	2 (2.1)	3 (0.7)
Includes children (<18 years)	Yes	155 (30.0)	35 (37.2)	120 (28.4)
Includes older adult (≥65 years)	Yes	217 (42.1)	23 (24.5)	194 (45.9)
Dirigidos a mujeres embarazadas	Yes	9 (1.7)	6 (6.4)	3 (0.1)

RSV, Respiratory Syncytial Virus; DMC, Data Monitoring Committee; NIH, National Institutes of Health. *Elimination of duplication of the coincident study of influenza and RSV. **Clinical trials can take place in more than one country. All cells show the absolute number of studies and the percentage of the total, *n* (%).

on considerations of low- and middle-income countries (7) and shown in different reviews on vaccines and monoclonal antibodies (8, 9), agrees with the high percentage of RSV vaccine research found over the years. However, no differences were found between countries according to income, as in other viral diseases (13). Similarly, the consolidated research of influenza clinical trials is consistent with the marked development of vaccines (4). This is reflected in the high percentage of clinical trials on phase 4 vaccines, unlike emerging infectious diseases such as COVID-19 (15–17).

We have not identified registered clinical trials on RSV aimed exclusively at the older adult population. In influenza, a minority of the studies addressed only older adult individuals. Approximately 1 in

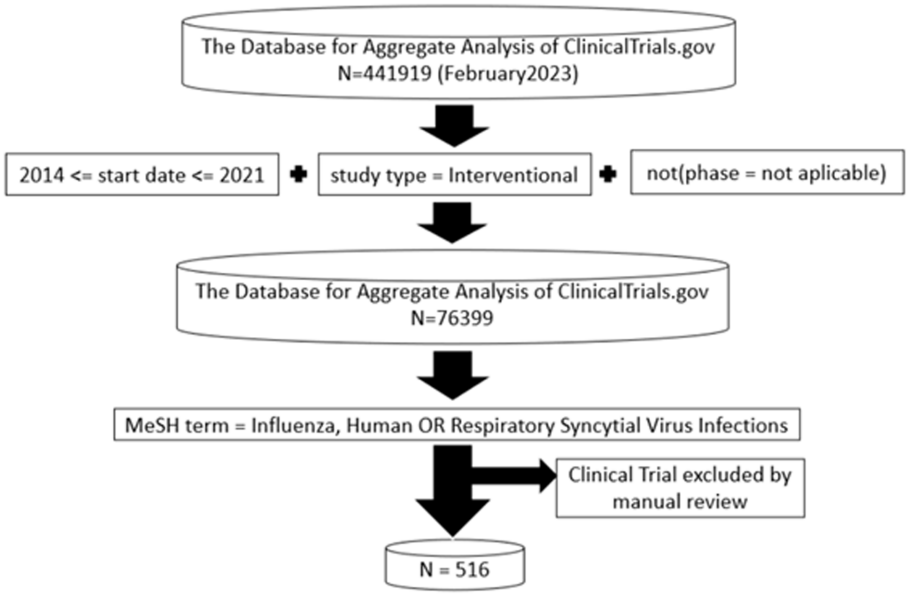

FIGURE 1
Trials dataset. Flow diagram depicting the derivation of the influenza virus and Respiratory Syncytal Virus.

TABLE 2 Design and methodology of clinical trials on influenza and RSV registered in ClinicalTrials.gov between 2014 and 2021.

	Level	Overall (*n* = 516)	RSV (*n* = 94)	Influenza virus (*n* = 423)
Phase	Early Phase 1	2 (0.4)	0 (0.0)	2 (0.5)
	Phase 1	129 (25.0)	41 (43.6)	88 (20.8)
	Phase 1/Phase 2	27 (5.2)	5 (5.3)	22 (5.2)
	Phase 2	125 (24.3)	33 (35.1)	92 (21.7)
	Phase 2/Phase 3	12 (2.3)	2 (2.1)	10 (2.4)
	Phase 3	113 (21.9)	13 (13.8)	101 (23.9)
	Phase 4	108 (20.9)	0 (0.0)	108 (25.5)
Allocation	Non-randomized	92 (17.8)	12 (12.8)	80 (18.9)
	Randomized	424 (82.2)	82 (87.2)	343 (81.1)
Assignment	Crossover	3 (0.6)	0 (0.0)	3 (0.7)
	Factorial	5 (1.0)	0 (0.0)	5 (1.2)
	Parallel	432 (83.9)	81 (86.2)	352 (83.4)
	Sequential	19 (3.7)	4 (4.3)	15 (3.6)
	Single Group	56 (10.9)	9 (9.6)	47 (11.2)
Number of arms	1	47 (9.1)	5 (5.3)	42 (9.9)
	2	223 (43.2)	45 (47.9)	178 (42.1)
	3	97 (18.8)	12 (12.8)	85 (20.1)
	4	65 (12.6)	13 (13.8)	52 (12.3)
	5	26 (5.0)	5 (5.3)	21 (5.0)
	6	28 (5.4)	7 (7.4)	21 (5.0)
	+ de 6	30 (5.8)	7 (7.4)	24 (5.7)
Enrollment	1–100	175 (34.4)	54 (59.3)	121 (28.9)
	100–1,000	253 (49.8)	26 (28.6)	227 (54.3)
	>1,000	80 (15.7)	11 (12.1)	70 (16.7)
Blinding	None (Open Label)	150 (29.1)	15 (16.0)	135 (32.0)
	Single	34 (6.6)	5 (5.3)	29 (6.9)
	Double	109 (21.2)	21 (22.3)	88 (20.9)
	Triple	64 (12.4)	18 (19.1)	46 (10.9)
	Quadruple	158 (30.7)	35 (37.2)	124 (29.4)
Study Protocol available	Yes	122 (23.8)	25 (25.6)	98 (23.2)
SAP available	Yes	122 (23.8)	25 (25.6)	98 (23.2)
Publicado en pubmed (February 2023)	Yes	139 (26.9)	23 (24.5)	116 (27.4)

RSV, Respiratory Syncytial Virus; SAP, Statistical Analysis Plan. All cells show the absolute number of studies and the percentage of the total, n (%).

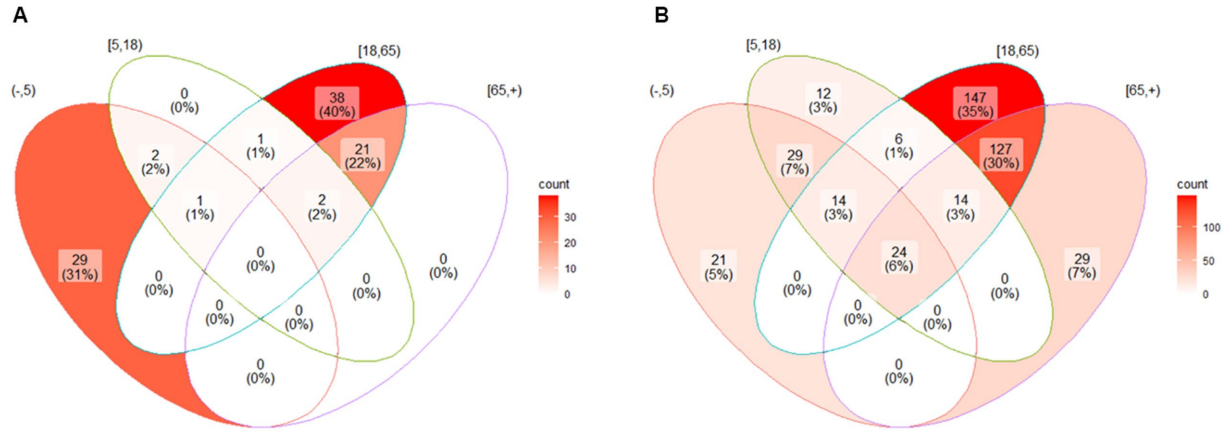

FIGURE 2
Venn diagram. The circles show the age strata where the clinical trials took place. The intersection of the circles shows the clinical trials with mixed age groups. The age groups considered are (1) less than 5 years old, (2) greater than and equal to 5 years old and less than 18 years old, (3) greater than or equal to 18 years old and less than 65 years old, and (4) greater than or equal to 65 years. The number of clinical trials and their percentage with respect to the total are presented for each of the strata.

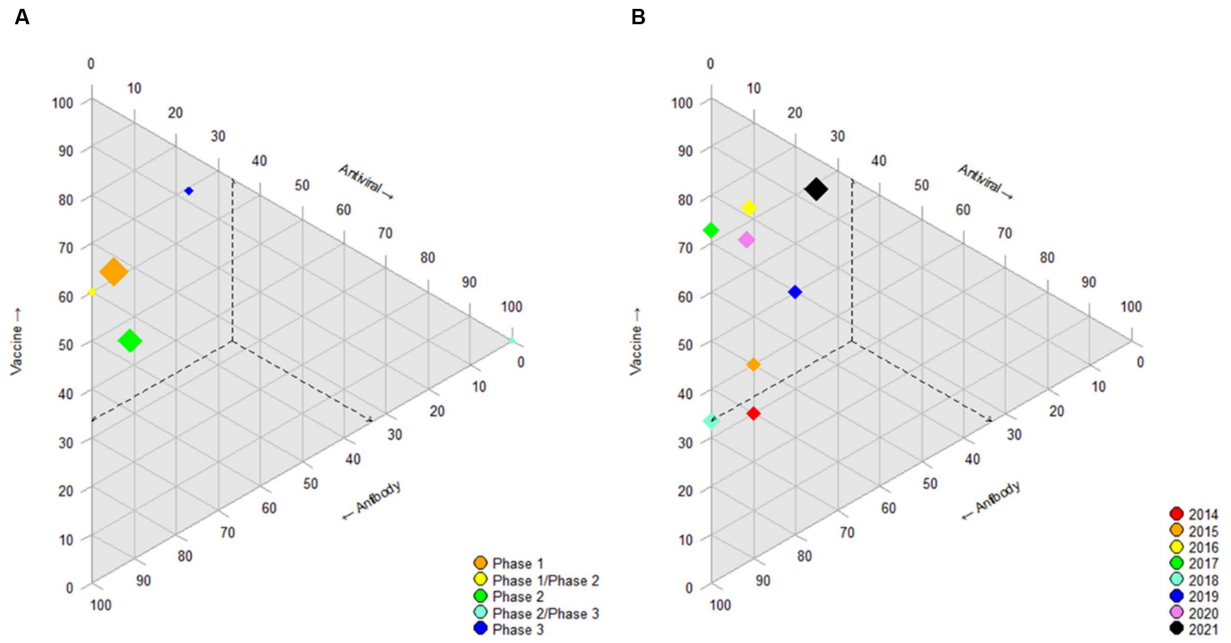

FIGURE 3
Ternary diagram. Ternary diagram representing the phase of clinical trial (or year) in accordance with their positions on each of the three axes for influenza virus. Each axis represents the percentage of intervention/treatment groups vaccine, antiviral and antibody. Other interventions or treatments are not shown. The dashed lines indicate the coordinates of the different phase (or year) leading to the point where intervention is located (as an example for interpretation). The size of the diamond represents the absolute number of trials in that category: a larger diamond size indicates a greater number of studies. **(A)** RSV-phase. **(B)** RSV year.

4 studies on RSV and 1 in 2 on influenza included the older adult population in some way. The development of specific vaccines for this age group with a high risk of hospitalization could reduce the total burden of viral respiratory diseases in clinics and hospitals during the winter months (20). Vaccines may show less efficacy in the older adult population, in whom they may be more necessary. Therefore, specific studies that include combined treatments could be convenient.

The study presents the limitations of the sources from which the data are collected (10, 12). First, the clinical trials for RSV and influenza viruses listed on ClinicalTrials.gov are not all clinical trials developed for those two viruses. However, all clinical trials developed in the United States must be registered in ClinicalTrial.gov; in addition, some journals, in order to publish the results of clinical trials, require registration in ClinicalTrials.gov within their editorial policy. Some

authors (13) have suggested that after manual review, approximately 80% of clinical trials registered in The International Clinical Trials Registry Platform (ICTRP) are also registered in ClinicalTrials.gov. Second, the information registered in ClinicalTrials.gov is entered manually by the research team of the clinical trial and is susceptible to errors and presents missing data (10, 12). For this reason, those inconsistencies identified by manual review were modified.

Research on RSV has shown variability in recent years in its focus on vaccines, antivirals or antibodies in the absence of lines that have demonstrated their efficacy. The clarification of the protection of newborns with the vaccination of pregnant women within clinical trials could modify the research in the coming years. Similarly, the development of new mechanisms of action (5), as well as the identification of vulnerable populations that may benefit more from the development of vaccines or drugs against influenza or RSV, such as cancer, immunosuppressed or high-risk subjects of cardiovascular events, are areas of future research.

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: clinicaltrials.gov.

Author contributions

DL, MM-O, and EC: conceptualization. DL: data curation, software, validation, and writing—original draft. DL and MM-O: formal analysis, methodology, and visualization. DL, AG-R, AL, GM, MR-R, and MM-O: funding acquisition. DL, AG-R, AL, GM, and MR-R: investigation. EC and MM-O: supervision. DL, AG-R, AL, GM, MR-R, EC, and MM-O: 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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Planning and development of an antimicrobial stewardship program in penitentiary facilities: strategies to optimize therapeutic prescribing and reduce the incidence of antibiotic resistance

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Introduction: In correctional facilities, due to the high incidence of bacterial infections, antibiotics are widely prescribed. As a result, it may occur a massive and improper use of antibiotics, which promotes the development of antibiotic-resistant bacteria. However, in literature, specific experiences, interventions or guidelines aimed to optimize their prescription within prisons are sporadic.

Objectives: In an Italian hospital where belong patients from four penitentiary institutions, a multidisciplinary team has implemented an antimicrobial stewardship project. The aim of the project was to reduce the incidence of antibiotic resistance in penitentiary institutions by optimizing and rationalizing antibiotic prescribing.

Methods: Following the analysis of microbiological prevalence and antibiotic consumption data within correctional facilities, the Antimicrobial Stewardship Team developed operational tools to support prison healthcare staff to manage properly antibiotic therapies.

Results: The analysis showed a gradual increase in antibiotic resistance: in 2021 the prevalence of resistant microorganisms was 1.75%, four times higher than in 2019. In contrast, between 2019 and 2021, antibiotic consumption decreased by 24%. Based on consumption data, pharmacy has drafted an antibiotic formulary for correctional facilities, supplemented with guidelines and data sheets, and also developed a prescription form for critical antibiotics.

Conclusion: Results showed an increasing incidence of antibiotic resistance within prisons, highlighting the need to establish a dedicated antimicrobial stewardship program. This project may impact positively not only on prisoners, but also for the entire community, as prisons can be considered as places of health education and promotion.

KEYWORDS

antimicrobial stewardship, antibiotic-resistant bacteria, bacterial infections, inmates, correctional facilities, prisons

Introduction

The significant incidence of bacterial infections in prison facilities is currently one of the main critical issues plaguing prison health care. HIV infection and viral hepatitis are the most common infectious diseases affecting people living in prison (PLP) (1), although local or systemic bacterial infections are also recurrent, such as skin and soft tissue infections, respiratory tract infections, urinary tract infections, sexually transmitted infections (STIs), tuberculosis, otolaryngological and dental infections (2, 3). Moreover, a massive and improper use of antibiotic drugs promotes the development of resistant or multidrug-resistant bacteria. Therefore, clinical management of infections is increasing difficult and the availability of effective agents is reduced (4, 5). Overall, PLP have a crucial role in the spread of antibiotic resistance. In fact, imprisonment associated to a compromised health status may expose PLP to an increased risk of contracting infections, which can be followed by an inappropriate use of antibiotics. The consequence is a wide spread of resistant microorganisms, both within prisons and then within the community at large, due to the rapid turnover of some inmates who often move from one correctional facility to a second prison (6). Currently, in the scientific literature there is little evidence compared to the trend of this phenomenon in the penitentiary setting and the development of best-practice tools useful to make in practice effective actions to prevent antimicrobial resistance (AMR) in prisons. In particular, in penitentiary institutions belonging to the Italian region where this study took place, no antimicrobial stewardship practice has ever been implemented to address the problem of AMR in the real life setting. According to WHO, antimicrobial resistance will be the leading cause of human death by 2050. Consequently, even in a context such as the prison setting, it is crucial to implement and foster concrete actions which may address this phenomenon between now and the coming decades.

Objectives

In order to counteract the spread of this phenomenon, a multidisciplinary team from an Italian hospital to which the four Milan penitentiary institutions belong, has implemented an antimicrobial stewardship project. The project aimed to provide operational tools to optimize prescription, rationalize the use of antibiotics and promote the safety of drug therapies by reducing the risk of prescription mistakes and antibiotic-related toxicity in prison, with the aim of decreasing the incidence of antibiotic resistance in this particular setting. Furthermore, this work aimed to analyze the microbiological prevalence and local consumption of antibiotics in Milan penitentiary setting between 2019 and 2021, describing all the interventions implemented for the development of a prison-specific antimicrobial stewardship program.

Methods

Setting and study design

The project has been developed through two steps and it has been addressed to the four penitentiary institutions in Milan (one remand house, San Vittore, two long-sentenced prisons, Opera and Bollate, and Beccaria, a juvenile prison). A multicenter retrospective epidemiological survey of the four penitentiary institutions has been carried out, to analyze microbiological prevalence and local antibiotic consumption data. Sample included prison population at Milan penitentiary facilities between 2019 (3665 PLP), 2020 (3346 PLP), and 2021 (3316 PLP). Data have been compared over this period, to assess trends in antibiotic consumption and in bacterial resistance. Based on results of the previous analysis, an antimicrobial stewardship program has been implemented. The program involved several health care professionals and provided for the development of operational tools aimed to support antibiotics prescription. Actions undertaken were addressed to a sample consisting of the population detained in Milan prisons since the beginning of the project. In particular, sample included 3540 adults, of which 192 women, 40 young male juveniles and 5 mothers with their 5 children. 40.5% of PLP were foreigners (7). The average age was 41 years, with a wide range of ages, due to the presence of juvenile prison and children up to 6 years hosted at the attenuated custodial institution (ICAM) for mother living in prison. The condition of incarceration at one of the Milan correctional institutions in the years under investigation was the inclusion criterion adopted. All PLPs who were no longer incarcerated by 2019, due to transfers to other prisons or release from prison, have been excluded.

Collection of microbiological prevalence data

The microbiology unit shared microbiological prevalence data, which have been obtained through the collection and analysis of microbiological sample reports from correctional facilities, referring to the years 2019, 2020, and 2021.

Collection of antibiotic consumption data

Antibiotic consumption data have been provided by the hospital pharmacy. In particular, the analysis focused on data for the years 2019, 2020, and 2021, which have been extracted from the hospital's management software. The data have been entered into a database containing the list of antibiotic drugs used in prisons, classified by Anatomical Therapeutic Chemical classification system (ATC) and marketing authorization code (MA code), dosage and pharmaceutical form. In addition, for each drug the database reported the Defined Daily Dose (DDD) value and the annual consumption, defined as the amount of antibiotic, expressed by the number of dosage units, used in penitentiary facilities in the period analyzed. According to the National Plan to Face Antimicrobial Resistance, approved in Italy in 2022, which assesses trends in territorial antibiotic consumption in DDD/1000 population per day (8), the following formula has been applied, to evaluate the consumption trend: DDD value of each antibiotic × the amount of antibiotic used × 1000/number of inmates. As a result, it has been obtained a unique indicator which provided a real and standardized representation of antibiotic consumption, compared with penitentiary population' size. Values have been summed by grouping the different active agents by ATC, in order to evaluate the overall consumption, according to the different classes of antibiotics.

Project development

The project included the development of an Antimicrobial Stewardship Team, including the following professional figures: infectious diseases specialist, pharmacist, nurse, microbiologist and health management physician. Team members attended a series of multidisciplinary meetings to analyze local epidemiological data, to identify the main purposes and working methods, and to periodically assess the advancement of the project.

After the collection and the review of microbiological prevalence and antibiotic consumption data, the Antimicrobial Stewardship Team addressed the following actions:

- based on antibiotic consumption data, the identification of active agents used in prisons, in order to draft a specific formulary for antibiotic therapies;
- the development of data sheets for each antibiotic included in the formulary, to guide physicians in prescribing and supporting nurses in the proper management of antibiotic therapies;
- the identification of critical antibiotics, which require approval by an infectious diseases specialist before their administration, and the development of a specific request form for their prescription;
- the drawing up of guidelines for the treatment of the most common infections in penitentiary institutions.

Results

Analysis of microbiological prevalence data

In 2019, 2020, and 2021 the microbiology unit analyzed a total of 742 microbiological samples coming from Milan prisons: 251 samples have been analyzed in 2019, 232 and 229 in 2020 and 2021, respectively. Most of them were respiratory or urinary samples. The results showed the presence of 7 antibiotic-resistant bacteria. In 2019, only a strain of *Proteus mirabilis* with a multiresistance profile to penicillins, cephalosporins, ciprofloxacin, colistin, fosfomycin, tigecycline, and to the trimethoprim-sulfamethoxazole association, has been identified. The bacteria infected a prisoner at the San Vittore penitentiary. In 2020, two microorganisms have been isolated in two people living in San Vittore prison: a β-lactamase-producing *Enterobacter hormaechei* resistant to penicillins and cephalosporins, and an extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* strain.

In 2021, the following microorganisms have been isolated in four PLP detained in Bollate and San Vittore prisons:

- an ESBL-producing *Escherichia coli* resistant to penicillins, cephalosporins, fluoroquinolones, and to the trimethoprim-sulfamethoxazole association;
- an ESBL-producing *Klebsiella pneumoniae*;
- a multiresistant *Morganella morganii* strain, specifically resistant to amikacin, penicillins, cephalosporins, ciprofloxacin, ertapenem and fosfomycin;
- an ESBL-producing *Escherichia coli* strain.

All microorganisms have been detected in PLP's urine and processed by urinoculture.

Antibiotic-resistant bacteria accounted for 0.94% of all microbiological isolates coming from Milan prisons, from 2019 to 2021. The analysis showed a progressive increase in antibiotic resistance: in 2019 and 2020 the prevalence was 0.39 and 0.86%, respectively, whereas in 2021 there was an increase of the prevalence up to 1.75% (Table 1).

Analysis of antibiotic consumption data

The analysis conducted by the hospital pharmacy showed a gradual decrease in antibiotic consumption within correctional facilities. In 2019, a total antibiotic consumption of 20600 DDD has been found. In 2020, this figure decreased by 12%, going to 18041 DDD. In 2021, the total consumption of antibiotics decreased further, settling around the value of 15670 DDD, 13% less than the previous year (Figure 1 and Table 2). Consistent with local and national data, this phenomenon was probably related to the lower incidence of infections among inmates detected during the COVID-19 pandemic (9).

Analyzing the consumption trend of the most widely used systemic antibiotics in Milan prisons, in 2021, compared with 2019, a lower use of the associations of penicillins and β-lactamase inhibitors has been observed. In particular, the consumption of amoxicillin-clavulanic acid tablets at the dosage of 875 mg + 125 mg decreased by 21%, going from 9914 DDD in 2019 to 7791 DDD in 2021. However, amoxicillin-clavulanic acid was still the most widely used antibiotic. Macrolides, such as clarithromycin and azithromycin, were also widely prescribed. In this case, the analysis showed a 23% reduction in consumption between 2019 and 2021, although there has been a sudden increase in 2020, especially for azithromycin (from 1246 DDD in 2019 to 2593 DDD in 2020, and then decreased to 1416 DDD in 2021). This significant increase in 2020 was likely due to the fact that azithromycin was initially recommended for the treatment of COVID-19 in non-hospitalized patients (8). Consumption of systemic fluoroquinolones in prisons amounted to 1772 DDD in 2021: it is noteworthy that compared to 2019, in 2020 it dropped by 24%, and in 2021 it decreased further by 11%. The most widely used fluoroquinolone agent was levofloxacin, whose consumption in 2019, 2020, and 2021 was, respectively, of 1853 DDD, 1219 DDD, and 1043 DDD. Among the broad-spectrum penicillins, the most widely prescribed antibiotic was amoxicillin 1000 mg. Its consumption was 2554 DDD in 2019, and it dropped to 1465 DDD in 2021, with an overall reduction of 43%. In contrast, the consumption of tetracyclines increased by 25%. In particular, the consumption of doxycycline increased from 655 DDD in 2019 to 826 DDD in 2021, with a slight decrease of 7% in 2020, compared

TABLE 1 Trend of antibiotic resistance in Milan penitentiary facilities in 2019, 2020, and 2021.

	2019	2020	2021
Analyzed samples	251	232	229
Multidrug-resistant bacteria isolated	1	2	4
Prevalence	0.39%	0.86%	1.75%

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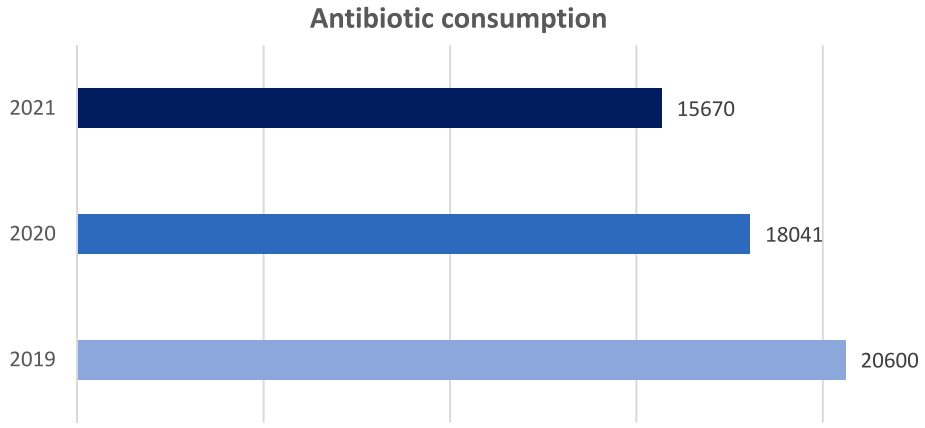

FIGURE 1
Trend of total antibiotic consumption in Milan correctional facilities from 2019 to 2021. Consumption: DDD × amount of antibiotic used × 1000/ number of inmates in the reference year.

TABLE 2 Number of adult people living in Milan prisons in 2019, 2020, and 2021.

Size of penitentiary population			
Prison	2019	2020	2021
Bollate	1347	1241	1209
San Vittore	984	921	930
Opera	1334	1184	1177
Total	3665	3346	3316

TABLE 3 Trends in antibiotic consumption grouped by ATC in Milan prisons between 2019 and 2021.

DDD/1000 PLP	Year		
ATC description	2019	2020	2021
Association of penicillins and beta lactamase inhibitors	9933	8367	7803
Macrolides	3218	3949	2483
Fluoroquinolones	2608	1987	1772
Broad-spectrum penicillins	2570	1673	1473
Tetracyclines	662	609	826
Third-generation cephalosporins	222	238	181
Associations of sulfonamides and trimethoprim	191	146	144
Nitrofurantoin	19	9	3
Other antibacterial agents	15	11	30
Aminoglycosides	5	7	6
Carbapenems	7	4	4
Glycopeptides	6	1	4
Beta lactamase-sensitive penicillins	4	3	2
Lincosamides			2
First-generation cephalosporins			1
Total	20600	18041	15670

to the previous year. Table 3 illustrates the consumption trends of all the classes of antibiotics used within the Milan prisons, between 2019 and 2021.

Implemented measures to promote appropriate prescribing and proper use of antibiotics

Based on these results, the pharmacy developed both a formulary of antibiotic therapies for correctional facilities and a request form aimed to address highly critical antibiotics' prescriptions. The antibiotic formulary was aimed at providing a useful tool for clinicians in the empirical choice of antibiotic therapy to deal with more critical infectious diseases which may occur in correctional institutions. All antibiotics used in Milan prisons have been listed and divided by pharmacological class and ATC. As part of the antimicrobial stewardship process, the use of this formulary requires specific training of prison medical staff, which is based on two key points: the need to collect biological samples for etiological identification before starting any antibiotic therapy, and the timely adaptation of the empirical therapy after the microbiological results. The prescription form aimed at ensuring greater control over the prescribing and use of antibiotics, through the direct involvement of the clinician and the pharmacist. It has been designed for prescribing antibiotics which do not belong to the first line of treatment, which have specific prescribing restrictions or that require special precautions in management and administration. Before their use, these agents required approval by an infectious diseases specialist. Some instances are glycopeptides such as vancomycin and teicoplanin, carbapenems, daptomycin, linezolid, colistin, tigecycline, intravenous ampicillin, dalbavancin and latest generation cephalosporins. The form included information about the requesting unit and the prescribing doctor, patient's data (including age and body weight), the required antibiotic, its dosage, duration of treatment, whether it was prescribed as monotherapy or in

combination with other medicines, its clinical indication, specifying whether the treatment was empiric or targeted and the name of the isolated microorganism, any previous antibiotic therapies. Eventually, the last section of the form required specifying whether the prescribed medication had been approved by an infectious diseases specialist. After verifying the request's appropriateness, according to antimicrobial prescribing criteria, the pharmacist can authorize it. The hospital pharmacy also prepared data sheets for each antibiotic included in the formulary, to provide support to healthcare professionals in prescribing and managing antibiotic therapies. Each data sheet contained information about pharmacokinetics, dosage, interactions, major side effects, methods of preparation and storage. The formulary has been supplemented with specific guidelines for the treatment of the most recurrent infections in prisons: skin and soft tissue infections, STIs, urinary tract infections, otorhinolaryngological infections and pulmonary infections. The guidelines have been developed by the pharmacy, in cooperation with the infectious diseases specialists working in prisons. They referred to both community and nosocomial infections, as PLP could develop infections before and/or during the incarceration. Moreover, they could also contract care-related infections during any hospitalization and, once discharged, they could continue their care within correctional facilities. They focused on antibiotic therapies, distinguishing between empiric therapies and following treatment lines, and indicating which active agents must be chosen once detected antibiotic resistance, specifying any particular contraindication or recommendation for allergic patients or pregnant women who live in prison.

Discussion

From a social perspective, prison communities are largely represented by individuals with great difficulties, such as homeless or people with mental disorders or drug addictions (10, 11). These people are at increased risk of infection (12). In addition, several risk factors related to detention setting, such as overcrowding, poor hygienic conditions, lack of effective preventive measures, promiscuity and poorly ventilated environments, may promote occurrence and transmission of infections (13, 14). Reduced availability of diagnostic tests, difficult access to microbiological tests and high mobility of PLP can delay diagnosis of infections, complicating identifications of infectious outbreaks, to stop transmission and to eradicate the disease (2). This increases risk of contagion and spread of new infections (2, 15). In a context of poor and difficult infection management, often there is a massive and improper use of antimicrobial drugs within correctional facilities, in particular of antibiotics, which causes the onset of antibiotic resistance. AMR commonly occurs in individuals with a compromised immune system, for example those with AIDS, cancer, or who have received an organ transplant (5). Other factors that may contribute to its development are close contact and promiscuity with strangers, especially if not vaccinated, drug abuse and smoking (5, 6, 14). In the community this phenomenon affects both men and women, while in prison mainly the male population, which represents the majority. In literature there are few data describing AMR and the inappropriate use of antibiotics within correctional institutions. Due to the lack of data in this context, it is crucial to compare results of this study with other findings, although this project could be innovative for the scientific community. However, limited evidence in literature is consistent with our results. In fact, a study which analyzed antibiotics prescribed for the treatment of acute upper respiratory tract infections (URTIs) and dental infections in Italian prisons showed that overprescription of antibiotics is widespread in the prison population, accounting for about 69.4% of the prescriptions (16). Another study found that antibiotics agents are prescribed in 67% of cases of respiratory infections diagnosed in prison settings, still underestimating the large use of these drugs and highlighting a low utilization of diagnostic tests, in particular microbiological tests (17). There may be several factors behind the overprescribing of antibiotics in prison facilities. For instance, most of the diagnoses of infections in prison facilities are based on clinical signs and symptoms, whereas microbiological examinations and diagnostic tests are less used. As a result, empirical prescription of broad-spectrum antibiotics is preferred over targeted therapies (16). Often prison internists prescribe broad-spectrum oral antibiotics, without requiring specialist or pharmacist advice. As a result, the incidence of inappropriate prescribing may increase the use of inappropriate antibiotics. Another reason could be related to a higher perceived risk of development of serious bacterial infections by PLP, as well as to a potential requirement for increased follow-up when antimicrobials are not prescribed. Furthermore, it has been reported that not prescribing antibiotics may be perceived by prison patients as a "non-cure," believing that these drugs are harmless (16, 18). In addition, the existence of an underlying chronic clinical condition in PLP is a further potential cause of bacterial resistance. In fact, patients with comorbidities are at high risk of developing antibiotic resistance, due to their vulnerability to infections and to the relative frequent exposure to antibiotic treatments (16, 19). Antimicrobial stewardship has proven to be an effective tool to rationalize antibiotic consumption, improving prescribing appropriateness (20, 21). Several actions have been implemented in hospitals and in different care settings (22). However, despite of the presence of a high infectious risk, effective measures in Italian prisons are lacking (23). Therefore, a dedicated antimicrobial stewardship project for Milan penitentiary facilities has been implemented, with a specific focus on antibacterial drugs. The results of this study showed a relatively low incidence of infections caused by multidrug-resistant microorganisms, as well as a gradual reduction in antibiotic consumption from 2019 to 2021. Nevertheless, it is noteworthy that prevalence of multidrug-resistant superbacteria infections in the three-year period analyzed followed an increasing trend, doubling from 2019 to 2020, and then again from 2020 to 2021. However, this study has some limitations. In fact, collected data may have been strongly affected by the COVID-19 pandemic, which led to a lower incidence of infections within prisons, probably due to the adoption of preventive measures such as the use of personal protective equipment and the implementation of hygiene and sanitation measures (24, 25). In addition, the high turnover of PLP resulting from new entries, releases or transfers to other prisons did not guarantee a defined and constant sample throughout the duration of the project. The project is still under development. Training meetings will be organized with medical staff, in order to promote the use of new prescription form and therapeutic formulary, as well as to educate and to sensitize prison staff on the proper management of antibiotic therapies. Microbiological diagnostic services will be improved to incentivize the prescribing of targeted antibiotic therapies based on the

antibiogram, thereby limiting the prescription of broad-spectrum empirical therapies. Eventually, some indicators related to antibiotic consumption and microbiological prevalence will be continuously monitored over time, with the aim to assess the effectiveness of the project.

Conclusion

The results of this study show that in Milan prisons, in recent years there has been a high number of empirical prescriptions, as well as a massive use of broad-spectrum antibiotics. This led to an increasing trend in the development of antibiotic-resistant bacteria. Therefore, it is crucial to establish an antibiotic stewardship program within correctional institutions, which provides supportive operational tools and defines a shared pathway aimed at improving the prescriptive appropriateness of antimicrobial therapies. In addition, the implementation of an antimicrobial stewardship project could be a significant initiative not only to improve PLP health conditions, but also to positively impact on the whole community. In fact, detention represents a health risk factor due to several determinants, such as social and cultural marginalization of penitentiary population, overcrowding, poor hygienic conditions and high turnover. However, prison often represents a contact point with the National Health System and an environment to prevent, diagnose, and treat infectious diseases.

Data availability statement

The original contributions presented in the study are included in the article/supplementary files. Further inquiries can be directed to the corresponding author.

Author contributions

LM, CD'A, and RR: conceptualization. LM, AZ, and CD'A: methodology. LM, CB, NC, and CD'A: investigation. LM, AZ, and RR: writing – original draft preparation. CD'A, CB, NC, and RL: writing – review and editing. CD'A, CB, NC, RL, and RR: visualization. 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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Applying Andersen's healthcare utilization model to assess factors influencing patients' expectations for diagnostic tests at emergency department visits during the COVID-19 pandemic

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Background: The uncertainties surrounding the COVID-19 pandemic led to a surge in non-urgent emergency department (ED) attendance among people presenting with upper respiratory tract infection (URTI) symptoms. These non-urgent visits, often manageable in primary care, exacerbated ED overcrowding, which could compromise the quality of ED services. Understanding patients' expectations and the reasons for these ED visits is imperative to mitigate the problem of ED overcrowding. Hence, we assessed the factors influencing patients' expectations for diagnostic tests during their ED visits for uncomplicated URTI during different phases of the pandemic.

Methods: We conducted a cross-sectional study on adults with URTI symptoms seeking care at four public EDs in Singapore between March 2021 and March 2022. We segmented the study period into three COVID-19 pandemic phases—containment, transition, and mitigation. The outcome variables are whether patients expected (1) a COVID-19-specific diagnostic test, (2) a non-COVID-19-specific diagnostic test, (3) both COVID-19-specific and non-COVID-19-specific diagnostic tests, or (4) no diagnostic test. We built a multinomial regression model with backward stepwise selection and classified the findings according to Andersen's healthcare utilization model.

Results: The mean age of participants was 34.5 (12.7) years. Factors (adjusted odds ratio [95% confidence interval]) influencing expectations for a COVID-19-specific diagnostic test in the ED include younger age {21–40 years: (2.98 [1.04–8.55])}, no prior clinical consultation (2.10 [1.13–3.89]), adherence to employer's health policy (3.70 [1.79–7.67]), perceived non-severity of illness (2.50 [1.39–4.55]), being worried about contracting COVID-19 (2.29 [1.11–4.69]), and during

the transition phase of the pandemic (2.29 [1.15–4.56]). Being non-employed influenced the expectation for non-COVID-19-specific diagnostic tests (3.83 [1.26–11.66]). Factors influencing expectations for both COVID-19-specific and non-COVID-19-specific tests include younger age {21–40 years: (3.61 [1.26–10.38]); 41–60 years: (4.49 [1.43–14.13])}, adherence to employer's health policy (2.94 [1.41–6.14]), being worried about contracting COVID-19 (2.95 [1.45– 5.99]), and during the transition (2.03 [1.02–4.06]) and mitigation (2.02 [1.03–3.97]) phases of the pandemic.

Conclusion: Patients' expectations for diagnostic tests during ED visits for uncomplicated URTI were dynamic across the COVID-19 pandemic phases. Expectations for COVID-19-specific diagnostic tests for ED visits for uncomplicated URTI were higher among younger individuals and those worried about contracting COVID-19 during the COVID-19 pandemic. Future studies are required to enhance public communications on the availability of diagnostic services in primary care and public education on self-management of emerging infectious diseases such as COVID-19.

KEYWORDS

emergency medicine, upper respiratory tract infection (URTI), COVID-19, diagnostic services, pandemic (COVID-19), emergency department (ED) utilization

Introduction

Hospital emergency departments (EDs) are vital components of the healthcare system as they provide immediate acute care for patients presenting with urgent medical conditions that may be life-threatening (1). However, growing reliance on emergency services has resulted in overcrowding in EDs globally (2, 3). Non-urgent ED attendances, which account for 9%—60% of ED visits, are among the main contributors to the problem of overcrowding in EDs (4, 5). Medical conditions that are manageable in the primary care setting account for a substantial proportion of non-urgent visits to the ED, resulting in overcrowding, long waiting times, increased healthcare-associated costs, high staff burden, and suboptimal use of hospital resources (2, 6). The quality of emergency services will be compromised if the rising numbers of non-urgent visits further strain the already overworked ED staff (2, 7, 8).

Patients seek non-urgent care in the ED for a myriad of reasons. These reasons include lack of access to primary healthcare facilities, lack of diagnostic services in primary care, referrals from primary care facilities, easy access to EDs, patient perceptions regarding the severity of their condition, and perceptions of better care in EDs (2, 3, 5, 9, 10). Efforts to mitigate ED overcrowding, such as teleconsultation, education on appropriate usage of ED services, and improving primary care access, have shown mixed results (11–13).

Despite modest success in efforts to alleviate the patient load pressure in the ED, the unprecedented coronavirus disease 2019 (COVID-19) pandemic has changed the health-seeking behavior of the public (14). The uncertainties surrounding the pandemic led to a surge in the number of patients presenting with URTI symptoms seeking emergency care worldwide, including Singapore (15–17). The emergency departments in public hospitals experienced a surge in demand regarding COVID-19-related issues, leading to healthcare worker fatigue and longer wait times (18, 19). Though these visits were acceptable during the initial stages of the pandemic, their urgency and relevance were reduced as the majority of the population was vaccinated through the National Vaccination Programme in Singapore (20). From 15 September 2021, the Ministry of Health advised low-risk vaccinated individuals diagnosed with COVID-19 to recover from home through the Home Recovery Programme or seek care from primary care facilities. Pre-pandemic, patients with uncomplicated URTI usually seek care in government-funded or private primary care clinics in their neighborhood to obtain a diagnosis. ED services are reserved for intermediate- to high-risk patients requiring urgent acute care (21).

Although the non-severity of COVID-19 among the highly vaccinated population rendered ED visits for uncomplicated URTI symptoms unnecessary, patients presenting with suspected COVID-19 and uncomplicated URTI symptoms continued to seek medical care at EDs in Singapore. The lack of awareness of national protocols devised for the public on self-management and the appropriate channels for seeking care, coupled with anxiety associated with contracting COVID-19, could have resulted in such ED visits (15).

With the evolving COVID-19 pandemic affecting the heath-seeking behavior of the public, it is imperative to understand patients' expectations and the reasons for these ED visits to optimize ED resources and design interventions that can reduce inappropriate ED visits. Anchoring on Andersen's healthcare utilization model, we assessed the factors influencing patients' expectations for diagnostic tests during their ED visits for uncomplicated URTI during the different phases of the pandemic.

Abbreviations: CCI, Charlson's co-morbidity index; ED, Emergency department; URTI, Upper respiratory tract infection.

Methods

Study design and setting

We conducted a cross-sectional survey on adults seeking medical care in the ED for uncomplicated URTI during the COVID-19 pandemic. Our study covered the EDs of four acute public hospitals, constituting 40% of the public hospitals in Singapore. Given the dynamic situation of the pandemic, we segmented the study period into three phases.

Phase 1: containment (15 March 2021–7 May 2021)

The containment phase includes the period when Singapore adopted a zero COVID-19 policy. All known COVID-19-positive cases were isolated, and the authorities conducted extensive contact tracing to break possible chains of transmissions.

Phase 2: transition (8 May 2021–31 August 2021)

The transition phase was when the Delta wave occurred in the community. The Delta strain's higher transmissibility rendered contact tracing and containment impractical. On 8 May 2021, the Multi-Ministry Taskforce announced new measures to bring down the rates of COVID-19 community transmission.

Phase 3: mitigation (1 September 2021–2 March 2022)

As vaccination coverage in the population increased to >70%, safe COVID-19 management measures were relaxed, and the COVID-19 home recovery program was introduced to ease the demand for healthcare services. We termed this phase "mitigation" as the government announced in September 2021 that each household would receive free antigen rapid test (ART) kits to self-manage COVID-19 at home.

Participants

We recruited adults who attended the EDs between March 2021 and March 2022. Patients must be diagnosed with an uncomplicated URTI (ICD-10 J00-J06) to meet the study inclusion criteria. We excluded patients admitted to the hospital and those with prior attendances to the ED within 30 days to avoid recruiting patients with complicated URTIs. COVID-19 suspects, identified through triage, were initially excluded from the study due to a default hospitalization policy. We included these patients from July 2021 onward following the revision of the national policy which encouraged home recovery for COVID-19 patients when more than 70% of the population had received at least one dose of the COVID-19 vaccination. A hospital admission was unnecessary when the presentation of COVID-19 symptoms became milder in the patient population. Consecutive patients were screened and monitored at triage; eligible patients were approached and recruited only after their medical consultation at the point of discharge from the emergency department.

Questionnaire

The questionnaire was interviewer-administered by trained data collectors to enable consistency across data collection. We validated the questionnaire with emergency physicians and other healthcare professionals and piloted the questionnaire in the ED to ensure its viability. In addition to collecting the patient's demographics (age, gender, race, nationality, and education level), we collected information on the health status of patients (smoking status and Charlson's co-morbidity index) and factors that could be associated with their health-seeking behavior (reasons for the ED visit, any prior healthcare consultation for URTI, employment status, and payment method). Participants can select the reasons for their ED visit from a list or indicate additional reasons as free text. The list of reasons for ED visits is shown in Table 1.

Outcomes

There were four outcome variables for this study: whether patients expected (1) a COVID-19-specific diagnostic test, (2) a non-COVID-19-specific diagnostic test (i.e., blood test, flu virus test, or chest X-ray), (3) both COVID-19-specific and non-COVID-specific diagnostic tests, or (4) no diagnostic test. COVID-19-specific diagnostic tests included SARS-CoV-2 antigen rapid test or polymerase chain reaction test.

Classification with Andersen's healthcare utilization model

We employed Andersen's healthcare utilization model to analyze the drivers of ED visits for uncomplicated URTI (22). This model describes the utilization of healthcare services as a function of three core factors:

(1) Predisposing factors—demographic and psychosocial features influencing health-seeking behavior,
(2) Enabling factors—factors facilitating access to healthcare services, and
(3) Individual needs—an individual's perception regarding his/her health condition and need for health services.

We classified demographic characteristics (age, gender, race, and nationality), health status of individuals, and psychosocial factors (advised by loved ones to visit ED, trust in ED's quality of care) under predisposing factors as these factors have an impact on an individual's health-seeking behavior. Factors that facilitated the utilization of ED services by patients were classified under enabling factors, and patients' concerns about their health condition were

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TABLE 1 Classification of data variables and reasons for visiting the ED according to Andersen's healthcare utilization model.

Andersen's category	Model variables	
Predisposing factors	Age	
	Gender	
	Race	
	Nationality	
	Education	
	Charlson's comorbidity severity	
	Smoking status	
	Reasons for visiting the ED	
	Advised by family/friends/colleagues to visit the ED	• My family member/friend/colleague/other people (not doctor) advised me to seek care at the emergency department.
	Trust that ED is high quality and thinks it is better than primary care clinics	• I think that the care that I will receive from the emergency department for my illness is better than that from GP clinics/polyclinics. • I trust the quality of care that this emergency department provides. • I want a more thorough checkup for my current illness. • Faster treatment
Enabling factors	Employment status	
	Payment method	
	Reasons for visiting the ED	
	Bill covered by an employer	• The medical bill for the emergency department visit is covered by my employer/insurance.
	Adhering to the employer's policy	• My company requires me to obtain a medical certificate from a public healthcare institution. • URTI protocol for healthcare workers • Full-time national servicemen URTI protocol.
	Referred by healthcare provider	• Singapore Civil Defense Force ambulance • Referred by specialists/other clinics. • Ministry of Health's advice on COVID
	Convenient	• I live/work close (within 3 km) to this emergency department. • This emergency department is open 24 h, and I can attend at my convenience/clinic closed. • I have previously attended/been admitted to this hospital and have medical records here.
Individual needs	**Reason for visiting the ED**	
	Having persistent symptoms/conditions for their illness	• I have a persistent cough/runny nose/sore throat/other respiratory symptoms. • I have a persistent fever. • My condition has not improved although I have consulted a GP/polyclinic doctor.
	Thinks that illness is severe	• My cough/runny nose/sore throat/other respiratory symptoms are very severe. • I am worried that I may have dengue. • I am worried I may have a serious infection. • I am worried I may have a serious disease. • I have a very high fever.
	Worried about contracting COVID-19	• I am worried that I may have COVID-19.

classified under individual needs. The reasons for visiting the ED were grouped to reduce the number of variables.

Analysis

We first performed univariate analyses on each independent variable to assess the differences between the outcome categories. Categorical variables were assessed using Pearson's chi-square test, while categories with a small number of variables were assessed using the Fisher–Freeman–Halton exact test. Continuous variables were assessed using non-parametric tests.

Next, we used the backward stepwise selection method to build a multinomial regression model. The initial model included variables with $p < 0.25$ from the univariate analyses. Variables were individually dropped from the model if it resulted in a lower

Akaike's information criteria and/or Bayesian information criteria value. The Charlson's co-morbidity index (CCI) was computed and classified into three categories (no co-morbidity—CCI 0, mild—CCI 1-2, moderate/severe—score CCI > 2) (23).

In addition, we present a bar chart to list the reasons our participants attend the ED. All analyses were performed with Stata version 15.0 (StataCorp LP, College Station, TX) and RStudio version 2022.02.3 (RStudio, PBC, Boston, MA).

Ethical approval

This study was approved by the National Healthcare Group Domain Specific Review Board in Singapore. NHG DSRB Ref: 2019/00174. Written consent was sought from participants for participating in this study.

Results

We screened 5,319 patients who visited the ED, of whom 1,234 were eligible. Of those eligible for the study, 683 (56%) consented to our study. Two participants were later excluded from the study as they did not meet the inclusion criteria after subsequent changes to their clinical statuses. We eventually analyzed the data of 681 participants.

Baseline characteristics and univariate analyses of respondents

The baseline characteristics and univariate analyses of participants' expectations for diagnostic tests are shown in Table 2.

Predisposing factors

The mean age of participants was 34.5 (Min: 21, Max: 88) years. Participants expecting only a COVID-19 diagnostic test had the lowest mean age (31.1 years), while those expecting non-COVID-specific diagnostic tests had the highest mean age (40.4 years). Half of the participants were men (49.8%), 46.1% were of the Chinese race, 73.1% were Singaporeans, and 32.9% had tertiary education (bachelor's degree and above). Most participants (91.2%) had no pre-existing co-morbidities, 78.1% were non-smokers, and a third (30.4%) had a prior (non-ED) consult for URTI. A significantly lower proportion of participants (17.2%) who expected only the COVID-19 test had a prior healthcare consult for the same episode of illness compared with other groups ($p < 0.001$). Half of the participants (52.6%) visited the ED because they trusted the ED to provide high-quality care and felt that ED care was better than primary care, and 28.2% reported that their close contacts (i.e., family, friends, and colleagues) advised them to seek care in the ED.

Enabling factors

Three-quarters (75.0%) of the participants were employed, and 16.2% were full-time national servicemen. A significantly higher proportion (82.2%) of employed participants were expecting only a COVID-19 diagnostic test ($p < 0.001$). Of the types of healthcare financing, 59.8% had employee benefits, 30.9% had to pay out-of-pocket, and the rest had some form of insurance or subsidy. A significantly higher proportion (73.9%) of participants with employee healthcare benefits were expecting only a COVID-19 diagnostic test ($p < 0.001$).

We grouped participants into three COVID-19 phases based on the date they visited the ED. 37.4% of participants visited the ED during the containment phase, 40.4% during the transition phase, and 22.2% in the mitigation phase. Almost half of ED attendees in the containment phase were not expecting any diagnostic test (50.7%), while half (49.0%) in the transition phase expected a COVID-19 test during their ED visit.

Of the enabling factors for which participants visited the ED for URTI, 28.6% indicated that their employer would cover their bill, 44.1% indicated that they were adhering to their employer's health policy, 23.2% were referred by a healthcare provider, and 68.4% cited convenience. Significantly lower proportions of participants not expecting any diagnostic tests in the ED visited the ED in accordance with their employer's health policy (16.4%) or had their hospital bill covered by their employer (16.4%) ($p < 0.001$ for both), while a significantly higher proportion of those expecting non-COVID-19-specific diagnostic tests (39.2%) were referred to the ED by other healthcare providers ($p < 0.001$).

Individual needs

Of participants visiting the ED to fulfill individual needs, 39.1% cited having persistent symptoms or illness, 42.4% thought that their illness was severe, and 30.8% were worried about contracting COVID-19. Most participants (90.8%) were satisfied with their ED visit. A significantly higher proportion (39.5%) of participants worried about contracting COVID-19 were expecting both COVID-19-specific and non-COVID-specific diagnostic tests ($p < 0.001$), while a significantly lower proportion (32.4%) of participants who perceived their illness as severe expected only a COVID-19 diagnostic test.

Determinants of expecting a diagnostic test in the ED during the COVID-19 pandemic

Expect only a COVID-19-specific test

Predisposing factors

Participants aged 21–40 were almost three times (adjusted odds ratio (aOR): 2.98, 95% confidence interval (CI) [1.04–8.55]) as likely as those aged above 60 to expect a COVID-19-specific diagnostic test during their ED visit (Table 3). Those without a prior clinical consultation for the same illness were also twice (2.10 [1.13–3.89]) as likely to expect a COVID-19-specific diagnostic test during their visit.

Enabling factor

Participants adhering to their employer's health policy to visit the ED for suspected COVID-19 were 3.7 times (3.70 [1.79–7.67]) as likely to expect only a COVID-19-specific diagnostic test.

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TABLE 2 Baseline characteristics and univariate analysis of patients by expectation for diagnostic services in the ED.

Baseline characteristics of respondents, n (%)	All patients	Not expecting a diagnostic test	Expects a COVID-19-specific test	Expects a non-COVID-19-specific test	Expects COVID-19-specific + non-COVID-19-specific tests	*P*-value
	(*N* = 681)	(*N* = 73)	(*N* = 296)	(*N* = 74)	(*N* = 238)	
Predisposing factors	n (%)	n (%)	n (%)	n (%)	n (%)	
Age, mean (Min, Max)	34.5 (21, 88)	38.2 (21, 77)	31.1 (21, 75)	40.4 (21, 73)	35.1 (21, 88)	<0.001^
Aged 21–40	517 (75.9%)	48 (65.8%)	252 (85.1%)	45 (60.8%)	172 (72.3%)	
Aged 41–60	123 (18.1%)	16 (21.9%)	34 (11.5%)	16 (21.6%)	57 (23.9%)	
Aged above 60	41 (6.0%)	9 (12.3%)	10 (3.4%)	13 (17.6%)	9 (3.8%)	
Male	339 (49.8%)	38 (52.1%)	154 (52.0%)	34 (45.9%)	113 (47.5%)	0.640
Race						
Chinese	314 (46.1%)	35 (47.9%)	146 (49.3%)	29 (39.1%)	104 (43.7%)	0.745
Malay	174 (25.6%)	18 (24.7%)	65 (22.0%)	21 (28.4%)	70 (29.4%)	
Indian	114 (16.7%)	12 (16.4%)	51 (17.2%)	15 (20.3%)	36 (15.1%)	
Other races	79 (11.6%)	8 (11.0%)	34 (11.5%)	9 (12.2%)	28 (11.7%)	
Nationality						
Singaporean	498 (73.1%)	49 (67.1%)	225 (76.0%)	52 (70.3%)	172 (72.3%)	0.303
Permanent resident	68 (10.0%)	8 (11.0%)	32 (10.8%)	5 (6.8%)	23 (9.7%)	
Others	115 (16.9%)	16 (21.9%)	39 (13.2%)	17 (23.0%)	43 (18.1%)	
Tertiary education	224 (32.9%)	20 (27.4%)	91 (30.7%)	28 (37.8%)	85 (35.7%)	0.348
Charlson's comorbidity severity						
No comorbidity	621 (91.2%)	64 (87.7%)	282 (95.3%)	61 (82.4%)	214 (90.3%)	0.021#
Mild	52 (7.6%)	8 (11.0%)	13 (4.4%)	11 (14.9%)	20 (8.4%)	
Moderate/Severe	8 (1.2%)	1 (1.4%)	1 (0.3%)	2 (2.7%)	4 (1.7%)	
Smoker	149 (21.9%)	23 (31.5%)	65 (22.0%)	10 (13.5%)	51 (21.4%)	0.071
Prior (Non-ED) consult for URTI	207 (30.4%)	34 (46.6%)	51 (17.2%)	37 (50.0%)	85 (35.7%)	<0.001
Reason: Advised by family/friends/colleagues to visit the ED	192 (28.2%)	15 (20.5%)	96 (32.4%)	17 (23.0%)	64 (26.9%)	0.115
Reason: Trust that ED is high quality and thinks it is better than primary care clinics	358 (52.6%)	38 (52.1%)	144 (48.6%)	41 (55.4%)	135 (56.7%)	0.294
Enabling factors						
Payment method	(*N* = 676)	(*N* = 72)	(*N* = 295)	(*N* = 73)	(*N* = 236)	
Employee benefits	404 (59.8%)	28 (38.9%)	218 (73.9%)	27 (37.0%)	131 (55.5%)	<0.001#
Government/private insurance	54 (8.0%)	9 (12.5%)	13 (4.4%)	10 (13.7%)	22 (9.3%)	
Out-of-pocket	209 (30.9%)	33 (45.8%)	62 (21.0%)	35 (47.9%)	79 (33.5%)	
Social subsidies	9 (1.3%)	2 (2.8%)	2 (0.7%)	1 (1.4%)	4 (1.7%)	
Employment						
Employed	511(75.0%)	60 (82.2%)	201 (67.9%)	55 (74.3%)	195 (81.9%)	<0.001

(Continued)

TABLE 2 (Continued)

Baseline characteristics of respondents, n (%)	All patients	Not expecting a diagnostic test	Expects a COVID-19-specific test	Expects a non-COVID-19-specific test	Expects COVID-19-specific + non-COVID-19-specific tests	*P*-value
	(*N* = 681)	(*N* = 73)	(*N* = 296)	(*N* = 74)	(*N* = 238)	
Not employed	60 (8.8%)	7 (9.6%)	16 (5.4%)	17 (23.0%)	20 (8.4%)	
NSF	110 (16.2%)	6 (8.2%)	79 (26.7%)	2 (2.7%)	23 (9.7%)	
Reason Bill covered by an employer	195 (28.6%)	12 (16.4%)	111 (37.5%)	16 (21.6%)	56 (23.5%)	<0.001
Reason: Adhering to the employer's health policy	300 (44.1%)	12 (16.4%)	170 (57.4%)	18 (24.3%)	100 (42.0%)	<0.001
Reason: Referred by healthcare provider	158 (23.2%)	23 (31.5%)	42 (21.4%)	29 (39.2%)	64 (26.9%)	<0.001
Reason: Convenient	466 (68.4%)	46 (63.0%)	220 (74.3%)	51 (68.9%)	149 (62.6%)	0.023
Individual needs						
Reason: Having persistent symptoms/conditions for their illness	266 (39.1%)	31 (42.4%)	99 (33.4%)	29 (39.2%)	107 (45.0%)	0.051
Reason: Thinks that illness is severe	289 (42.4%)	34 (46.6%)	96 (32.4%)	40 (54.1%)	119 (50.0%)	<0.001
Reason: Worried about contracting COVID-19	210 (30.8%)	13 (17.8%)	94 (31.8%)	9 (12.2%)	94 (39.5%)	<0.001
COVID-19 phases						
Containment	255 (37.4%)	37 (50.7%)	105 (35.5%)	34 (46.0%)	79 (33.2%)	<0.01
Transition	275 (40.4%)	16 (27.4%)	145 (49.0%)	21 (28.4%)	93 (39.1%)	
Mitigation	151 (22.2%)	20 (27.4%)	46 (15.5%)	19 (25.7%)	66 (27.7%)	

[#] Fisher–Freeman–Halton test.
[^] Kruskal–Wallis test.
NSF, Full-time national servicemen.

Individual needs

Participants who thought their illness was not severe were 2.5 times (2.50 [1.39–4.55]) as likely as those who perceived their illness to be severe to expect a COVID-19-specific diagnostic test. Those worried about contracting COVID-19 were also 2.3 times (2.29 [1.11–4.69]) as likely to expect a COVID-19-specific diagnostic test.

COVID-19 phases

Compared with the containment phase, participants in the transition phase were 2.3 times (2.29 [1.15–4.56]) as likely to expect only a COVID-19-specific diagnostic test.

Expect a non-COVID-19-specific test

Enabling factor

Non-employed participants were 3.8 times (3.83 [1.26–11.66]) as likely as employed participants to expect non-COVID-19-specific diagnostic tests. All other factors were not statistically significant.

Expect both COVID-19-specific and non-COVID-19-specific tests

Predisposing factor

Participants aged 21–40 and 41–60 were 3.6 (3.61 [1.26–10.38]) and 4.5 times (4.49 [1.43–14.13]) as likely as adults above 60 years old to expect both COVID-19-specific and non-COVID-19-specific diagnostic tests during their ED visit (Table 3).

Enabling factor

Participants adhering to their employer's health policy to visit the ED for suspected COVID-19 were almost three times (2.94 [1.41–6.14]) as likely to expect both COVID-19-specific and non-COVID-19-specific diagnostic tests.

Individual needs

Participants were almost three times (2.95 [1.45– 5.99]) as likely to expect both COVID-19-specific and non-COVID-19-specific diagnostic tests if they were worried about contracting COVID-19.

COVID-19 phases

Compared with the containment phase, participants in the transition (2.03 [1.02–4.06]) and mitigation (2.02 [1.03–3.97])

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TABLE 3 Multinomial logistic regression of expectation for ED diagnostic services.

Andersen's category	Model variables	Expects a COVID-19-specific test	Expects a non-COVID-19-specific test	Expects a COVID-19-specific + non-COVID-19-specific tests	VIF
	(Base: Not expecting any tests)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	Adjusted OR (95% CI)	
	Age				
Predisposing	Aged above 60	Ref	Ref	Ref	
	Aged 41–60	2.35 (0.73, 7.60)	0.92 (0.28, 2.99)	4.49 (1.43, 14.13)*	3.66
	Aged 21–40	2.98 (1.04, 8.55)*	0.89 (0.32, 2.49)	3.61 (1.26, 10.38)*	3.50
	Prior (non-ED) healthcare consult for URTI				
Predisposing	No prior consult	2.10 (1.13, 3.89)*	0.89 (0.44, 1.79)	1.12 (0.62, 2.03)	1.20
	Employment				
Enabling	Employed	Ref	Ref	Ref	
	Not employed	2.38 (0.79, 7.20)	3.83 (1.26, 11.66)*	1.74 (0.60, 5.08)	3.21
	Full-time national servicemen	1.99 (0.76, 5.25)	0.33 (0.06, 1.81)	0.85 (0.31, 2.37)	3.40
	Payment method				
Enabling	Out-of-pocket	Ref	Ref	Ref	
	Employee benefits	1.80 (0.92, 3.52)	1.06 (0.48, 2.36)	1.46 (0.76, 2.81)	1.60
	Government/private insurance	0.85 (0.31, 2.31)	0.84 (0.29, 2.44)	1.18 (0.47, 2.95)	1.20
	Social subsidies	0.36 (0.04, 3.20)	0.24 (0.02, 3.11)	0.64 (0.09, 4.47)	1.12
	Employers' policy				
Enabling	Adheres to employers' policy	3.70 (1.79, 7.67)**	1.95 (0.80, 4.79)	2.94 (1.41, 6.14)*	1.12
	Perception of illness severity				
Individual need	Thinks that illness is severe	0.40 (0.22, 0.72)*	1.36 (0.68, 2.70)	0.83 (0.47, 1.47)	1.13
	Concerns about contracting COVID-19				
Individual need	Worried about contracting COVID-19	2.29 (1.11, 4.69)*	0.51 (0.19, 1.36)	2.95 (1.45, 5.99)*	1.32
	COVID-19 phases				
	Containment	Ref	Ref	Ref	
	Transition	2.29 (1.15, 4.56)*	1.37 (0.60, 3.16)	2.03 (1.02, 4.06)*	1.34
	Mitigation	1.04 (0.51, 2.11)	0.96 (0.42, 2.18)	2.02 (1.03, 3.97)*	1.30

*$p < 0.05$, **$p < 0.001$.
VIF, Variance inflation factor.

phases were twice as likely to expect both COVID-19-specific and non-COVID-19 specific diagnostic tests during their ED visit.

Reasons for patients with URTI to visit the ED

Figure 1 shows the reasons for visiting the ED during different phases of the COVID-19 pandemic. The reasons are grouped by the three determinant components of Andersen's healthcare utilization model. A larger proportion of participants reported more reasons during the containment and transition phase of COVID-19 compared with the mitigation phase.

The top enabling factor for visiting the ED for URTI throughout all phases of the COVID-19 pandemic is the convenience of living or working near the ED. More than half of the participants cited this reason during the containment (57.6%) and transition (65.1%) phases, while 27.1% cited this reason during the mitigation phase. Similarly, more participants cited a "requirement for obtaining a medical certificate" and healthcare financing (i.e., bill covered by employer or insurance) during the containment and transition phase compared with the mitigation phase. The proportion of participants citing "bill covered by their employer or insurance" was notably lower during the mitigation phase (2.6%) compared with the other phases (∼30– 40%). A fifth of participants cited that they were "referred to the ED by a primary care doctor" consistently over the three COVID-19 phases, while a quarter of them (25.8%) cited "employer's policy for healthcare workers to visit the ED for URTI symptoms" during the transition phase.

The perceived need to visit the ED did not vary too much across the COVID-19 phases, except that the worry of contracting COVID-19 was more pronounced during the transition phase. Reasons such as having "persistent URTI symptoms" and "worried about contracting COVID-19" were the top two perceived needs for visiting the ED.

The top predisposing factor for visiting the ED during all phases of the COVID-19 pandemic is the trust in the quality of care the ED provides. This reason was pronounced during the containment and transition phase compared with the mitigation phase (>40% vs. 16.5%). Other notable reasons include a desire to "seek a more through check-up", "advised by friends/ family/ colleagues" to visit the ED and thinks that "ED care is better than primary care".

Discussion

Our study has provided invaluable insights into patients' changing expectations for diagnostic tests during ED visits for uncomplicated URTI during the COVID-19 pandemic. Applying Andersen's healthcare utilization model enabled us to ascertain factors influencing patient expectations for ED visits, which would guide future pandemic preparedness planning in preventing the overwhelming use of ED resources. We observed that being younger and not having a prior medical consultation for URTI in other healthcare institutions were significant predisposing factors influencing expectations for a COVID-19-specific diagnostic test. Enabling factors for expecting a COVID-19-specific diagnostic test include adherence to employment policy and the COVID-19 pandemic phase, while individual needs include non-perception of illness severity and worry about contracting COVID-19.

Adults aged 60 years and below were more likely than their older counterparts to expect a COVID-19-specific test or a combination of COVID-19-specific and non-COVID-19-specific diagnostic tests during their ED visit for uncomplicated URTI. There is growing evidence of young adults having disproportionately higher non-urgent ED visits (3). Studies in other countries have found that convenience, concern about the seriousness of symptoms and desire for reassurance, influence from friends and family, and negative perceptions about alternatives, such as primary care providers, are reasons young adults seek non-urgent ED care (3). We found similar reasons, such as the desire to seek a more thorough check-up, being advised by friends, family, or colleagues to visit the ED, and thinking that ED care is better than primary care. We also found that the top predisposing reason for visiting the ED during the COVID-19 pandemic was trust in the quality of ED care. With the ED equipped with services that many primary care facilities lack, such as X-ray and blood test services (24), patients can receive a more thorough examination by visiting the ED. As such, patients may deem ED services superior to primary care facilities (25), which likely contributed to the surge of non-urgent ED visits for URTI during the containment and transition phases of the COVID-19 pandemic in Singapore.

We found that non-employed participants were 3.8 times more likely to expect non-COVID-19-specific diagnostic tests during their ED visit. These non-employed participants were likely retired older adults presenting to the ED with non-specific symptoms that are hard to differentiate from acute symptoms (26). Some patients may prefer specialist care and easy access to radiologic and laboratory diagnostic tests in a one-stop center such as an ED (27). Furthermore, patients may also perceive the all-inclusive (medical consultation and diagnostic tests) fixed ED cost to be lower than the combined charges of consultation fees and individual diagnostic tests in primary care clinics.

The employer's health policy was a significant enabling factor for patients expecting only COVID-19-specific tests and both COVID-19-specific and non-COVID-19-specific diagnostic tests at the ED. This finding was unsurprising as newly devised URTI protocols for healthcare workers encouraged them to seek care at their respective institutions if they experienced URTI symptoms, for which employee benefits covered the costs. Hence, the convenience of accessing ED services and reduced medical fees in the form of employee benefits, insurance, or subsidies made visiting the ED a natural choice for many healthcare workers (3, 25).

Expectations for ED diagnostic tests were dynamic across the COVID-19 pandemic phases. Twice as many participants expected either only a COVID-19-specific diagnostic test or both COVID-19-specific and non-COVID-19-specific tests during the transition phase compared with the containment phase. However, the expectation for both COVID-19-specific and non-COVID-19-specific diagnostic tests remained twice as high during the mitigation phase as the containment phase. Notably, the expectation for only a COVID-19-specific diagnostic test was

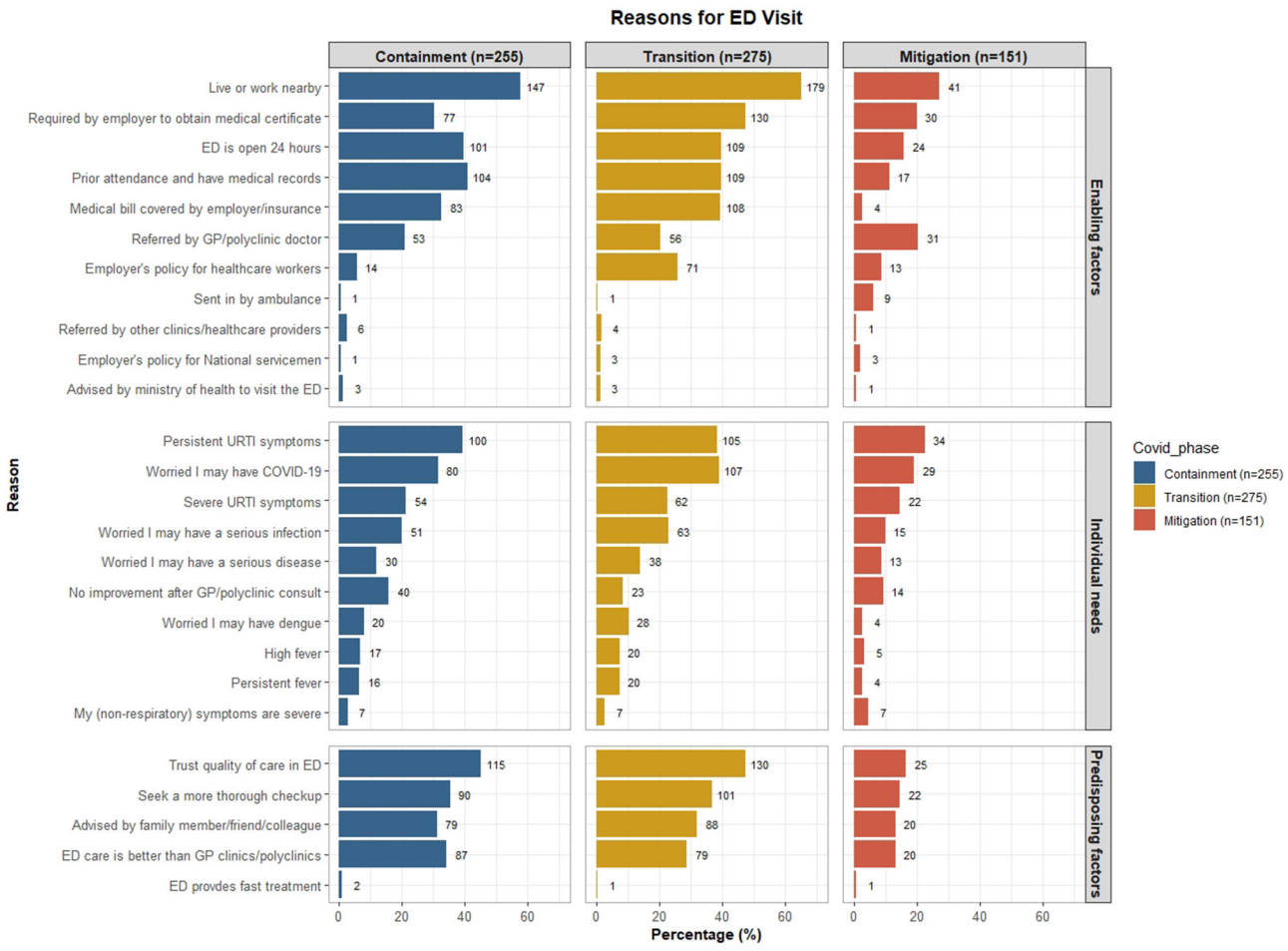

FIGURE 1
Reasons for visiting the ED during different phases of the COVID-19 pandemic, grouped by the three determinant components of Andersen's healthcare utilization model. Participants can list more than one reason.

similar between the mitigation and containment phases. The change in expectations regarding COVID-19-specific tests in the mitigation phase is likely due to the availability of COVID-19 self-test kits in the community, which became more readily available with the nationwide distribution of the kits to every household by the Singaporean government.

As anticipated, patients worried about contracting COVID-19 were more likely to expect a COVID-19-specific diagnostic test. However, those who thought that their illness was severe were less likely to expect any diagnostic test. This observation could be attributed to the wide range of patient expectations when seeking emergency care. Patients may have greater expectations for the care and medical services of the ED visit beyond mere diagnostics services (28, 29). As evident from our findings, patients have many reasons for visiting the ED, such as having persistent URTI symptoms and worries about a severe infection. These individual needs exist across the COVID-19 pandemic phases and may not be related to the pandemic. Therefore, unnecessary ED visits may persist even after the COVID-19 pandemic, and broader considerations are required to design interventions for mitigating inappropriate ED use.

The strengths of our study include measuring the changes in the population's healthcare-seeking behavior over the various phases of the pandemic and surveying actual URTI patients in the ED to obtain real-world responses. To the best of our knowledge, this is the first study analyzing the reasons behind ED visits in patients with uncomplicated URTI during the pandemic, and the findings would be useful for addressing gaps in public health communications during the pandemic. However, our study is limited by not assessing the arrival time of patients attending the ED. Patients visiting the ED after office hours may have different reasons for making non-urgent ED visits and hence have differing expectations in the ED. There may also be recall bias as this was a self-reported cross-sectional study, although the bias was likely to be minimal as all participants completed the survey at the ED visit. We could not comprehensively assess the motivations for which participants attended the ED, which could shed light on other changing needs across the pandemic phases. Future studies could take

a mixed-methods approach to enhance the interpretation of our findings.

Despite the milder disease presentation of COVID-19 in a highly vaccinated population and the government's encouragement of people with mild COVID-19 symptoms to visit primary care clinics, individuals with URTI symptoms continue to visit the ED with the expectation of receiving a COVID-19 diagnostic test during the mitigation phase. These patients could be confused about the dynamic national COVID-19 policies communicated to them over the course of the evolving pandemic (30). Although the government's communications on COVID-19 measures were frequent and transparent, their approach toward managing the pandemic was reactive rather than proactive (31). The constantly changing rules on safe management measures inadvertently caused confusion and significant anxiety among the public (30). Therefore, besides redirecting patients to primary care facilities such as public health preparedness clinics, strengthening the healthcare system's adaptability to changes and improving public communications are essential measures to enhance national preparedness for the next pandemic. For instance, tailoring public communication strategies to the different pandemic phases, such as encouraging young people to seek care at primary care clinics during the transition phase of the pandemic, might help to divert resources to those who need the service more.

Conclusion

In conclusion, patients' expectations for diagnostic tests during ED visits for uncomplicated URTI were dynamic across the COVID-19 pandemic phases. With the widespread availability of self-test kits during the mitigation phase, expectations for diagnostic tests shifted to having both COVID-19-specific and non-COVID-19-specific tests. Expectations were also higher among younger individuals and those worried about contracting COVID-19 during the pandemic. Enabling factors such as convenience, the requirement to obtain a medical certificate, and adherence to employers' health policy for URTI were pronounced during the transition phase. Other factors, including the public's perception of better care for URTI at EDs than at primary care clinics, were beyond the pandemic and should be addressed post-pandemic. Future work is required to enhance public communications on the availability of diagnostic services in primary care and public education on self-management of emerging infectious diseases such as COVID-19.

Data availability statement

The datasets presented in this article are not readily available because the data contains sensitive information. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving humans were approved by National Healthcare Group Domain Specific Review Board in Singapore. 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

ZH contributed to conceptualization, methodology, formal analysis, data curation, writing—original draft, project administration, and supervision. KN contributed to data collection, writing—original draft, and writing—review and editing. WK, HL, YW, and HT contributed to project administration and writing—review and editing. ES, JO, and LP contributed to writing—review and editing. AC contributed to conceptualization, methodology, supervision, funding acquisition, 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.

Publisher's note

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Investigation of two norovirus outbreaks linked to drinking water contaminated with multiple GII strains in a rural county—Chongqing, China, 2021

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Backgrounds: Norovirus is leading cause of non-bacterial gastroenteritis outbreaks globally, characterized by different strains prevalent in different countries and regions.

Methods: Cases were defined as individuals experiencing diarrhea ≥3 times/24 h, and/or vomiting ≥2 times/24 h in two villages between January 28 and February 9, 2021. Investigations were conducted to identify causes. Cases were interviewed using a standardized in-person form to collect data on potential risk factors. A retrospective cohort study was conducted to investigate the role of the spring water supply as the outbreak source. Residents from neighboring villages with different water sources served as the unexposed population. Stool specimens, rectal swabs, and water samples were tested using quantitative real-time Polymerase Chain Reaction, with subsequent sequencing performed on pathogen-positive specimens.

Results: Village-specific attack rates were 21.93% (123/561) and 26.99% (88/326), respectively. Evidence from both epidemiological and laboratory tests was consistent. Drinking spring water was statistically associated with the two outbreaks (RR = 41.8 and 79.2, respectively). In both outbreaks, stool specimens, rectal swabs, and water samples tested positive for norovirus. Specifically, GII.2 (P16) and GII.17 (P17) were identified in outbreak A, and GII.4 Sydney (P16) and GII.1 (P16) in outbreak B.

Conclusion: These two independent gastroenteritis outbreaks share similarities, both being linked to norovirus GII strains. The contaminated spring drinking water was identified as the probable source and was promptly closed and subjected to disinfection procedures. These findings reinforce the importance of implementing sanitation and environmental disinfection measures in rural areas, especially during the periods of increased rainfall.

KEYWORDS

norovirus, GII strains, outbreak, drinking water, rural county, China

1 Introduction

Human norovirus is one of the most prevalent pathogens causing acute gastroenteritis worldwide (1). It is extremely contagious, with an estimated infectious dose of as minimal as 18 viral particles (2). Norovirus can be transmitted through various routes, including waterborne, foodborne, or person-to-person contact. Multiple transmission routes involving in one norovirus outbreak is not rare (3–7). The transmission route in a norovirus outbreak varies based on the setting and geographical region. In China, 93% of the norovirus outbreaks occurred in schools, the dominant transmission route was person-to-person contact, accounting for 63% of all norovirus outbreaks reported to the National Public Health Emergency Event Surveillance System from 2014 to 2017, and waterborne transimission only accounted for 3.4% (8).

In China, the GII (genogroup II) was mostly detected genogroup in norovirus outbreaks. From October 2016 to September 2018, the most commonly reported strain in norovirus outbreaks was GII.2 (P16) (9). From 2014 to 2018, multiple GII strains were identified in norovirus outbreaks, and the dominant GII strains showed temporal variations in different cities (10–12). For instance, in Jiangsu, China, the GII.2 (P16) strain was mostly identified in norovirus outbreaks from January 2015 to December 2018 (10). In Huzhou, China, the predominant strains in norovirus outbreaks changed from GII.4 variants to GII.17 (P17) in 2014–2015, followed by GII.3 (P12) in 2015–2016, and later GII.2 (P16) in 2016–2018 (11). In Guangdong, China, from 2013 to November 30, 2017, the majority of reported norovirus outbreaks were typed as GII, with GII.17 strain and GII.2 strain peaked during winter seasons of 2014–2015 and 2016–2017, respectively (12).

In 2011, the first norovirus infectious diarrhea case was reported in Chongqing, China. From 2011 to 2016, a total of 121 norovirus epidemic events (clustered epidemics and outbreaks) were reported in Chongqing. These events involved 1,637 cases, with no reported fatalities. Nearly all epidemic events occurred in schools, and 80% were transmitted through person-to-person contact (13). Among the 121 epidemic events, the GII.2 strain was identified as the predominat pathogen through sequencing analysis (13). The data from the National Surveillance System for Disease Control and Prevention indicated that, from 2017 to 2022, an average of approximately 40 norovirus outbreaks were reported annually in Chongqing.

Many norovirus outbreaks and clustered epidemics are reported in China annually. Previous studies have predominantly focused on the long-term epidemiological characteristics of these outbreaks in China or the distribution of norovirus genotypes (9, 14). However, relatively few studies have conducted on norovirus outbreak responses. In early February 2021, a county in Chongqing confronted a sudden surge of cases, with reported dozens of villagers in two separate villages experiencing diarrhea and vomiting for unknown reasons. In response to these outbreaks, investigations were conducted. This paper aims to elucidate the two gastroenteritis outbreaks, identify the causes, detect possible associations between the two outbreaks, and provide scientific information for preventing further transmission.

2 Methods

2.1 Case definition

In early 2021, two separate gastroenteritis outbreaks occurred in county A were reported repeatedly to Chongqing Municipal Center for Disease Control and Prevention (CDC). To control outbreaks, epidemiological investigations were conducted immediately by an investigation team including epidemiologists, physicians, and laboratory technicians.

For both outbreak A and outbreak B, a probable case was defined as an individual who had experienced diarrhea ≥3 times per 24 h, and/or vomiting ≥2 times per 24 h from January 28 to February 6 and from February 2 to February 9, 2021, respectively.

A laboratory-confirmed case in both outbreaks was defined as a probable case in which a stool specimen, an rectal swab, or a vomit sample was tested positive for norovirus using quantitative real-time polymerase chain reaction (RT-PCR).

2.2 Case finding

Case finding was conducted in each villager's home and local village clinics. Villagers with symptoms of diarrhea and/or vomiting within the previous 3 days during each outbreak were investigated for case confirmation. To enhance the accuracy of case identification, doctors in the local village clinics were interviewed to obtain clinical symptoms, onset time, and other related information of treated patients in recent days during each outbreak. Cases were interviewed in person with a standardized form to collect clinical and epidemiology data. This included name, age, gender, occupation, address, time of onset, symptoms (diarrhea, vomiting, stomach cramping, abdominal distension, etc.), whether to use the centralized water supply and other related information.

2.3 Environmental investigation

We inspected the environmental hygiene regarding water sources and the living environments of the cases. For the water sources of the two villages, their coverage of residents, disinfection facilities and frequency, and the overall sanitation were examined carefully. For the cases' homes in each outbreak, investigations regarding water used for drinking and cooking, and water purifiers (manufacture of water bands and equipment) were conducted. Other public activities related to water use, such as banquets, gatherings, and washing clothes in the public pool were also investigated.

2.4 Retrospective cohort study

Evidence from the descriptive epidemiology investigation and environmental investigation indicated that water source was the most probable risk factor for both outbreaks. To verify this hypothesis, a retrospective cohort study was conducted, using residents from incriminated villages and neighbor villagers with different water sources to identify risk factors for each outbreak.

2.5 Sample collection and preprocessing

Stool specimens, rectal swabs, and vomit samples from cases were collected. Water samples from the water sources, reservoirs (containing water from different water sources), and the homes of the cases were also collected.

For the nucleic acid extraction from water samples, we used 8 mL of lysis buffer (QVL Lysis Buffer) to lyse 2 mL of water sample according to the ratio in the kit (OMEGA E.Z.N.A Viral RNA Kit) for 10 min, added 6 mL of absolute ethanol, and mixed well. The above liquid was filtered through a cellulose acetate membrane filter column (HiBind RNA Mini Column) using a suction filtration pump, and then the relevant operations were carried out according to the kit steps, and the final obtained RNA was equivalent to concentrating about 14 times.

For the nucleic acid extraction from cases' samples, we used an automatic nucleic acid extraction instrument (SSNP-3000A) and matched supporting reagents. Rectal swabs were directly added with 200 uL reagent samples for nucleic acid extraction, about 3 g of stool specimens were added with 10 mL phosphate-buffered solution, thoroughly mixed and centrifuged, and 200 uL of supernatants were taken for nucleic acid extraction.

2.6 Laboratory detection

The samples were tested twice using a commercial RT-PCR kit (Norovirus GI/GII Nucleic Acid Detection Kit from Jiangsu Bioperfectus Technologies Co., Ltd.).

For the nucleic acid of the samples tested positive for norovirus by RT-PCR, part of the region including the polymerase region and capsid region was amplified using the Prime Script One-Step RT-PCR Kit Ver.2. The amplified product was sent to the Beijing Genomics institution for sequencing.

The successfully sequenced nucleic acid sequences were spliced, edited, and organized by DNA STAR software, and the nucleic acid sequences were compared on the NCBI website,[1] and the sequence with the highest homology known sequence types was used as typing results. Phylogenetic analysis was performed using MEGA 7 software.[2] All reference sequences were downloaded from Genbank, sequence alignment was performed using Clustalw, the phylogenetic tree was constructed by the Neighbor-Joining method, and bootstrap was used to test 1,000 times repeatedly.

2.7 Statistical analysis

The demographic characteristics and clinical symptoms of cases were displayed with proportions. Chi-square tests or Wilcoxon rank sum tests were conducted to compare differences in attack rates, gender, age, and occupation among cases between the cases in the two outbreaks. A p-value of less than 0.05 (two-tailed) was determined to be statistically significant. All statistical analyses were performed using Excel and Epi info (version 7.2.5.0). ArcGIS software was used to visualize the locations of the two outbreaks.

1 https://www.ncbi.nlm.nih.gov/

2 https://www.megasoftware.net

3 Results

3.1 Epidemiological characteristics

Two norovirus outbreaks occurred in two separate villages of county A in Chongqing, China, in late January and early February of 2021. The two villages are located 34 miles apart and there were no common activities recently among villagers from the two villages. In outbreak A and outbreak B, 561 villagers and 328 villagers shared the same spring water supply, respectively (Supplementary Figure S1).

A total of 123 cases (including nine laboratory-confirmed cases) and 88 cases (including 37 laboratory-confirmed cases) were reported in the two outbreaks, with attack rates of 21.93% (123/561) and 26.99% (88/326). The median age was 47.0/41.5 years, males accounted for 52.85%/51.14%, and farmers accounted for 85.37%/78.41%, with no statistically significant differences in age ($p = 0.427$), gender ($\chi^2 = 0.06$, $p = 0.806$), occupation ($\chi^2 = 6.61$, $p = 0.359$), and attack rates ($\chi^2 = 2.92$, $p = 0.087$) of the two outbreaks. Seventy-four household cluster cases were identified in outbreak A and 39 in outbreak B. The major clinical symptom of cases was diarrhea (95.93%) and vomiting (59.09%) in two outbreaks, respectively. Detailed information is shown in Table 1.

Both epidemic curves showed a continuous transmission mode, indicating cases exposing continuously to the source of infection. Outbreak A lasted from 31st January to 6th February 2021, the index cases showed symptoms on 31st January and the onset time peaked on February 2, 2021. In outbreak B, the onset time of the first case and the last case was 10:00 on February 5 and 05:30 on February 9, 2021; and the onset time peaked on February 7, 2021. The epidemic curves of the two outbreaks are presented in Figure 1.

3.2 Environmental investigation

The two villages use different water sources for their daily life. In outbreak A, there are three spring water sources in the village, water sources A1 and A2 on a hillside and water source A3 located near a creek. These three water sources converge into a large reservoir. In general, water sources A1 and A2 supplied drinking water to local villagers. Water source A3 was reused from January 24, 2021, because a large number of migrant workers returned home leading to unsatisfied water demands. On January 27, the water from the large reservoir was discharged through the ditches to households. Due to a lower altitude of water source A3, sewage overflowed from the ditches and formed large puddles less than one meter away from water source A3. On January 29 and 30, rainfalls caused the sewage from puddles to flow to water source A3. What's worse, the large reservoir had not been dredged and disinfected for over 10 years.

In outbreak B, there are also three water sources (B1–B3), all of which are spring water sources. The three water sources flow into one reservoir that had not been cleaned and disinfected for over 7 years. Before the occurrence of outbreak B, there was heavy rainfall at midnight on February 4, 2021. The layouts regarding the water sources and water supply systems for the two outbreaks are shown in Supplementary Figure S2.

TABLE 1 Epidemiological characteristics of the two norovirus outbreaks occurred in early 2021.

Characteristics	Outbreak A (n = 123)	Outbreak B (n = 88)	$\chi 2$	p
Demographic characters				
Age (range, median)	2–84 yrs., 47 yrs	10 mons–83 yrs., 41.5 yrs		0.427[c]
Gender (male, %)	65, 52.85	45, 51.14	0.06	0.806
Occupation (peasant, %)	105, 85.37	69, 78.41	6.61	0.359
Clinical symptoms (n, %)				
Diarrhea[a]	118, 95.93	45, 51.14		
Vomiting[b]	46, 37.40	52, 59.09		
Stomach cramping	59, 47.97	—		
Abdominal distension	—	27, 30.68		
Transmission route	Waterborne, person-to-person	Waterborne, person-to-person		
Start date of the outbreak	January 31st	February 5th		
End date of the outbreak	February 6th	February 9th		
Duration (days)	7	5		
Number of cases	123	88		
Number of persons at risk	561	326		
Attack rate	21.93	26.99	2.92	0.087
Confirmed case	9	32		
Household cluster case	74	39		

yrs, years; mons, months; "—" no cases were reported.
[a]Diarrhea ≥3 times per 24 h.
[b]Vomiting ≥2 times per 24 h.
[c]p-value obtained from Wilcoxon rank sum test.

3.3 Retrospective cohort study

A retrospective cohort study was conducted to examine the role of the spring water supply as the outbreak source. Residents in neighboring villages with different water sources served as the unexposed population. In outbreaks A and B, the attack rate of the exposed group was markedly higher than the unexposed group ($p < 0.001$). The results of the retrospective cohort study concluded that contaminated drinking water was the risk factor for both outbreaks. Table 2 shows detailed information.

3.4 Laboratory detection findings

In outbreak A, a total of nine rectal swabs and one stool specimen from cases, three swab samples from cases' houses, and six water samples were detected positive for norovirus GII. In outbreak B, a total of 52 samples were detected positive for norovirus GII, including 37 rectal swabs, five stool samples, five swab samples from cases' houses, and five water samples. The laboratory results are exhibited in Supplementary Tables S1, S2.

3.5 Genotyping and polygenetic analysis

In outbreak A, only two rectal swabs have successfully identificated, revealing the presence of two distinct strains: GII.17 (P17) and GII.2 (P16). The GII.17 (P17) strain was highly homologous (99.59%) to a 2016 Brazilian isolate (MH746992.1), while the GII.2 (P16) strain was highly homologous (99.25%) to a 2017 Zhejiang isolate (MH806429.1).

In outbreak B, a total of 16 samples (13 rectal swabs and three stool specimens) revealed the presence of two strains. Fourteen samples (11 rectal swabs and three stool specimens) were identified as GII.4 Sydney (P16) strain, all of which have high homology (99.01%–99.82%) with a 2019 Beijing isolate (OL336386.1). Two rectal swabs were identified as GII.1 (P16) strain which is highly homologous (97.99%–99.10%) to a 2018 Zhejiang isolate (OK217108.1). The phylogenetic tree of the sequences obtained in the two outbreaks is shown in Figure 2.

3.6 Control measures

To address the two outbreaks, a series of prompt and effective response measures were conducted. Firstly, the centralized spring water supply in these affected villages was promptly discontinued. Secondly, qualified cleaning and standardized disinfection (with chlorine-containing disinfectant) regarding water sources, reservoirs, and cases' homes were conducted. Thirdly, an emergency surveillance system for diarrhea cases was activated to detect potential infections in this county. Finally, gatherings, such as playing mahjong and banquets, were minimized. Additionally, health education regarding norovirus during the outbreak response was strengthened, including hand hygiene, the management of feces from humans and animals and cases' excreta, to prevent contamination of drinking water sources.

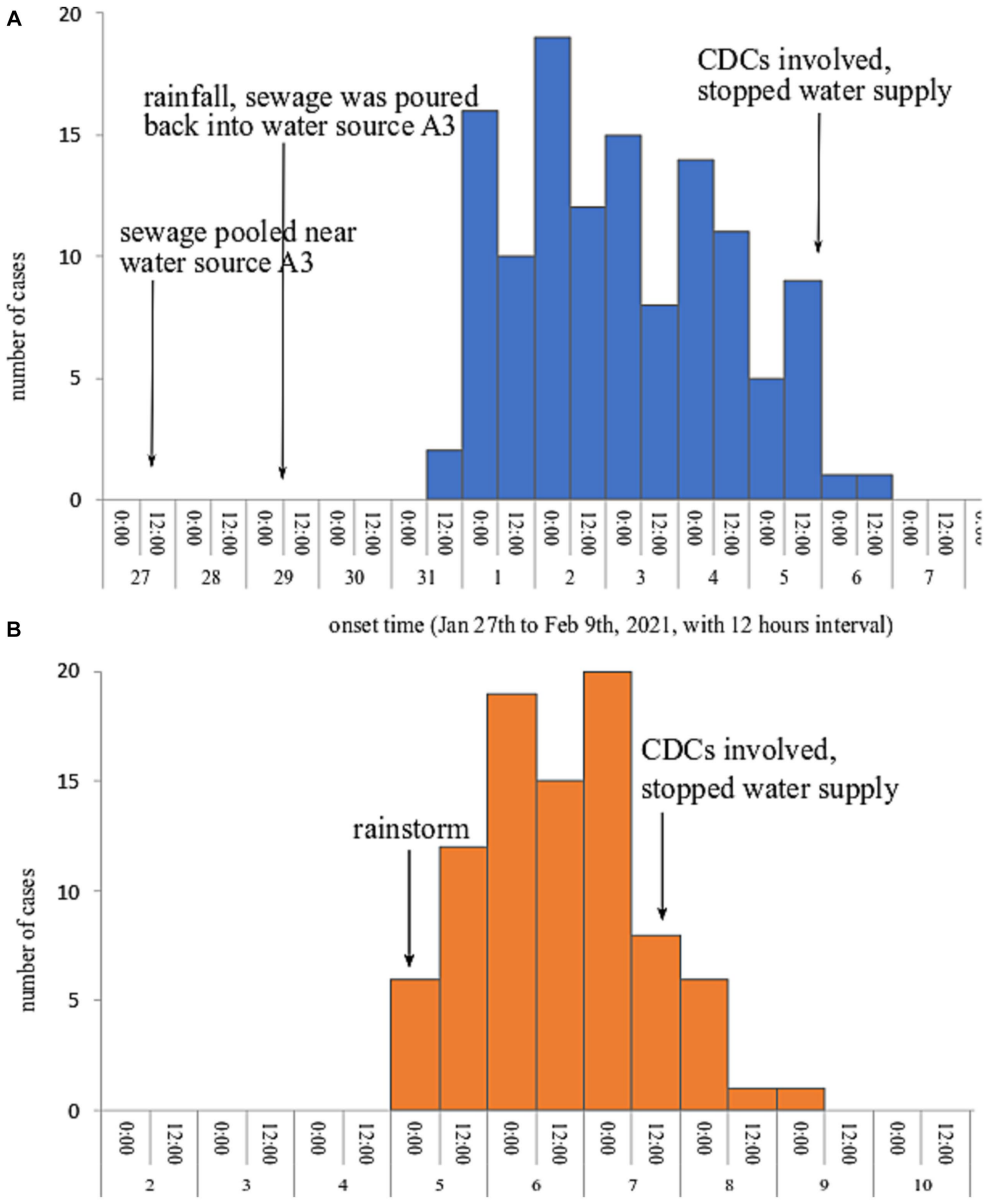

FIGURE 1
Epidemic curves of the two outbreaks occurred in county A in early 2021.

TABLE 2 Analysis of risk factors of the two norovirus outbreaks.

Contaminated drinking water	Exposed group		Unexposed group		RR	95% CI
	Cases	Total	Cases	Total		
Outbreak A	123	561	0	188	41.85	5.89–297.39
Outbreak B	88	326	0	290	79.23	11.11–565.12

RR, risk ratio; RR was calculated by adding one in each exposure group; CI, confidential interval.

4 Discussion

Norovirus causes the most outbreaks of non-bacterial gastroenteritis in human beings, which is affected by many climate factors, including low temperature (−6.6°C to 20°C), relative humidity (10% to 66%), and rainfall (1 day to 3 months) (15). Norovirus in groundwater can remain detectable for over 3 years and can remain infectious for at least 61 days (16). In this study, there were rainfalls before the two outbreaks. Thus, rainfalls might lead norovirus in nature to contaminate the spring water sources. Studies have shown that norovirus could persist long in the water environment (17, 18), and cold temperatures could further

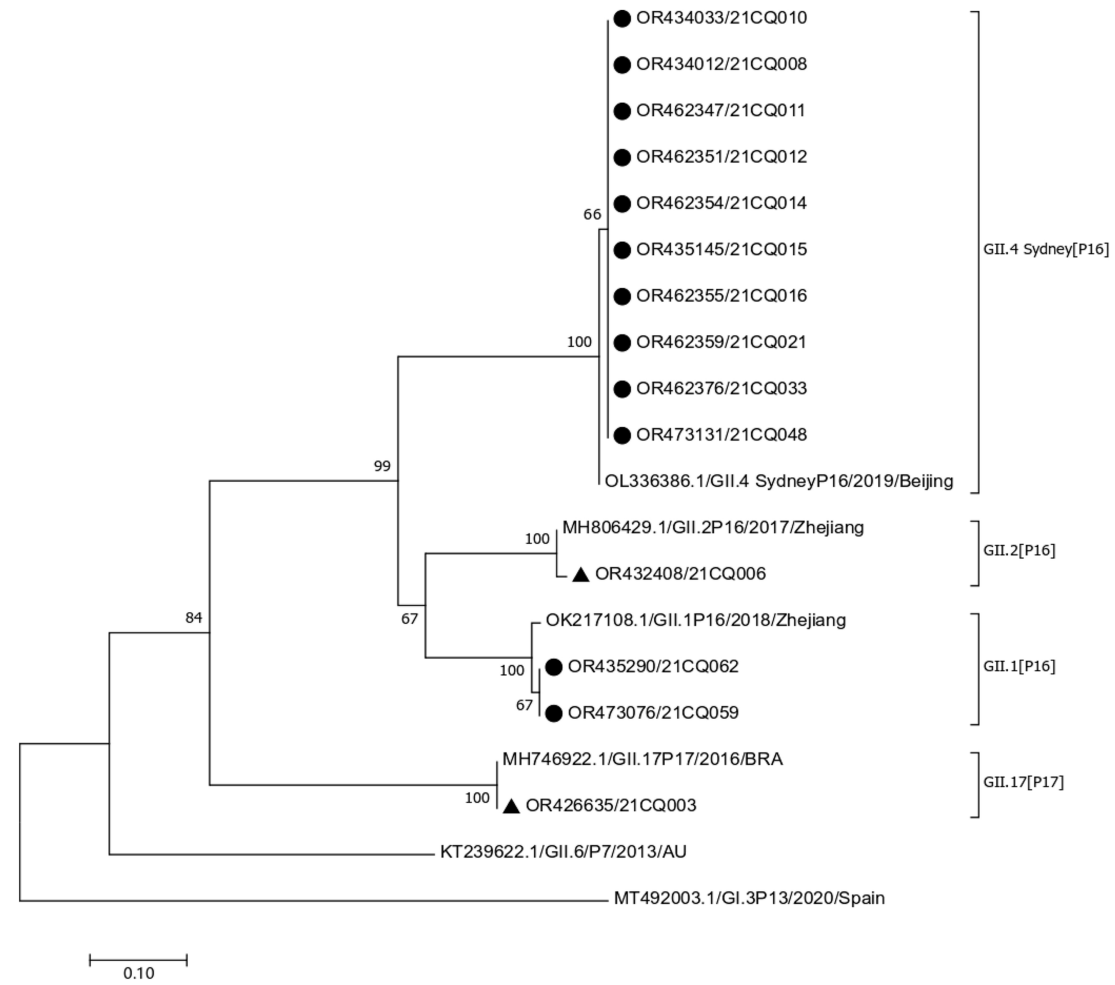

FIGURE 2
Phylogenetic tree based on partial nucleotide sequences in the polymerase-capsid region. "▲," sequences obtained from outbreak A, "●," sequences obtained from outbreak B.

prolong its viability (19). At the same temperature, the persistence of norovirus was longer in drinking water than in wastewater. During the two outbreaks, the local temperature was below 15°C from January 27 to February 11, 2021, which might extend the persistence of norovirus in drinking water, potentially leading to the emergence of the two outbreaks. The rainfall and low temperature in the two villages contributed to the epidemic of both norovirus outbreaks.

Contaminated drinking water is a common source of norovirus outbreaks among individuals (20, 21). In our study, water samples collected from the water source and cases' homes both detected positive for norovirus, clearly indicating that drinking water has been contaminated by norovirus. In this remote county, there was poor sanitation, unqualified water purification equipment, and even limited or no access to municipal water. The local villagers had to depend on these natural water sources for their livelihood. Then villagers would be exposed when contaminated water is used for drinking, cooking, or entertainment. These high-risk factors (rainfall, low temperature, poor sanitation, unqualified water purification, and limited access to municipal water) increased the possibility of exposure to contaminated drinking water for villagers.

Our finding revealed that multiple norovirus GII strains are distributed widely in contaminated water sources and implicated cases of different GII strains-associated outbreaks had no significant differences in age and gender. In recent years, GII.4 was the predominant genotype of norovirus gastroenteritis in China, GII.P17, GII.17 strains, and GII.2 had a rapid increase (22–25), also GII.2 (P16) was the main genotype causing norovirus outbreaks from October 2016 to September 2019 (26). Waterborne and person-to-person transmissions were observed in our two outbreaks, which aligns with previous studies that have revealed norovirus GII transmition by food, water (27), or person-to-person contact (24).

In both outbreaks, evidence from epidemiological investigation, environmental investigation, and laboratory detection consistently indicated the epidemiological source and pathogenic etiology. Diarrhea and vomiting are the most common clinical symptoms of norovirus illness, which both were prevalent in both outbreaks. Besides, the epidemiological investigation and environmental investigation indicated that drinking water was the risk factor for each outbreak. Most importantly, PCR tests and sequencing analysis proved that contaminated drinking water was the source of the outbreaks.

Nevertheless, two limitations should not be ignored in this study. First, because of limited laboratory conditions, the concentration of the water sample was completely manual, with a theoretical enrichment of approximately 14-fold, but it had not been verified. Second, owing to the quality of the samples, the water samples in both outbreaks could not be successfully sequenced and were only detected as the GII strains. This limitation cannot directly indicate the connection between contaminated drinking water and cases. It highlights the critical importance of sample quality in future investigations.

In conclusion, the two acute gastroenteritis outbreaks that occurred in a remote county were independent. Both outbreaks were linked to contaminated drinking water and norovirus GII strains. Cases from different GII strains-associated outbreaks showed no significant differences in age, gender, and occupation. It revealed potential safety hazards of spring water supply in this rural county, reinforcing the importance of good sanitation (hand hygiene, the management of feces from humans and animals and cases' excreta) and environmental disinfection (regular water source disinfection) in rural areas, especially during the rainy seasons.

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

As an immediate response to a public health emergency, investigations were initiated upon the occurrence of an outbreak. In accordance with Article 12 of Chapter I of the Law of the People's Republic of China on the Prevention and Control of Infectious Diseases (28) (available at: http://en.nhc.gov.cn/2019-03/05/c_74526.htm), all related units and individuals must accept the investigation, inspection, and sample collection by the investigation offices and provide relevant information truthfully. Therefore, ethical approval and participant consent were exempted from these investigations for public health emergency response.

All samples and data collected from participants were used exclusively for this study, with investigators ensuring the confidentiality of participants' responses and the sample data. Data analyses were conducted anonymously, without disclosing any personal information about the participants. Research involving human participants, human material, and human data had been performed under the Declaration of Helsinki.

Author contributions

TL: Data curation, Formal analysis, Investigation, Visualization, Writing – original draft, Writing – review & editing. JP: Data curation, Funding acquisition, Methodology, Visualization, Writing – review & editing. QL: Conceptualization, Methodology, Project administration, Resources, Writing – review & editing. BL: Data curation, Formal analysis, Investigation, Writing – review & editing. YY: Data curation, Investigation, Visualization, Writing – review & editing. CY: Data curation, Investigation, Resources, Writing – original draft, Writing – review & editing. DY: Data curation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing. WT: Conceptualization, Investigation, Project administration, Resources, Writing – review & editing. LQ: Conceptualization, Formal analysis, Funding acquisition, Investigation, 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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2023.1259584/full#supplementary-material

SUPPLEMENTARY FIGURE S1
Laboratory results of samples collected from two outbreaks.

SUPPLEMENTARY FIGURE S2
Dataset used in this study.

SUPPLEMENTARY TABLE S1
Location of villages for the two outbreaks occurred.

SUPPLEMENTARY TABLE S2
Layout of water resources with potential contaminant risks of the two outbreaks.

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Detection of COVID-19 epidemic outbreak using machine learning

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Background: The coronavirus disease (COVID-19) pandemic has spread rapidly across the world, creating an urgent need for predictive models that can help healthcare providers prepare and respond to outbreaks more quickly and effectively, and ultimately improve patient care. Early detection and warning systems are crucial for preventing and controlling epidemic spread.

Objective: In this study, we aimed to propose a machine learning-based method to predict the transmission trend of COVID-19 and a new approach to detect the start time of new outbreaks by analyzing epidemiological data.

Methods: We developed a risk index to measure the change in the transmission trend. We applied machine learning (ML) techniques to predict COVID-19 transmission trends, categorized into three labels: decrease (L0), maintain (L1), and increase (L2). We used Support Vector Machine (SVM), Random Forest (RF), and XGBoost (XGB) as ML models. We employed grid search methods to determine the optimal hyperparameters for these three models. We proposed a new method to detect the start time of new outbreaks based on label 2, which was sustained for at least 14 days (i.e., the duration of maintenance). We compared the performance of different ML models to identify the most accurate approach for outbreak detection. We conducted sensitivity analysis for the duration of maintenance between 7 days and 28 days.

Results: ML methods demonstrated high accuracy (over 94%) in estimating the classification of the transmission trends. Our proposed method successfully predicted the start time of new outbreaks, enabling us to detect a total of seven estimated outbreaks, while there were five reported outbreaks between March 2020 and October 2022 in Korea. It means that our method could detect minor outbreaks. Among the ML models, the RF and XGB classifiers exhibited the highest accuracy in outbreak detection.

Conclusion: The study highlights the strength of our method in accurately predicting the timing of an outbreak using an interpretable and explainable approach. It could provide a standard for predicting the start time of new outbreaks and detecting future transmission trends. This method can contribute to the development of targeted prevention and control measures and enhance resource management during the pandemic.

KEYWORDS

COVID-19, prediction, machine learning, early detection, outbreak

1 Introduction

The coronavirus disease (COVID-19) pandemic is caused by the novel coronavirus SARS-CoV-2, which has spread rapidly and affected human lives worldwide. Since the start of the pandemic non-pharmaceutical interventions (NPIs) such as wearing masks, social distancing, and pharmaceutical vaccination have been implemented to control the spread of the virus. However, the emergence of new variants of the virus has raised concerns about their potential for increased transmission. The pandemic continues to impact human lives, and it is crucial to control it and reduce its transmission.

Predictions can be made in several ways. One common approach is to use mathematical models that consider factors such as the rate of transmission, number of cases, and effectiveness of control interventions such as social distancing and vaccination. These models can predict future trends in COVID-19 transmission dynamics and estimate the number of cases and deaths (1–3). Mathematical models are widely used for predicting infectious diseases, but they can be difficult to adapt to various external factors such as social distancing or the emergence of new variants (4, 5).

Another approach is to use machine learning (ML) methods to detect changes in the trend of transmission and potential outbreaks (6–9). Shahid et al. (6) predicted the confirmed cases, deaths, and recoveries of COVID-19 in 10 major countries using ARIMA, SVR, LSTM, and Bi-LSTM. Chakraborty et al. (10) performed short-term forecasts of future COVID-19 cases in Canada, France, Republic of Korea, the United Kingdom, and India, using a hybrid forecasting approach based on the ARIMA and wavelet-based models. Katragadda et al. (9) explored the COVID-19 spread growth in America by comparing the mobility of local people and visitors, and forecasted the number of cases using various ML models.

Investigating the start point of infectious disease outbreaks and analyzing the transmission dynamics of epidemics is critical for several reasons. First, understanding the source of an outbreak can help identify the underlying cause of the disease and prevent future outbreaks. Second, analyzing the transmission dynamics of epidemics can provide important information on how the disease spreads and who is at risk. This information can then be used to develop effective preventive and control measures. Third, investigating the start point of an outbreak and analyzing the transmission dynamics can help determine the scope and severity of the outbreak. This information is important for determining the level of response required to control an outbreak and to protect public health. Therefore, understanding the start point of infectious disease outbreaks and analyzing transmission dynamics is essential for the effective investigation, prevention, and control of outbreaks.

Early detection (ED) methods and warning systems for epidemics are important to prevent and control the spread of the virus. Shi et al. (11) developed statistical models combining least absolute shrinkage and selection operator with the ARIMA model to forecast the spread of dengue pandemic in Singapore. Several studies have used statistical methods for the ED of infectious disease outbreaks using statistical methods (11–13). ML has been proposed as a useful tool for ED of COVID-19 outbreak (14–16). Martinez-Velazquez et al. (14) detected the COVID-19 outbreak using self-reported symptom data and evaluated the performance of models using 15 ML classifiers, such as decision tree, neural network, Support Vector Machine (SVM), and Random Forest (RF).

Korea experienced five reported outbreaks from March 2020 to October 2022. The start times of outbreaks were not clearly determined, as different start dates were reported, as summarized in Supplementary Table S1. Here, we investigated national COVID-19 outbreaks without considering regional factors, as the country's size is not very large (17). Additionally, policy decisions related to COVID-19 are managed at the national level by the Korea Disease Control and Prevention Agency (KDCA). No explainable standards were recommended to determine the start time of the COVID-19 outbreak. In this study, we aimed to develop a method to detect early COVID-19 outbreaks or identify potential early outbreaks using ML by analyzing epidemiological data in the Republic of Korea.

2 Methods

The method used to detect the emergence of the COVID-19 outbreak is illustrated in Figure 1. We propose a novel method using the risk index and machine learning, without requiring any new developments in the machine learning method. This approach enables us to interpret the transmission trend using the risk index function and various data.

2.1 Epidemiological data

We analyzed epidemiological data on reported cases of COVID-19 from February 18, 2020 to October 31, 2022, provided by KDCA (18) in the Republic of Korea, shown in Supplementary Figures S1A,B. The proportions of delta and omicron variants were obtained from covariance data (19, 20). We computed the number of delta variant cases and omicron cases by multiplying the daily COVID-19 cases with proportional data (18–20).

Previous studies mentioned that enhanced social distancing was a crucial intervention to prevent the spread of COVID-19 transmission in Korea (21–23).

We used collected data on social distancing measures among NPIs from a press release by KDCA (24), where we divided the levels of social distancing into four categories based on their intensity (distancing level 1 to 4) (25–27). Supplementary Table S2 summarizes the important times to change the level of social distancing. The higher the level, the more stringent the control intervention implemented. In addition, Supplementary Figure S1C and Supplementary Table S3 show the proportion of days of the week on the yearly number of COVID-19 cases.

2.2 Ethical considerations

The data are presented in Supplementary Table S3. The datasets were fully anonymized and did not include any personally identifiable information. Thus, ethical approval was not required for this analysis.

2.3 Overview of the estimation of transmission trend of COVID-19 epidemic

Figure 1 shows a schematic of the detection of early outbreaks. Figure 1A shows newly reported COVID-19 cases and several outbreaks in Korea, along with the proportion of variants. Figures 1B,C shows a new method for estimating the start time of the new outbreak.

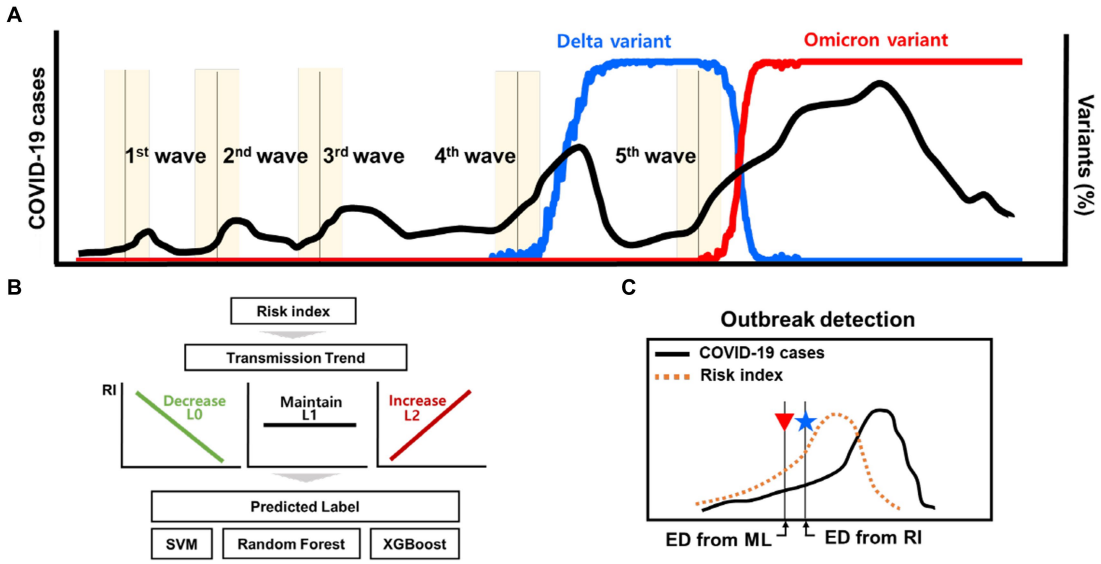

FIGURE 1
Schematic for the outbreak detection of COVID-19 outbreak. **(A)** The reported dates of the new COVID-19 outbreaks and the proportion of variants. **(B)** Transmission trend is estimated using ML techniques of classification. **(C)** Detection of new outbreak using the risk index and ML techniques.

2.4 Sample data

2.4.1 Define calibration and prediction periods

The daily number of COVID-19 cases was collected for specific periods of k days. Let $I(t)$ denote the number of COVID-19 cases on day t. The first sample data of the cases is defined as $s_1 = \{I_1(1), I_1(2), \ldots, I_1(k)\}$, where $I_\omega(t)$ denotes $I(t)$ on the ω-th sample data. The sample data comprise two partitions of time periods: a calibration period, excluding the most recent x days, and a prediction period, including the most recent x days to predict the most recent x days, where the length of the calibration period is $y = k - x$ and the length of the prediction period is x, as shown in Figure 2A.

In other words, the sample data s_1 can be expressed as $s_1 = s_1^{\mathrm{C}} \cup s_1^{\mathrm{P}}$, where $s_1^{\mathrm{C}} = \{I_1(1), I_1(2), \ldots, I_1(y-1), I_1(y)\}$ denotes the sample data for the calibration period and $s_1^{\mathrm{P}} = \{I_1(y+1), \ldots, I_1(k)\}$ denotes the sample data for the prediction period. In general, for the time window $\omega \in \{1, \ldots, n\}$ with a total of n sample data, the ω-th sample data of the cases are defined as $s_\omega = \{I_\omega(\omega), I_{\text{É}}(1+\omega), \ldots, I_\omega(k-1+\omega)\}$.

The time interval for each ω-th sample data is defined as $T_\omega = \{\omega, 1+\omega, \ldots, k-1+\omega\}$. T_ω comprises the time period for the calibration period (T_ω^{C}) and the time period for the prediction period (T_ω^{P}), expressed by $T_\omega = T_\omega^{\mathrm{C}} \cup T_\omega^{\mathrm{P}}$, where the time periods are defined as $T_\omega^{\mathrm{C}} = \{\omega, 1+\omega, \ldots, \tau_\omega\}$ and $T_\omega^{\mathrm{P}} = \{\tau_\omega + 1, \ldots, k-1+\omega\}$, and $\tau_\omega = \omega - 1 + y$ is the final time of the calibration period.

Moreover, for each ω-th sample data, μ_ω^{C} and $\sigma_\omega^{\mathrm{C}}$ denote the mean and standard deviation of s_ω^{C} for the calibration period, respectively. Likewise, μ_ω^{P} and $\sigma_\omega^{\mathrm{P}}$ are the average number and standard deviation of s_ω^{P} for the prediction period, respectively.

In the present study, we set the calibration period to 21 days (i.e., $k = 35, x = 14$) and the time window as 1 day from February 18, 2020, to October 31, 2022. The sample data of the cases consisted of 953 sets (i.e., n =953), which comprised 667 training data and 286 test data (the ratio of train data to test data was assumed to be 7:3), where all sample data of the cases were defined as $S = \{s_1, s_2, \ldots, s_{953}\}$. We considered various periods, where the calibration periods ranged from 14 to 28 days and the predication periods ranged from 7 to 21 days, assuming that the calibration periods were longer than the prediction periods.

2.4.2 Normalization and regression analysis

We normalized the sample data from s_ω to $\hat{s}_\omega$ using the min-max normalization. Moreover, we applied the linear regression model to the sample data for the calibration period ($\hat{s}_\omega^C$) and prediction period ($\hat{s}_\omega^P$), where $\hat{s}_\omega = \hat{s}_\omega^C \cup \hat{s}_\omega^P$. Here, $\beta_\omega^{\mathrm{C}}$ and $\beta_\omega^{\mathrm{P}}$ denote the slopes obtained from the linear regression model for the samples $\hat{s}_\omega^{\mathrm{C}}$ and $\hat{s}_\omega^{\mathrm{P}}$, respectively, which are defined as the increment rates. $\mu^{\mathrm{C}} = \{\mu_\omega^{\mathrm{C}}\}$ denotes the vector of the mean number of COVID-19 cases during the calibration period. $\mu^{\mathrm{P}} = \{\mu_\omega^{\mathrm{P}}\}$ denotes the vector of the average number of COVID-19 cases during the prediction period. That is, the regression analysis for each sample data ω as follows:

$$\begin{cases} \hat{s}_\omega^{\mathrm{C}} = \alpha_\omega^{\mathrm{C}} + \beta_\omega^{\mathrm{C}} t, & t \in T_\omega^{\mathrm{C}} \\ \hat{s}_\omega^{\mathrm{P}} = \alpha_\omega^{\mathrm{P}} + \beta_\omega^{\mathrm{P}} t, & t \in T_\omega^{\mathrm{P}} \end{cases}$$

where $\alpha_\omega^{\mathrm{C}}, \alpha_\omega^{\mathrm{P}}$ are intercept values of the linear regression model for calibration period and prediction period, respectively. $\sigma^{\mathrm{C}} = \{\sigma_\omega^{\mathrm{C}}\}$ denotes the vector of the standard deviation of the COVID-19 cases for the calibration period. $\sigma^{\mathrm{P}} = \{\sigma_\omega^{\mathrm{P}}\}$ denotes the vector of the standard deviation of COVID-19 cases for the prediction period. "*Week*" represents the day of the week, corresponding to final time of the calibration period (τ_ω). "*Delta*" denotes the number of delta variant and "*Omicron*" denotes the number of omicron variant. "*Policy*" denotes the level of NPIs implemented in Korea.

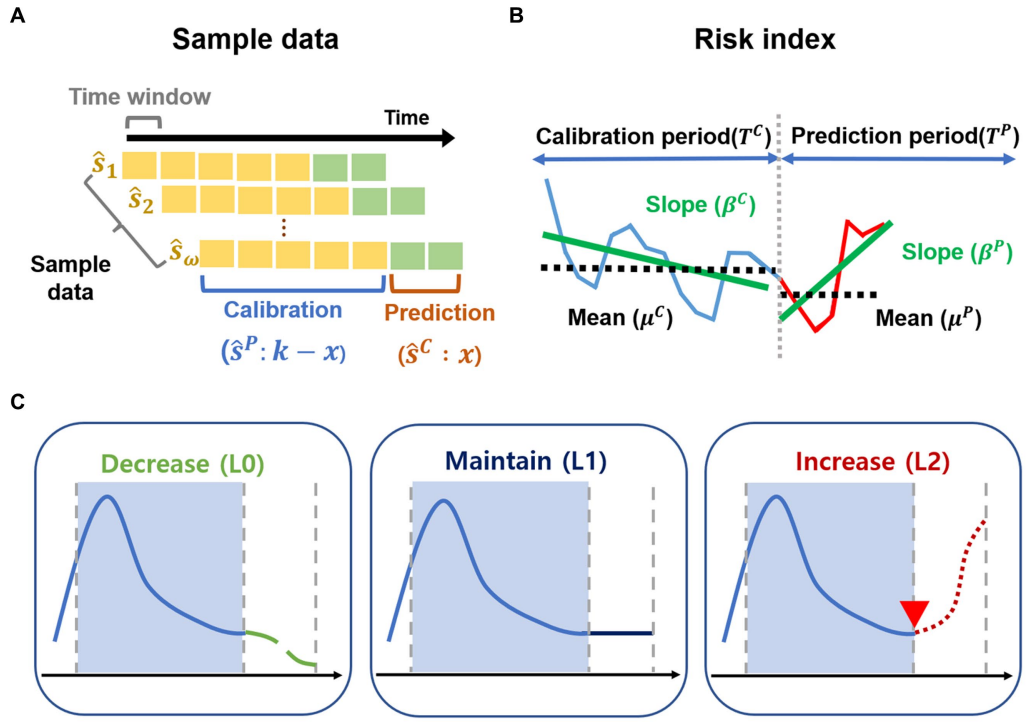

FIGURE 2
Sample data and risk index. **(A–C)** Outline of the methods. **(A)** The sample data are generated for the calibration period and prediction period from February 2020 to October 2022. **(B)** Risk index for transmission trend is developed. **(C)** Transmission trends are grouped as decrease (L0), maintain (L1), increase (L2) using risk index.

2.5 Development of risk index and labeling for transmission trend

In the present study, we developed a method for early detection of potential infectious disease outbreaks by estimating the starting point of such outbreaks. Previous studies have focused on detecting outbreaks early through statistical or machine learning techniques based on data such as the number of COVID-19 cases, NPIs, and variant viruses in (11–16). As an alternative new approach, we aimed to quantify the risk potential to indicate the increasing trends and changes of transmission trends from calibration period to prediction period.

2.5.1 Definition of risk index

We proposed a quantitative representation of these changes as the risk index, which can be used to classify the risk of potential outbreaks, as described in Figure 2B. For each ω-th sample data, we selected two functions of f and g for transmission trend changes, which consist of the mean of COVID-19 cases (μ_ω^{C}, μ_ω^{P}) and the increment rate ($\beta_\omega^{\mathrm{C}}$, $\beta_\omega^{\mathrm{P}}$) for calibration period and prediction period, respectively. c_1 and c_2 represent the positive scaling parameters of the functions f and g. The risk index $[\mathrm{RI}(\tau_\omega)]$ is expressed as follows.

$$\mathrm{RI}(\tau_\omega) = f(\omega)\, g(\omega) = \sinh\left(c_1 \left(\frac{\mu_\omega^{\mathrm{P}} - \mu_\omega^{\mathrm{C}}}{\mu_\omega^{\mathrm{C}}} \right) \right) e^{c_2\left(\beta_\omega^{\mathrm{P}} - \beta_\omega^{\mathrm{C}}\right)}. \quad (1)$$

(i) **Change of the mean of COVID-19 cases**: The function f represents the rate of change to describe how much the COVID-19 cases have increased during the prediction period based on the calibration period. The function f denotes the hyperbolic sine (sinh) function of relative difference between μ_ω^{P} and μ_ω^{C} divided by μ_ω^{C}. If $\mu_\omega^{\mathrm{P}} > \mu_\omega^{\mathrm{C}}$, the function f exhibits positive exponential growth. Otherwise, the function f becomes negative exponential decay.

(ii) **Change of the increment rate of COVID-19 cases**: The function g represents the change of the increment rate for transmission trend to describe how much the slope in prediction period ($\beta_\omega^{\mathrm{P}}$) has increased from the slope in calibration period ($\beta_\omega^{\mathrm{C}}$) for the linear regression model. The function g is defined as an exponential function of the difference between $\beta_\omega^{\mathrm{P}}$ and $\beta_\omega^{\mathrm{C}}$. If $\beta_\omega^{\mathrm{P}} > \beta_\omega^{\mathrm{C}}$, the function g has positive exponential growth with $g > 1$. Otherwise, the function g becomes exponential decay with $0 < g \leq 1$.

We defined the risk index as the product of two functions. For example, one sample shows $\mu_\omega^{\mathrm{P}} > \mu_\omega^{\mathrm{C}}$ and $\beta_\omega^{\mathrm{P}} > \beta_\omega^{\mathrm{C}}$. Then, the function f exhibits positive exponential growth. The function g amplifies the function f because of $g > 1$. However, another sample shows $\mu_\omega^{\mathrm{P}} > \mu_\omega^{\mathrm{C}}$ and $\beta_\omega^{\mathrm{P}} < \beta_\omega^{\mathrm{C}}$. Then, the function f exhibits positive exponential growth. The function g plays a role in decreasing the function f because of $0 < g \leq 1$.

2.5.2 Labeling for transmission dynamics using risk index

We calculated the values of risk index for each sample data point ($S = \{s_1, s_2, \ldots, s_{953}\}$). We uniformly divided the values of risk index $\{\mathrm{RI}(\tau_\omega)\}_{\omega \in \{1,\ldots,n\}}$ into three groups and determined labels as

decrease (L0), maintain (L1), and increase (L2) in the transmission trend. We used a dataset with a similar size for each class (or label) as demonstrated in the previous study (28).

For instance, in the groups with small values of risk index, $\mathrm{RI}(\tau_\omega)$, indicating L0, we interpreted that the transmission trend would decrease for the prediction period, compared to that in the calibration period. Supplementary Figures S2A–C shows examples of the sample data labeled in L0, L1, and L2, respectively.

2.6 Machine learning approaches to estimate the transmission trend

We used eight features to estimate the transmission trends using ML techniques. Table 1 summarizes the features of the training and testing sample data.

We applied ML techniques such as SVM, RF, and XGB (29–31). SVM is a supervised learning ML model used for classification. SVM uses support vectors to define decision boundaries and classifies unclassified points by comparing them with the corresponding decision boundaries.

SVM can be considered a model that adds a constraint condition to the perceptron-based model to find the most stable decision boundary. RF is a type of ensemble learning method used for classification and regression. It learns multiple decision trees in parallel to output classification or average predictions. A feature of RF is that the trees have slightly different characteristics due to their randomness. This property results in the decorrelation of the predictions of each tree, thereby improving the generalization performance. In addition, randomization makes the forest robust to noise data. XGB is an ensemble model that uses the boosting technique in a number of decision trees, which represents Extreme Gradient Boosting. XGB is characterized by the implementation of parallel learning to support Gradient Boost, an algorithm implemented using the existing boosting technique. In addition, XGB has a strong resistance to overfitting owing to its regularization function.

Grid search methods were used to determine the best performing hyperparameters for the three models. We used a 10-fold cross validation of the training data to determine the best performance. As a result of applying the grid search method to the three ML methods, the regularization parameter, gamma, and kernel in SVM were 50, 0.3, and the radial basis function, respectively. The number of trees and maximum depth of the RF and XGB algorithms were 85 and 14, and 110 and 7, respectively. Supplementary Table S4 summarizes the range of parameters used in the grid search process. We divided the training and test data into the same ratio for label 0, label 1, and label 2. To evaluate the performance of the three models, we show confusion matrices and receiver operating characteristic (ROC) curves for the test data and compare the accuracy of the three models with *F*1-score and AUC for L0, L1, and L2. We used Python language version 3.10 and scikit-learn version 1.1.3. In addition, we used *SVC*, *RandomForestClassifier*, *XGBClassifier* functions of scikit-learn to simulate the three classification algorithms.

TABLE 1 Description of features for training the sample data.

Features	Description
$_{\propto}\mathrm{C}$	Average number of COVID-19 cases for calibration period
σ^{C}	Standard deviation of COVID-19 cases for calibration period
β^{C}	Slope obtained from the linear regression model of COVID-19 cases for calibration period
Week	Start day of the week for calibration period
$Delta^{\mathrm{C}}$	Average number of Delta variant for calibration period
$Omicron^{\mathrm{C}}$	Average number of Omicron variant for calibration period
$Policy^{\mathrm{C}}$	Average level of NPIs for calibration period
$Policy^{\mathrm{P}}$	Average level of NPIs for prediction period

2.7 Outbreak detection method

Determining the start time of the new outbreak is important for controlling the spread of COVID-19. Supplementary Table S5 lists the start time of the reported outbreaks in Korea, including the important characteristics of each outbreak. In this study, we propose a new approach to detect a new outbreak, which we called as "estimated outbreak," described in Figure 2C. We compared the reported outbreaks with the estimated outbreaks.

Estimated outbreaks have two approaches. First, we determined the estimated outbreak using the risk index. We defined the start time of the new outbreak as the first day when L2 designated from risk index (RI) was maintained for at least 14 days. The start time of the early outbreak estimated from RI is denoted by ED from RI. Second, we determined the estimated outbreak using the machine learning methods. We defined the start time of the new outbreak as the first day when label 2, estimated from ML methods, was maintained for at least 14 days, denoted by ED from ML. There are three ED from ML methods; (i) ED from SVM, (ii) ED from RF, and (iii) ED from XGB. Here, 14 days is the duration of the maintenance. Republic Korea's COVID-19 prevention policy is established after more than 2 weeks, which is why we designated a 2 weeks period. We varied the duration of maintenance between 7–28 days.

Moreover, we analyzed the performance of the proposed methods around ED from RI. To do that, we compared the start time of estimated outbreaks during the 4 weeks, 2 weeks before and after the ED from RI. We defined and set the warning period and the interval for comparing the performance of the ML methods to be 4 weeks.

2.8 Data availability

We developed the proposed method in Python 3.10 and made the codes using source data freely available on GitHub at https://github.com/modeling-computation/covid-19_outbreak/.

3 Results

3.1 Estimation of the transmission trend

Supplementary Figure S2 shows examples of the sample data with three labels. We calculated the correlation between the labels and the scaling parameters in Eq. (1). The labels, which were classified using

the risk index, accurately reflected the trend of increase, maintenance, and decrease in Supplementary Figure S3. We set the scaling parameters to 0.01 because the correlation was high (0.6) when c_1 and c_2 were 0.01, as Supplementary Figure S3A shows. Supplementary Figure S3B displays the correlations between the labels and all eight features described in Table 1. The slope (β^C) and standard deviation of the COVID-19 cases (σ^C) for the calibration period had a strong correlation with labels. Supplementary Figure S3C illustrates the range of the risk index for each label using a box plot. The box plot clearly indicates that high values of the risk index correspond to label 2.

Figure 3 evaluates the performance of ML methods such as SVM, RF, and XGB. Figures 3A–C presents confusion matrices for each method. The most critical errors occur when either predicting L2 when the actual label is L0, or predicting the L0 when the actual label is L2. RF and XGB did not make any of these errors, while SVM had two such cases. Figures 3D–F depicts the ROC curve for each class. The area under the curve (AUC), which measures accuracy in the ROC curve, was found to be close to 1 for all three ML methods. Table 2 summarizes the accuracy of the ML methods. The accuracies of SVM, RF, and XGB were higher than 0.94, with values of 0.9441, 0.9580, and 0.9545, respectively. The prediction of the *F*1-score for L0 (Decrease) or L2 (Increase) was particularly accurate, with values of 0.95 and higher.

Figure 4 shows the feature importance in RF and XGB. The features of standard deviation (σ^C), the increment rate (β^C), and mean ($\propto^C$) of the COVID-19 cases for the calibration period were important for both methods. The control intervention ($Policy^C$) also had a high rank of importance in RF, and the delta variant ($Delta^C$) was an important feture in XGB.

We conducted a sensitivity analysis by changing the calibration period from 14 to 28 days and the prediction periods from 7 to 21 days, as Supplementary Table S6 indicates. The results showed that the highest accuracy was achieved with a calibration period of 21 days and a prediction periods of 14 days.

3.2 Estimation of the start time for outbreaks

Korea experienced several outbreaks between March 2020 and October 2022. Figure 5A shows the number of COVID-19 cases from 9 June 2021 to 7 July 2021 for an estimated outbreak. The black dashed line in Figure 5A represents the reported outbreak. The asterisks in Figure 5B (★) presents the ED from RI. The shaded areas indicate the labels as L0 (green), L1 (yellow), and L2 (red) according to the risk index. We determined the start time of the new outbreak when the label remained at L2 for 2 weeks, which was the duration of maintenance. Therefore, the ED from RI for this outbreak was 23 June 2021. Figure 5C compares the ED from RI with the ED from ML. The ED from RF and ED from XGB showed the same dates as the ED from RI.

Figure 6 summarizes all estimated outbreaks. Figure 6A displays the number of COVID-19 cases with the five reported outbreaks. We obtained seven estimated outbreaks, numbered (1)–(7), based on ED from RI in Figure 6B. Black dashed lines in Figure 6B indicate the reported outbreaks. This method declared the ED a few days earlier than the start time of reported outbreaks. There were seven estimated outbreaks, including the 1st and 5th ones [(1) and (5)], while there were only five reported outbreaks.

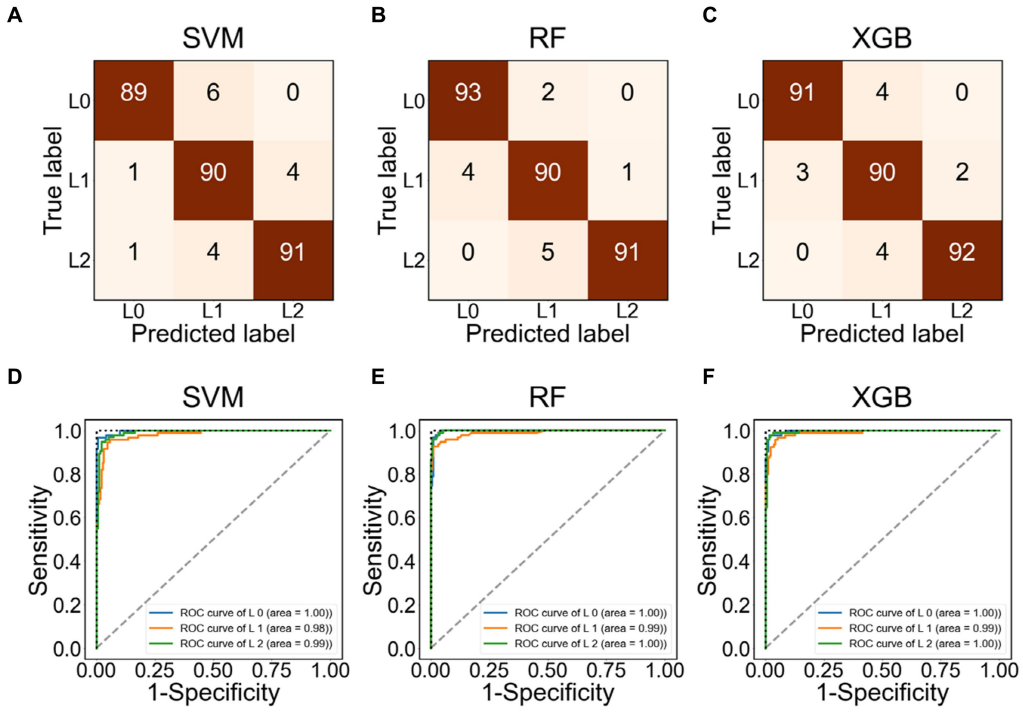

FIGURE 3
Confusion matrix and ROC curve using the test data labeled as L0, L1, L2. **(A–C)** Confusion matrix using SVM, RF, and XGB, respectively. **(D–F)** ROC using SVM, RF, and XGB, respectively.

TABLE 2 Accuracy of test data in three ML methods.

Estimator	Accuracy	*F*1-score		
		Label 0 (L0: decrease)	Label 1 (L1: maintain)	Label 2 (L2: increase)
SVM	0.9441	0.9570	0.9231	0.9529
RF	0.9580	0.9688	0.9375	0.9681
XGB	0.9545	0.9630	0.9326	0.9684

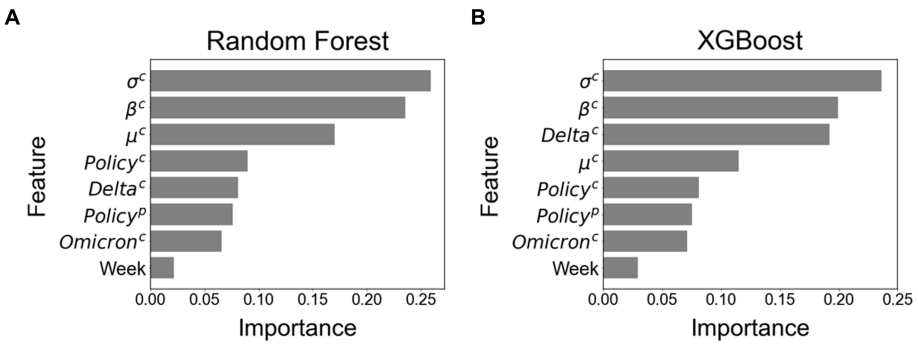

FIGURE 4
Feature importance among all eight features. **(A)** Feature importance using Random Forest. **(B)** Feature importance using XGBoost.

Figure 6C shows the specific results of each outbreak using ML methods. The figure also displays the COVID-19 cases (black solid line) and the risk index (blue dashed line). The ED from RI and the ED from ML predicted the same start dates of the (2), (3), (6), and (7) outbreaks. However, for the (1), (4), and (5) outbreaks, the ED from RI and the ED from ML differed by only 1 day. This means that both methods predicted almost identical start dates.

Table 3 summarizes the accuracy of the results between the reported and estimated outbreaks. We compared the accuracy of ML on the start time of outbreaks (1)–(7). We examine the results during the warning period, which was between 2 weeks before and after the ED from RI. The overall accuracy was high, ranging from 80% to 100%. Regarding the warning period for 4 weeks, RF showed the most accurate estimation with 100% accuracy, except for (1) and (5) outbreaks. This implies that RF detected the ED better for the rapid increase in a trend than other ML methods such as SVM and XGB.

Supplementary Figure S4 compares ED from RI with ED from ML by different durations of maintenance. When the duration changed to 7 or 21 days, there was no significant difference in the results. However, starting from 28 days, some outbreak detection points were not identified for a few outbreaks.

So far, we have used the training and testing datasets with a random 7:3 split ratio. Here, we conduct a simulation to assess the applicability of our approach for future prediction of the transmission trend. We divide the data into the train data from February 2020 to April 2022, when the omicron variant became prominent, and the test data from May to October 2022. We obtain sufficiently high accuracy on the test data as 0.8647 for RF and 0.8529 for XGB, even though those values decrease by approximately 5%–10%, compared to predictions made with randomly shuffled data. We need to figure out if our estimation can capture the fact that the start time of the 7th outbreak falls within the test data period.

Figure 7 shows the result of the estimation using the train data (February 2020–April 2022) and the test data (May 2022–October 2022). Based on the ED from RI results, the start time of the 7th outbreak was determined to be on 24 June 2022. In comparison, the machine learning predictions yielded the following results: the ED from SVM and the ED from XGB were 4 days later and 2 days earlier, respectively. However, the ED from RF accurately predicted the exact same day. Therefore, this result confirms that our approach can effectively predict the early outbreaks.

4 Discussion

In the present study, we aimed to propose a machine learning-based method to predict the transmission trend of COVID-19 and to detect the start time of new outbreaks by analyzing epidemiological data in the Republic of Korea. To do so, we first, evaluated the performance of ML methods such as SVM, RF, and XGB in estimating the transmission trend. We developed a risk index to measure changes in the transmission trend, which were categorized into three groups: decrease (L0), maintain (L1), and increase (L2). We achieved a high accuracy (over 94%) in predicting the classification of transmission trends. Specifically, the SVM, RF, and XGB methods yielded accuracies of 0.9441, 0.9580, and 0.9545, respectively, as shown in Figure 3 and Table 2.

Second, we estimated new outbreaks from March 2020 to October 2022 in Korea. We proposed a new method for identifying the start time of new outbreaks when the label 2 is sustained for at least 14 days, which means the duration of maintenance is set to be 14 days. According to this standard, we estimated outbreaks using two approaches: (i) ED from RI, (ii) ED from ML. We obtained seven estimated outbreaks, numbered (1)–(7) based on ED from RI, as shown in Figure 6 and Table 3, while there were only five reported outbreaks. This means that the proposed method could be applied to

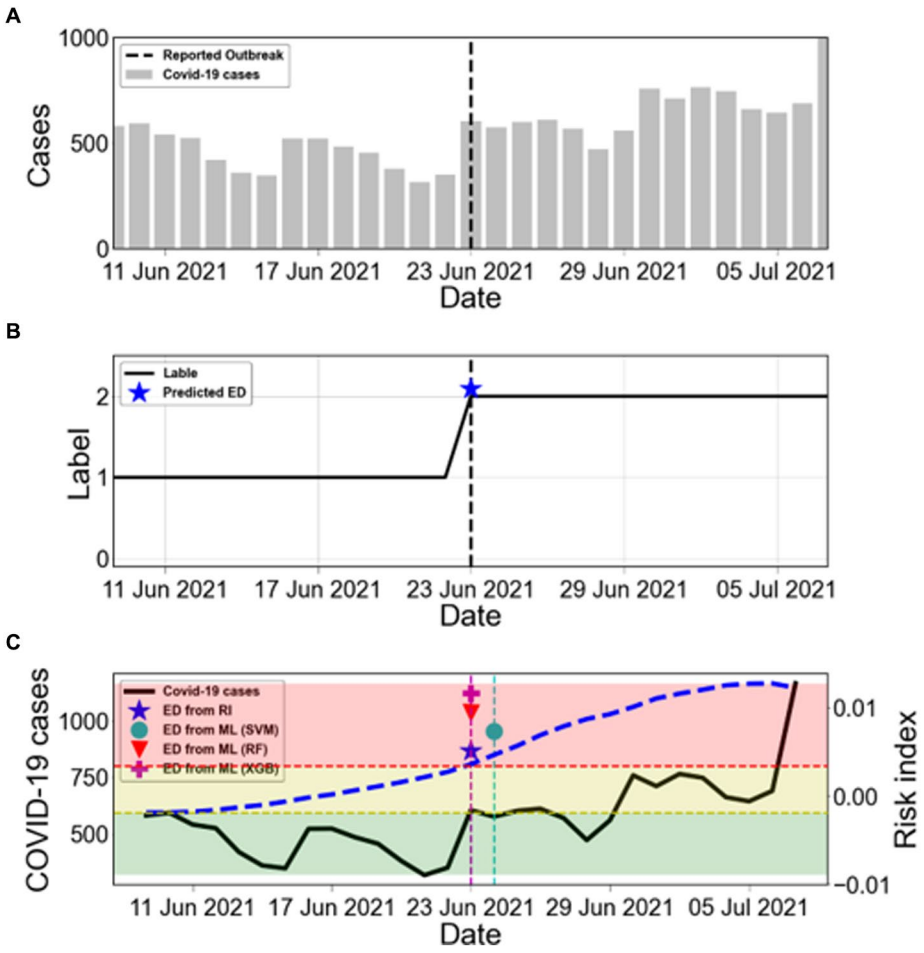

FIGURE 5
Estimation of the start time of COVID-19 outbreaks. **(A)** The bars show the COVID-19 cases from 9 June 2021 to 7 July 2021. The black dashed line marks the reported outbreak. **(B)** The label is obtained from the risk index. The blue asterisk (★) represents ED from RI. **(C)** Comparison between ED from RI and ED from ML during the warning period from ED from RI. The black solid line shows the number of COVID-19 cases (left *y*-axis). The blue dashed line shows the calculated risk index (RI) (right *y*-axis). The results of ED from ML are marked as SVM (●), RF (▼), and XGB (+). The shaded areas indicate the labels as L0 (green), L1 (yellow), and L2 (red) according to the risk index.

detect minor outbreaks such as (1) and (5). We found that both the ED from RI and the ED from ML accurately predicted the same start dates for the (2), (3), (6), and (7) outbreaks. For the (1), (4), and (5) outbreaks, the ED from RI and the ED from ML differed by only 1 day. This indicates that both methods predicted start dates that were nearly identical. Additionally, we compared the accuracy of ED from ML in predicting the start time of outbreaks (1)–(7) during the warning period, which is the time period before and after 2 weeks from the ED from RI. The overall accuracy was high, ranging between 80%–100%. RF and XGB achieved the highest accuracy for outbreak detection, with 100% accuracy, except for the (1) and (5) outbreaks.

Third, we conducted a sensitivity analysis in our study, which included two components: (i) we evaluated the impact of different calibration periods (ranging from 14 to 28 days) and prediction periods (ranging from 7 to 21 days), with the calibration period being longer than the prediction period. Based on our analysis, we determined that the highest accuracy was obtained when using a calibration period of 21 days and a prediction period of 14 days, as presented in Supplementary Table S6. (ii) We varied the duration of maintenance for L2 between 7 and 28 days, as shown in Supplementary Figure S4. We observed that there was no significant difference in the results when the duration was changed to 7 or 21 days. However, when the duration was extended to 28 days, some outbreak detection points were missed for a few outbreaks.

This study has several limitations. First, previous studies (32, 33) have shown that vaccination reduces the number of severe cases. However, this study did not consider the effect of vaccination. We assumed that vaccination had a greater impact on reducing the number of infected patients than on the occurrence of outbreaks. Thus, we did not consider vaccination because we aimed to predict the occurrence and trend of outbreaks using classification methods.

Second, there is a limitation of insufficient data available, as COVID-19 has only had a period of 2 years of circulation compared to diseases such as influenza and norovirus that exhibit long-term epidemic patterns, which have been studied using ML to predict the start time of outbreaks in (34, 35). To overcome this, we analyzed the pattern of COVID-19 transmission in Korea and successfully extracted features that were highly related to the labels listed in Table 1. Consequently, we were able to achieve high accuracy in predicting the trend of epidemic patterns in three categories: increase, maintain, and decrease.

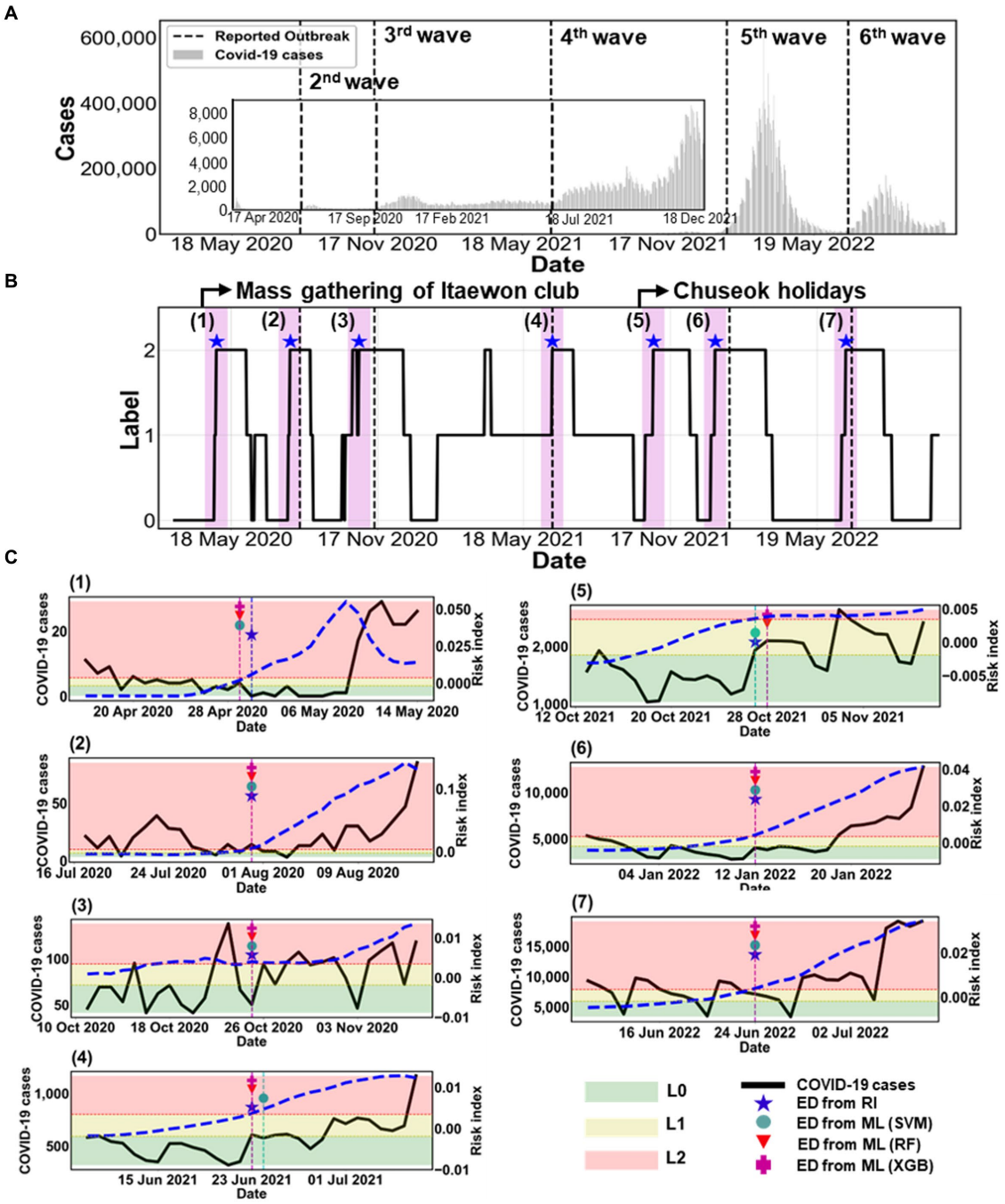

FIGURE 6
Comparison of estimated outbreaks. **(A)** The epidemic curve is shown from 18 February 2020 to 31 October 2022. The black dashed lines mark five reported outbreaks, described in Supplementary Table S5. **(B)** The label is obtained from the risk index in the black solid line. The blue asterisk (★) represents ED from RI. The magenta shaded region indicates the warning period from ED from RI. **(C)** Comparison between ED from RI and ED from ML during the warning period from ED from RI for (1)–(7) estimated outbreaks. The black solid line shows the number of COVID-19 cases (left *y*-axis). The blue dashed line shows the calculated RI on the right *y*-axis. ED from ML are marked as SVM (●), RF (▼), and XGB (+). The shaded areas indicate the labels as L0 (green), L1 (yellow), and L2 (red) according to the risk index.

Despite these limitations, our study proposes a novel approach for estimating the start time of new outbreaks using machine learning methods and a risk index function, which has not been previously studied. Our approach offers several advantages and potential applications. In previous studies (14, 36), only the data on the number of infected patients were utilized for predictions of COVID-19 transmission. However, we incorporated various data, including the intensity changes in NPIs policies implemented by the Korean government and the prevalence of variant viruses (especially delta and omicron). Thus, our interpretation is comprehensive by analyzing the epidemiological data.

TABLE 3 Comparison of the accuracy of the test data between the reported outbreak and estimation of ED using ML method (ED from ML).

	Reported outbreak[a]	ED from RI	ED from ML					
			ED from SVM		ED from RF		ED from XGB	
Estimated outbreak	Date	Date	Date	Accuracy	Date	Accuracy	Date	Accuracy
(1)	—	2020-04-30	2020-04-29	0.923	2020-04-29	0.923	2020-04-29	0.923
(2)	2020-08-12	2020-07-31	2020-07-31	0.857	2020-07-31	1.000	2020-07-31	1.000
(3)	2020-11-13	2020-10-25	2020-10-25	0.857	2020-10-25	1.000	2020-10-25	1.000
(4)	2021-06-23	2021-06-23	2021-06-24	0.889	2021-06-23	1.000	2021-06-23	1.000
(5)	-	2021-10-27	2021-10-27	1.000	2021-10-28	0.833	2021-10-28	0.833
(6)	2022–01–30	2022-01-12	2022-01-12	0.857	2022-01-12	1.000	2022-01-12	1.000
(7)	2022-07-01	2022-06-24	2022-06-24	1.000	2022-06-24	1.000	2022-06-24	1.000

There are seven outbreaks estimated from ML methods during a 4 weeks, denoted by (1)–(7). The date of ED from RI and ED from ML shows the timing of the early outbreak from the estimation.

[a]Reported outbreak represents the start time of the outbreaks, summarized in Supplementary Table S5.

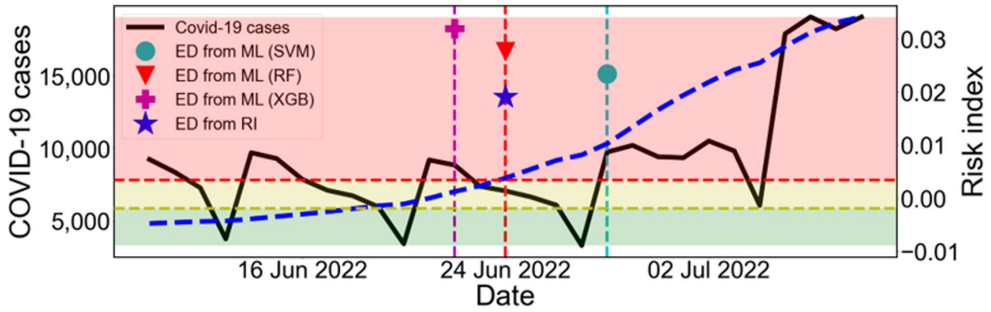

FIGURE 7
Estimation of the outbreak using the train data (February 2020–April 2022) and the test data (May 2022–October 2022). Comparison between ED from RI and ED from ML during the warning period from ED from RI. The black solid line shows the number of COVID-19 cases (left *y*-axis). The blue dashed line shows the calculated RI (right *y*-axis). The results of ED from ML are marked as SVM (●), RF (▼), and XGB (+). The shaded areas indicate the labels as L0 (green), L1 (yellow), and L2 (red) according to the risk index.

We newly suggested a risk index to quantify the changes of transmission trend. The risk index indicates the change of the transmission trend, which can be used to classify the risk of potential outbreaks. This measurement is a mathematically interpretable novel measurement that was not used in previous research. Using this metric, we are able to classify sample data into three distinct patterns (Increase, Maintain, Decrease) and assign labels accordingly.

Moreover, the variability in NPI intensity can be contingent on policy decisions. This means that by adjusting the NPI levels during the prediction period, we can anticipate shifts in future patterns of infection. This has the potential to assist in determining effective policy steps. In essence, our proposed predictive method can be utilized as a scientific foundation for establishing policy levels.

Previous research (14, 36) showed that the prediction accuracy for early detection of outbreak exhibited around 60%–80% even though the proposed methods were different. However, in the current study, employing machine learning techniques for the categorization on test data yielded a significantly higher accuracy of approximately 94%. Notably, a higher accuracy was achieved specifically for the Increase category (L2). By incorporating various datasets and utilizing the novel risk index for categorizing infection patterns, our proposed method contributed to achieving robust predictive performance even with limited data.

Overall, our study highlights the strength of our approach in accurately predicting the timing of an outbreak using an interpretable and explainable method. This method is also applicable to other infectious diseases and can contribute to the development of targeted prevention and control measures, facilitating better management of resources during the pandemic. It would enable healthcare providers to respond more effectively to COVID-19. Our proposed method identified outbreaks using machine learning-based approaches and can be further improved by collecting more data and establishing appropriate criteria for classes in future studies.

5 Conclusion

In conclusion, this study proposed a novel method for detecting the start time of new outbreaks and predicting transmission trends using machine learning-based approaches and a risk index function. The method achieved high accuracy in estimating the classification of transmission trends and successfully identified outbreaks with an interpretable and explainable method. The accuracy of SVM, RF, and XGB was higher than 0.94, with RF achieving the highest accuracy for outbreak detection. The method provides a standard for

predicting the start time of new outbreaks, enabling healthcare providers to respond more effectively to COVID-19 transmission. Overall, the study demonstrates the strength of machine learning-based approaches in accurately predicting the timing of outbreaks, ultimately improving patient care and reducing the burden on healthcare systems.

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

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements.

Author contributions

GC and JP: analyzed the data. GC, JP, YC, HA, and HL: drafted and revised the manuscript and interpreted the results. 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.2023.1252357/full#supplementary-material

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Clinical features of omicron SARS-CoV-2 variants infection associated with co-infection and ICU-acquired infection in ICU patients

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Background: Although the decreasing rate of hospital admission in the omicron wave has led countries to loosen control, still the patients requires ICU admission. It is common for viral respiratory infections to be co-infected with bacteria. However, the difference between co-infection and ICU-acquired infection on their clinical characteristics and outcomes during the Omicron wave was little reported.

Methods: Clinical and microbiological data were collected from ICU patients with omicron infection between April 1st, 2022, and May 31th, 2022 and a comprehensive comparative study of the clinical characteristics and endpoint were conducted.

Results: The Omicron SARS-CoV-2 variants-infected patients requiring intensive care had high rates of co-infection (42.55%). Additionally, the ICU COVID-19 patients with co-infection showed more severe clinical features compared to those with ICU-acquired infection. Furthermore, Multivariate Cox analysis demonstrated that co-infection (hazard ratio: 4.670, $p = 0.018$) was a significant risk factor for poor outcomes in ICU patients with COVID-19. Besides, Kaplan–Meier survival curve analysis revealed that COVID-19 patients with co-infection had a significantly shorter 28-Day survival time compared to those with ICU-acquired infection ($p < 0.001$). Finally, our investigation identified a significant association between the presence of *Candida app.* in the broncho-alveolar lavage and an elevated risk of mortality (OR: 13.80, $p = 0.002$) and invasive ventilation (OR: 5.63, $p = 0.01$).

Conclusion: Co-infection is prevalent among patients requiring intensive care and is linked to unfavorable outcomes in the Omicron wave. Consequently, more attention may be needed for the empirical antibacterial treatment in ICU patients within the COVID-19 Omicron variant, especially anti-fungi.

KEYWORDS

COVID-19, Omicron, co-infection, ICU-acquired infection, *Candida app*

1 Introduction

Coronavirus disease 2019 (COVID-19) may induce the incidence of acute respiratory distress syndrome (ARDS), requiring intensive care unit (ICU) admission and invasive or noninvasive mechanical ventilation. Up to now, there are several SARS-CoV-2 variants, including Alpha, Beta, Gamma, Delta, and Omicron, the newest variant, which was first confirmed in November 2021 in South Africa (1). In comparison with the previous four variants, the Omicron variant has the highest mutation rate, with 50 mutations accumulating in its genome (2). As many countries are becoming dominated by the Omicron strain, COVID-19 prevention and control have become more challenging due to its fast-spreading property and immune escape (3). Currently, Omicron and its sub-variants caused successive outbreaks, with many countries currently experiencing their more than once wave. Importantly, although most patients in Omicron wave are asymptomatic, or symptomatic people who could heal on their own, still some patients require ICU admission. Therefore, more efforts are needed to investigate the clinical characteristics of critically ill patients with Omicron SARS-CoV-2 variants infection.

The presence of respiratory viral infections may increase the risk of serious bacterial and fungal infections, leading to increased mortality, and thus co-infection caused by respiratory pathogens including bacterial and fungal is a challenging issue in COVID-19. Chen and colleagues recorded a high co-infection with both bacterial and fungal in COVID-19 patients in 2019 (4). Lansbury et al. showed that 7% of hospitalized COVID-19 patients suffered a bacterial co-infection and 14% needed intensive care (5). In support of these, Buehler et al. also found that COVID-19 patients with bacterial pulmonary superinfection had a severer disease course, particularly a lower probability of being alive and free of invasive mechanical ventilation at study day 28 (6). However, the mortality rates from COVID-19 co-infection have varied widely from twofold compared to non-co-infected COVID-19 patients, to having no effect on mortality among co-infected ICU patients (7–9). Besides, it should be noted that all these results were obtained from patients with non-Omicron variants of SARS-CoV-2. Given these controversial results, investigation on the co-infection and ICU-acquired infection may be necessary for the clinical decision of antimicrobial treatment in COVID-19 therapy in the ICU.

In this study, we aimed to describe the demographic characteristics, hematological parameters, and pathogen infections in ICU patients with Omicron SARS-CoV-2 variants infection and comparison of clinical features of between co-infection or ICU-acquired infection.

2 Materials and methods

2.1 Study design and participants

This retrospective study was conducted in the Shanghai Municipal Center for Disease Control and Prevention, one of the institutions designated to treat SARS-CoV-2 infections. All cases were confirmed to be infected with COVID-19 by performing real-time reverse transcription PCR testing. After excluding the patients who had not provided their consent, from April 1st, 2022 to May 31th, 2022, a total of 47 COVID-19 patients needed ICU care were included in the study. Considering that COVID-19 outbreak in Shanghai during this period was attributed to the heightened contagiousness of Omicron, therefore, these cases in our study were suspected to be infected with Omicron SARS-CoV-2 variants, although virus genotyping was not conducted to identify the Omicron COVID-19 strain. The protocols in this study was approved by the Ethical Committee of the Shanghai Tenth People's Hospital (SHYS-IEC-5.0/22 K131/P01) and has also been registered on chictr.org.cn (ChiCTR2300070486).

2.2 Study definition

Clinical classification at admission was based on the criteria of the Guidelines for the Diagnosis and Treatment of COVID-19 of China (9th version), and disease severities were classified as mild, moderate, severe, and critical. Co-infection was defined as bacterial/fungal infections, which were detected within the first 48 h of ICU admission. ICU-acquired infection was considered bacterial/fungal infections, which occurred >48 h after ICU admission. The definition for pathogen detection was based on at least one positive result detected in broncho-alveolar lavage, blood, or urine which were collected through the electronic patient records.

2.3 Clinico-pathological parameters of study participants

Demographic information, clinical details, and 28-day mortality were obtained through the electronic patient records. The following data were collected: age, gender, initial symptoms, comorbidities, laboratory tests (within the first 48-h), microbiologic results were detected by metagenomics next generation sequencing (broncho-alveolar lavage, blood, and urine), and the type of mechanical ventilation. To ensure accuracy, the data was collected and reviewed by two researchers independently.

2.4 Statistical analysis

Continuous and categorical variables were presented as the median interquartile range (IQR) and N (%), respectively. Chi-squared test or Fisher's exact test was applied for categorical data, and Wilcoxon rank-sum test was applied for continuous variables between the Co-infection group and the ICU-acquired infection group. Multivariate Cox proportional-hazard analysis was used to estimate the independent prognosis factors in COVID-19 patients, and comorbidities including hypertension, diabetes, cardiovascular disease, nervous system diseases, chronic respiratory disease, and cancer were included as covariates. The Kaplan–Meier method and log-rank test were used to compare the prognosis of COVID-19 patients in two groups. Univariate logistic regression model was used to calculate the death and invasive ventilation risk associated with each isolated pathogen. For statistical difference of mean of different immunity and inflammatory markers in COVID-19 co-infection groups Krusk al-Wallis test was used. SAS software 9.4 (SAS Institute Inc., Cary, NC, USA) was adopted for the statistical analyses. $p < 0.05$ was considered significant while p-values between 0.05 and 0.10 was considered borderline statistically significant.

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TABLE 1 Main characteristic of older adults ICU patients with Omicron SARS-CoV-2.

Variables	Total (*N* = 47)	Co-infection (*N* = 20)	ICU-acquired infection (*N* = 27)	*p* Value
Age (years)	79.72 (74.00–89.00)	85.50 (76.5–90.5)	77.00 (71.0–88.0)	0.061
Gender				0.689
Male	29 (61.70)	13 (65.00)	16 (59.26)	
Female	18 (38.30)	7 (35.00)	11 (40.74)	
Clinical classification				**0.037**
Severe cases, *n* (%)	20 (42.55)	5 (25.00)	15 (55.56)	
Critically ill type, *n* (%)	27 (57.45)	15 (75.00)	12 (44.44)	
Comorbidities				
Hypertension	28 (59.57)	14 (70.00)	14 (51.85)	0.210
Diabetes mellitus	16 (34.04)	8 (40.00)	8 (29.63)	0.458
Cardiovascular disease	27 (57.45)	14 (70.00)	13 (48.15)	0.134
Nervous system diseases	25 (53.19)	10 (50.00)	15 (55.56)	0.706
Chronic respiratory disease	29 (61.70)	14 (70.00)	15 (55.56)	0.314
Cancer	9 (19.15)	5 (25.00)	4 (14.81)	0.380
Initial symptoms				
Fever	21 (44.68)	8 (40.00)	13 (48.15)	0.579
Cough	23 (48.94)	9 (45.00)	14 (51.85)	0.642
Nausea/vomiting	9 (19.15)	4 (20.00)	5 (18.52)	0.898
Dyspnea	21 (44.68)	12 (60.00)	9 (33.33)	0.069
Hemoptysis	4 (8.51)	1 (5.00)	3 (11.11)	0.458
Fatigue	22 (46.81)	11 (55.00)	11 (40.74)	0.333
Anorexia	25 (53.19)	11 (55.00)	14 (51.85)	0.831
Mechanical ventilation				**0.003**
Non-invasive ventilation	30 (63.83)	8 (40.00)	22 (81.48)	
Invasive ventilation	17 (36.17)	12 (60.00)	5 (18.52)	
Length of ICU stay (days)	16 (11, 31)	14 (7.5–26.5)	24 (14–32)	0.079
Day-28 mortality				**0.001**
Alive	29 (61.70)	7 (35.00)	22 (81.48)	
Death	18 (38.30)	13 (65.00)	5 (18.52)	

ICU = intensive care unit.
Data are median (interquartile range [IQR]) or n/N (%). p values were calculated by chi-squared test, Fisher's exact test or Mann–Whitney U test, as appropriate.

3 Results

3.1 Demographic and clinical characteristics

According to the results of pathogen detection within the first 48 h of ICU admission. All the participants included in our study were divided into the co-infection (n = 20) or the ICU-acquired infection (n = 27). The baseline characteristics of the patients are summarized in Table 1. In all the participants, 29 (61.70%) patients were males with a median age of 79.72 years (Rang: 74.00–89.00). The patients in the co-infection group were borderline significantly older than the ICU-acquired infection group (85.50 vs. 77.00, $p = 0.061$). In terms of clinical classification, 20 (42.55%) patients were severe type, and 27 (57.45%) patients were critically ill type. When we classified by co-infection or ICU-acquired infection, there was a significantly higher percentage of patients with critically ill type in the co-infection group than in the ICU-acquired infection group (75.00% vs. 44.44%, $p = 0.037$). In addition, the most common symptom at the beginning of the disease was fever (44.48%), cough (48.94%), dyspnea (44.68%), fatigue (46.81%), and anorexia (53.19%). A borderline difference on dyspnea was found between co-infection and ICU-acquired infection (60.00% vs.33.33%, $p = 0.069$).

Furthermore, in all the participants, 30 patients received non-invasive ventilation while 17 patients received invasive ventilation. When we classified the type of mechanical ventilation as invasive ventilation or not, there was a significant difference between co-infection and ICU-acquired infection ($p = 0.003$). The length of ICU stay for patients with co-infection was 14 days (Range, 7.5–26.5) while that for patients with ICU-acquired infection was 24 days (Range, 14–32), and further analysis revealed that there was a borderline significant difference in the length of ICU stay between

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TABLE 2 Laboratory findings of patients with SARS-CoV-2 Omicron infection at admission.

Parameters	Normal Range	Total (*N* = 47)	Co-infection (*N* = 20)	ICU-acquired infection (*N* = 27)	*p* Value
Blood routine examination					
White blood cells,x10^9/L	3.5–9.5	10.38 (6.10–14.89)	12.79 (7.64–14.73)	8.93 (5.72–14.91)	0.424
Lymphocytes,x10^9/L	1.1–3.2	0.77 (0.39–1.01)	0.49 (0.28–0.69)	0.88 (0.60–1.05)	**0.006**
Platelets,x10^9/L	125–350	180 (145–248)	169.50 (108.50–212)	193.50 (158–249)	0.263
Hemoglobin, g/L	115–150	106 (88–127)	107 (198.50–120.50)	104 (84–129)	0.739
Biochemical examination					
C-reactive protein, mg/L	0–10	52.55 (25.80–111.07)	76.40 (40.48–132.01)	49.22 (21.38–97.91)	0.254
Alanine aminotransferase, U/L	7–40	19.00 (13.00–46.00)	20.50 (17.50–37.50)	19.00 (13.00–46.00)	0.334
Aspartate aminotransferase, U/L	13–35	32 (21–49)	37.50 (30.50–58.50)	28 (20–45)	**0.044**
Total bilirubin, μmol/L	3.4–20.5	13.95 (9.40–21.00)	14.10 (8.90–26.10)	13.05 (10.50–19.40)	0.595
ALP, U/L	50–135	91.50 (69–112)	96 (76–133)	88 (65–95)	0.099
Creatinine, μmol/L	41–81	75.10 (52.60–120.00)	92.95 (64.60–161.25)	61.10 (49.10–103.90)	0.085
Uric acid, μmol/L	150–350	308.02 (168.96–421.65)	369.43 (176.75–468.96)	234.32 (168.96–375.51)	0.177
Urea, mmol/L	3.1–8.8	8.97 (5.89–13.30)	10.29 (8.26–19.30)	6.45 (5.30–10.00)	**0.032**
Lactate dehydrogenase, U/L	120–250	332.5(254–391)	351.50 (257–473.50)	324 (253–366)	0.308
K^+, mmol/L	3.5–5.3	3.90(3.40–4.26)	3.95(3.45–4.23)	3.9(3.40–4.30)	0.534
Na^+, mmol/L	137–147	139(135–144)	141(136–144)	139(134–143)	0.262
Coagulation function					
Prothrombin time, sec	11–14.5	14.95 (13.50–16.50)	15.40 (13.90–16.65)	14.90 (13.20–16.50)	0.425
Fibrinogen, g/L	2.0–4.0	4.68 (3.63–5.71)	4.82 (3.18–6.17)	4.57 (3.64–5.20)	0.407
D-dimer, μg/mL	0–0.50	2.50 (1.32–6.03)	2.15 (1.03–6.65)	2.81 (1.42–6.03)	0.623
Immune/inflammatory factors					
$CD3^+$, cell/μL	690–2,540	339.02 (161.94–706.30)	231.47 (136.87–413.29)	631.62 (279.85–711.24)	**0.032**
CD4+, cell/μL	410–1,590	231.11 (107.23–402.39)	190.53 (102.01–293.85)	256.83 (195.59–419.52)	0.232
CD8+, cell/μL	190–1,140	75.98 (41.08–286.27)	54.49 (22.28–77.60)	158.17 (67.10–321.33)	**0.008**
CD4/CD8 ratio	0.90–3.60	2.51 (1.31–4.49)	3.76 (2.73–5.41)	1.51 (1.19–2.75)	**0.004**
TNF-α, pg./mL	< 16.50	0.34 (0.13–1.70)	0.23 (0.13–1.81)	0.34 (0.06–1.70)	0.728
IL-6, pg./mL	< 5.40	6.29 (0.00–29.64)	9.58 (6.02–54.61)	0.00 (0.00–29.64)	**0.022**
IL-10, pg./mL	< 12.90	13.83 (8.55–144.76)	34.37 (12.79–693.04)	9.86 (2.79–44.64)	**0.034**

ICU = intensive care unit; ALP = alkaline phosphatase; K+ = Potassium ion; Na+ = Sodium ion; TNF-α = Tumor necrosis factor-α; IL-6 = Interleukin-6; IL-10 = Interleukin-10.
Data are median (interquartile range [IQR]) or n/N (%). *p* values were calculated by chi-squared test, Fisher's exact test or Mann–Whitney U test, as appropriate.

these two groups ($p = 0.079$). Besides, 18 patients (38.30%) were death and 29 (61.70%) alive in day-28. Thirteen patients with the co-infection were dead (65%) while 5 patients in the ICU-acquired group (18.52%) were dead in day 28. The proportions of mortality patients were significantly different between the two groups ($p = 0.001$).

3.2 Hematological parameters of ICU patients with SARS-CoV-2 omicron variant and co-infection or ICU-acquired infection

Laboratory findings of the participants were described in Table 2. There were no differences in the levels of white blood cell count (12.79 [7.64–14.73] vs. 8.93 [5.72–14.91], $p = 0.424$), C reactive protein (CRP) (76.40 [40.48–132.01] vs. 49.22 [21.38–97.91], $p = 0.254$), and hemoglobin (107 [198.50–120.50] vs. 104 [84–129], $p = 0.739$) between the co-infection and ICU-acquired infection groups. However, that the absolute numbers of lymphocytes count (0.49 [0.28–0.69] vs. 0.88 [0.60–1.05], $p = 0.006$) were significantly lower in co-infection patients than that in ICU-acquired infection patients. Compared with the ICU-acquired infection, the co-infection patients had significantly higher AST (37.50 [30.50–58.50] vs. 28 [20–45], $p = 0.044$) and serum urea (10.29 [8.26–19.30] vs. 6.45 [5.30–10.00], $p = 0.032$) levels. Meanwhile, serum ALP levels (96 [76–133] vs. 88 [65–95], $p = 0.099$) and creatinine levels (92.95 [64.60–161.25] vs. 61.10 [49.10–103.90], $p = 0.085$) also tended to be higher in the co-infection group with a borderline significance.

Considering that immunity and inflammatory responses are closely associated with the clinical manifestations of COVID-19 and outcomes (10, 11), the levels of peripheral $CD3^+$, $CD4^+$, and $CD8^+$ T

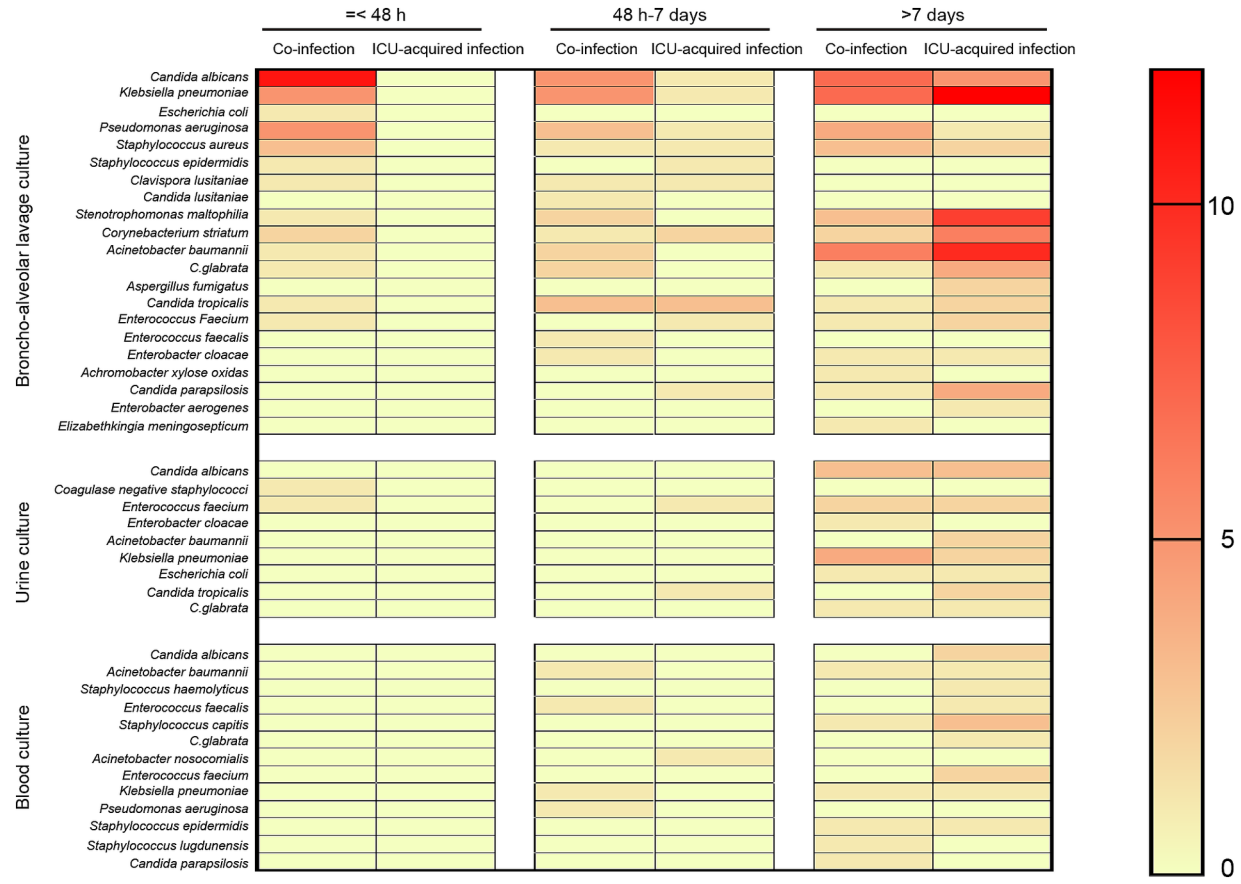

FIGURE 1
Distribution of infection pathogens positive results stratified by days. The potential pathogens were detected by metagenomics next generation sequencing (broncho-alveolar lavage, blood, and urine) and stratified by days (≤ 48 h, 48 h – 7 days, and ≥ 7 days) following ICU admission.

cells as well as the levels of TNF-α, IL-6, and IL-10 were also determined in this study. Compared with ICU-acquired infection patients, co-infection groups had lower levels of $CD3^+$ T cells (231.47 [136.87–413.29] vs. 631.62 [279.85–711.24], $p = 0.014$). No significant differences observed in CD4+ cells, but co-infection patients had lower levels of $CD8^+$ T cells (54.49 [22.28–77.60] vs. 158.17 [67.10–321.33], $p = 0.008$), and higher CD4/CD8 ratio (3.76 [2.73–5.41] vs. 1.51 [1.19–2.75], $p = 0.004$) than that of ICU-acquired infection patients. Furthermore, compared with ICU-acquired infection patients, co-infection group had a significantly higher levels of serum IL-6 (9.58 [6.02–54.61] vs. 0.00 [0.00–29.64], $p = 0.022$) and IL-10 (34.37 [12.79–693.04] vs. 9.86 [2.79–44.64], $p = 0.034$) (Table 2). Collectively, all these laboratory tests indicated that ICU patients with co-infection had severer inflammatory responses as well as worse liver and kidney function index compared with those with ICU-acquired infection.

3.3 Identification of pathogen infections In omicron patients with ICU care

The list of identified potential pathogens by metagenomics next generation sequencing (broncho-alveolar lavage, blood, and urine) in co-infection and ICU-acquired infection patients stratified by days following ICU admission were shown in Figure 1. Fifteen potential pathogens were identified from 20 patients in the co-infection group within the first 48 h of ICU admission. And the most common co-pathogen in broncho-alveolar lavage were *Candida albicans* (n = 11), *Klebsiella pneumonia* (n = 5), *Pseudomonas aeruginosa* (n = 5), and *Staphylococcus aureus* (n = 3) and 11 patients had more than one coinfected bacterium. Furthermore, two patients were also detected to be infected with *Coagulase-negative staphylococci* and *Enterococcus Faecium* in urine, respectively.

Beyond 48 h of hospital admission to the end of ICU stay, 21 potential co-pathogens were identified in broncho-alveolar lavage, 8 potential co-pathogens were identified in urine, and 13 potential co-pathogens were identified in blood. When we stratified by days, 16, 2, and 5 potential pathogens were identified in broncho-alveolar lavage, urine, and blood, respectively from days 3–7 and 17, 8, 11 potential pathogens were identified in broncho-alveolar lavage, urine, and blood, respectively from day 8 onwards (Figure 1).

3.4 Co-infection Is associated with poor outcomes In ICU patients with omicron variants infection

Results of multivariate analysis on the risk of severe clinical events in these 47 patients are shown in Table 3. In the multivariate-adjusted

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TABLE 3 Multivariate analysis for the risk of severe events in older adults ICU patients with SARS-CoV-2 Omicron.

Clinical factors	Day-28 mortality			Mechanical ventilation		
	HR	95% CI	*p* Value	HR	95% CI	*p* Value
Age	1.050	0.990–1.114	0.106	1.044	0.986–1.105	0.144
Sex	0.787	0.247–2.505	0.685	0.966	0.268–3.487	0.958
Hypertension	1.419	0.432–4.662	0.564	0.555	0.161–1.910	0.350
Diabetes	0.633	0.204–1.964	0.429	0.638	0.184–2.213	0.478
Co-infection	**4.670**	**1.298–16.802**	**0.018**	**5.715**	**1.553–21.035**	**0.009**
Cardiovascular diseases	1.141	0.371–3.503	0.818	1.108	0.332–3.697	0.867
Chronic respiratory diseases	0.790	0.233–2.682	0.705	0.754	0.206–2.760	0.669
Nervous system diseases	2.141	0.544–8.429	0.276	1.372	0.384–4.900	0.627
Cancer	1.660	0.362–7.611	0.514	0.673	1.053–2.950	0.599

HR = hazard ratio; CI = confidence interval.

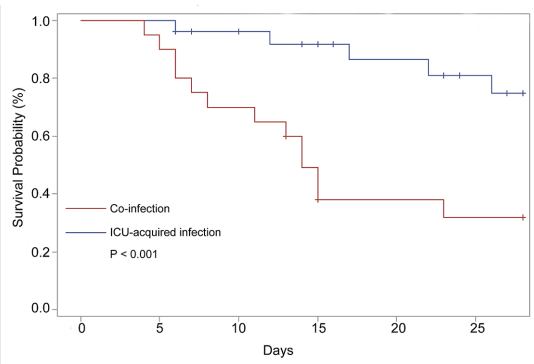

FIGURE 2
Comparison of 28-Day survival of the COVID-19 patients need ICU care with co-infection and ICU-acquired infection. The data from 47 patients contributed to these analyses. N = 20, co-infection; N = 27, ICU-acquired infection.

Cox proportional hazards model, co-infection was observed to be a meaningful factor associated with the occurrence of outcomes of the patients. The ICU patients with co-infection (HR = 4.670, 95% CI: 1.298–16.802, $p = 0.018$) had a significant increased risk of day-28 mortality.

Furthermore, risk factors for the type of mechanical ventilation were also assessed. We found that the older adults ICU patients with co-infection (HR = 5.715, 95%CI: 1.553–21.035, $p = 0.009$) also had a significantly increased risk of invasive ventilation. The survival curve for clinical outcomes based on co-infection shown in Figure 2 indicates that the patients with co-infection had poor outcomes compared with those with ICU-acquired infection ($p < 0.001$).

3.5 *Candida* spp. Is a significant risk factor for death In ICU patients with omicron variants infection

In most co-infections, more than one micro-organism was isolated from broncho-alveolar lavage. The results showed that the presence of *Candida* spp. in the broncho-alveolar lavage was associated with an increased risk of death (OR: 13.80, $p = 0.002$) and invasive ventilation (comparing with co-infection patients who not infected with *Candida* spp.) (OR: 5.63, $p = 0.01$). However, these associations were not observed when *Klebsiella* spp., *Staphylococcus* spp., *Acinetobacter* spp., or *Pseudomonas* spp. existed in the broncho-alveolar lavage (Table 4). Furthermore, when we stratified the co-infection patients as co-infection with or without *Candida* spp., we discovered that compared with ICU-acquired infection, the patients co-infected with *Candida* spp. showed significantly lower lymphocyte numbers, $CD3^+$, $CD4^+$, and $CD8^+$ T cells, along with a higher ratio of CD4/CD8. Conversely, no differences were observed between ICU-acquired infection and co-infection without *Candida* spp. (Figure 3).

4 Discussion

According to our knowledge, this is the first study to be conducted in China on the comparison of clinical features of associated with co-infection and ICU-acquired infection in ICU patients following Omicron variant infection. This work describes four major novel findings. First, our results showed that the COVID-19 patients need ICU care in the Omicron wave had high rates of co-infection. Second, we found that the ICU patients with co-infection had more seriously clinical features and higher day-28 mortality compared with those with ICU-acquired infection. Thirdly, we observed that co-infection was an independent meaningful factor associated with the occurrence of invasive ventilation and day-28 mortality in ICU patients following Omicron variant infection. Finally, we found *Candida app*. in the broncho-alveolar lavage was significantly associated with an increased risk of death in ICU COVID-19 patients. Collectively, our data suggested that co-infection is common in ICU patients and associated with poor outcomes in the Omicron wave. More attention may be needed for the empirical antibacterial treatment in ICU patients within the COVID-19 Omicron variant, especially anti-fungal.

Several studies have investigated the effects of co-infection on their clinical characteristics and outcomes in COVID-19 patients. For example, Garcia-Vidal and colleagues found that compared with COVID -19 patients without infection, both hospital-acquired super infection and community-acquired co-infection patients had worse outcomes (12). Baskaran et al. also indicated that patients with co-infections were more likely to die in ICU compared to those

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TABLE 4 Risk of death associated with isolation of bacteria and fungi from COVID-19 patients.

Genus	Day-28 mortality		Mechanical ventilation	
	OR (95% CI)	*P*-value	OR (95% CI)	*P*-value
Candida spp.	13.80 (2.60, 7.28)	0.002	5.63 (1.46, 5.75)	0.01
Klebsiella spp.	Undefined[a]	0.967	1.20 (0.18, 8.00)	0.851
Staphylococcus spp.	Undefined[a]	0.963	0.87 (0.14, 5.31)	0.877
Acinetobacter spp.	Undefined[a]	0.967	8.92 (0.91, 8.84)	0.061
Pseudomonas spp.	5.33 (0.55, 5.88)	0.149	2.99 (0.45, 2.06)	0.257

[a]Undefined because odds ratio (OR) could not be calculated with a zero cell. OR and 95% confidence interval (CI) were calculated using univariate logistic regression model. Data were calculated considering the isolated micro-organisms individually.

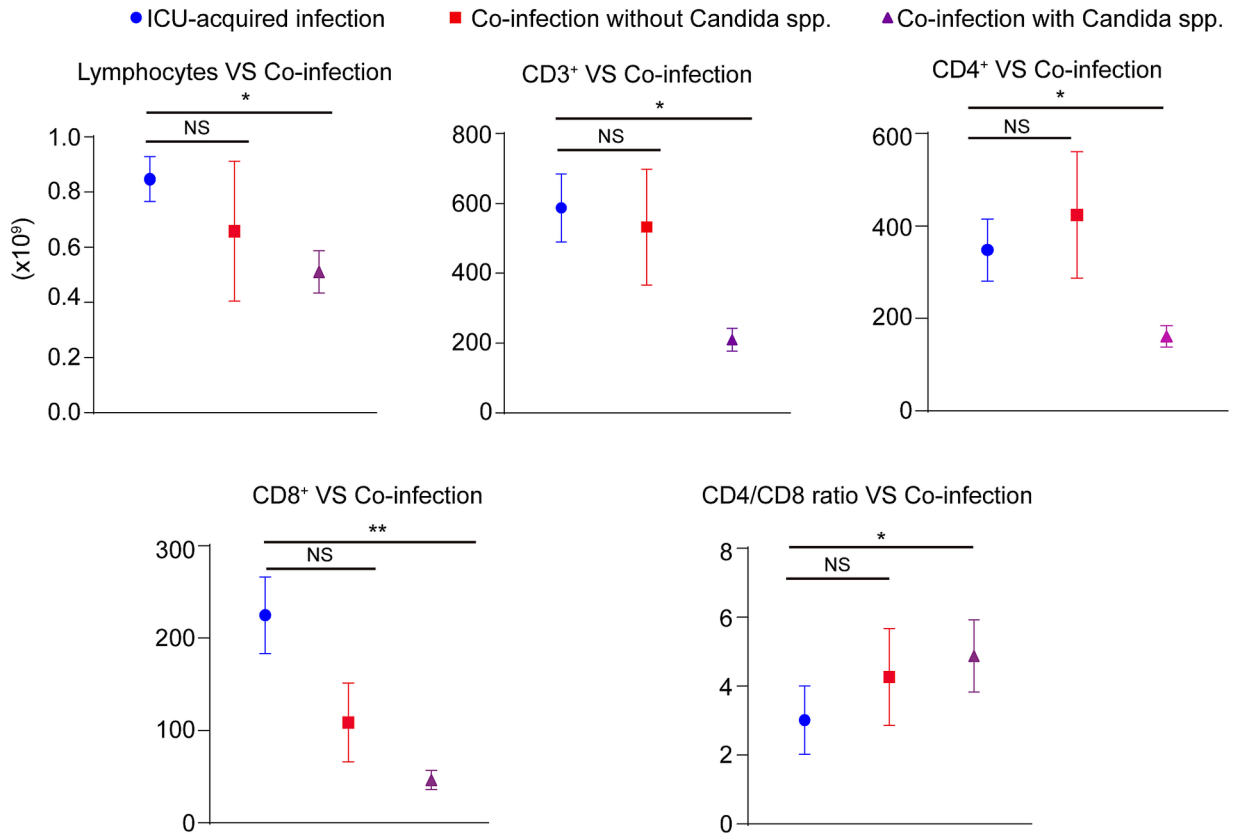

FIGURE 3
The role of immunity and inflammatory markers in COVID-19 patients. The absolute numbers of lymphocytes count as well as the levels of peripheral CD3⁺, CD4⁺, and CD8⁺ T cells were obtained through the electronic patient records. Stratifing the COVID-19 patients as ICU-acquired infection, co-infection patients with *Candida* spp. infection, and co-infection without *Candida* spp. infection. NS; NO significant, $*p < 0.05$, $**p < 0.01$.

without co-infections (13). However, few studies have elucidated the contrasting clinical characteristics and outcomes in patients with co-infection and ICU-acquired infection, especially with Omicron SARS-CoV-2 infection. As we know, the advanced age has been regarded as one of the main COVID-19 risk factors for co-infection and age-related immune system senescence is considered to be the major reason for increased susceptibility to infection (14, 15). Interestingly, in our study, compared with the ICU-acquired infection group, the elders with omicron infection in the co-infection group were associated with higher age. Furthermore, the co-infection patients displayed more symptoms of dyspnea, worse clinical classification at admission, and a higher percentage of individuals using invasive ventilation. All these clinical features suggested that co-infected patients were more likely to develop a severe condition. Besides, at the follow-up endpoint, we compared the length of ICU stay and day-28 mortality in two groups. We observed that there was a borderline difference in the length of ICU stay between the co-infection and ICU-acquired infection patients. The short length of stay in co-infected patients indicated that the co-infected older adults with omicron infection might present considerably fast disease progression. Moreover, co-infections resulted in 13 patients' death representing a 65% mortality rate on the day-28, which was significantly higher than ICU-acquired infections. Our data showed that there was a higher percentage of patients in the co-infection

group (60%) than in the ICU-acquired infection group (18.52%) treated by invasive ventilation ($p = 0.003$). Therefore, our study highlighted that although the virulence of the omicron variant is reduced, it still has a poor prognosis in the older adults, especially with co-infection.

Viral infection triggers an innate immune response and immunopathology is the main mechanism in the genesis and progression of COVID-19 (16). As we know, $CD3^+$ and $CD8^+$ T cells are crucial in T-cell antigen recognition and resistance to viruses or other stimuli (11). Previously, it has been reported that $CD3^+$ and $CD4^+$ T cell count were independent prognostic factors for death in older adults with severe community-acquired pneumonia and sepsis (17, 18). Recently, Wan et al. and Liu et al. showed that the counts of CD8+ cells were remarkably decreased in severe and critical patients with COVID-19 (19, 20). In addition, several studies showed that patients with critical or severe COVID-19 presented low $CD3^+$ T cell count (21, 22). Besides, the increased higher ratio of CD4/CD8 was associated with the inflammatory status of COVID-19 (23). The CD4/CD8 ratio was considered a marker for the early identification of those likely to require intervention in the ICU (24). These reports revealed that the reduced lymphocyte count was related to the occurrence of critical COVID-19 cases. In the present study, the results showed that co-infection patients had lower levels of lymphocyte, $CD3^+$, and $CD8^+$ T cells, and a higher ratio of CD4/CD8 in comparison with ICU-acquired infection patients, which may contribute to the severer symptoms and worse clinical outcomes in co-infection patients.

The excessive release of cytokines and chemokines in COVID-19 results in a cytokine storm ensues (25, 26). Cytokine storm refers to a pathological state characterized by uncontrolled systemic inflammation, which is caused by the excessive cytokines production, leading to multi-organ failure and even death (27). Several cytokines, such as IL-1β, IL-6, IL-8, IL-10, TNF-α, interferon (IFN)-γ, play crucial roles in the pathogenesis of cytokine storm (28). Previous studies have highlighted the relationship between cytokine storm and severity of COVID-19, and cytokine storm is verified as a significant contributor to mortality associated with the disease (29). Prevention and mitigation of the cytokine storm may be a promising strategy to save patients with severe COVID-19 (29). Infections and tissue injuries are defended against by IL-6. However, the overproduction of IL-6 when fighting against SARS-CoV-2 may result in systemic inflammatory responses (30). There is increasing evidence indicating that IL-6 is an early biomarker of lung damage and is closely associated with prolonged mechanical ventilation, increased morbidity, and mortality in lung diseases (31, 32). For example, the increased IL-6 levels in serum and brochoalveolar lavage fluid have been found in asthmatic patients (33, 34). Huang et al. Have found that IL-6 is a strong predictor of the frequency of chronic obstructive pulmonary disease exacerbation within 1 year (35). In addition, the essential role of IL-6 in sepsis-induced acute lung injury and pulmonary arterial hypertension has also been observed (36, 37). IL-10 is highly abundant in influenza infection, especially during the adaptive immune response (37). Patients in the ICU with COVID-19 have higher peripheral IL-10 levels than those in non-ICUs (38). Furthermore, targeting IL-6 and IL-10 has been proposed for treating ARDS in COVID-19 patients based on its immunoregulatory functions (39). However, the difference between co-infection and ICU-acquired infection on the inflammatory response in ICU COVID-19 patients with omicron infection is still unclear. In addition, we found that the ICU patients with co-infection had significantly higher levels of IL-6 and IL-10 than those with ICU-acquired infection. These results are apparently in line with previous reports suggesting IL-6 and IL-10 are disease severity predictors (25, 40). Besides, co-infections also cause hematological and biochemical imbalance, worsening the general clinical condition. These results indicated that the patients with co-infection had severer inflammatory responses, which might contribute to the worse prognosis of Omicron infection.

It is common for viral respiratory infections to be co-infected with bacteria. They are the main causes of difficult diagnosis, poor outcomes, increasing morbidity and mortality, and greater healthcare costs (41). There are many pathogens that may cause respiratory co-infections, including bacteria, viruses, fungi, etc. In the current study, the most common bacteria in broncho-alveolar lavage in the older COVID-19 patients within 48 h were *Klebsiella pneumonia*, *Pseudomonas aeruginosa*, and *Staphylococcus aureus*. The predominant late pathogens observed in the broncho-alveolar lavage were *Klebsiella pneumonia*, *Stenotrophomonas maltophilia*, and *Corynebacterium striatum*. Consistent with previous results, these pathogens commonly caused hospital- and ventilator-acquired pneumonia, especially in ICU. Bacterial infections are indeed more prevalent in critically ill patients, but fungal infections are also deadly. Our data showed that in both co-infections and ICU-acquired infections, *Candida* spp. was detected with a high prevalence. As we know, *Candida* spp. is a part of the human microbiota, and it is difficult to differentiate infection from colonization. Therefore, this might be one cause for the higher rate of co-infections in our study. In line with our results, Silva et al. also reported a high prevalence of *Candida* spp., which was the main fungus causing infections in critically ill patients (42). It is noteworthy that interventions for patients with COVID-19 in ICU increased the opportunity of infections, including corticosteroids, broadspectrum antibacterial, and mechanical ventilation. Therefore, the increased risk of death may indicate that *Candida* spp. might act as the pathogen, which should not be ignored. To ascertain the essential role of co-infection on the progression of COVID-19, the Kaplan–Meier analysis and survival curves were conducted, and the results indicated that the COVID-19 patients with bacterial/fungal co-infection had a significantly poorer survival rate than ICU-acquired infection. Furthermore, multivariate Cox analysis also showed that co-infection was an independent meaningful factor associated with the occurrence of severe events, including 28-day mortality and the type of mechanical ventilation. Interestingly, further analysis showed that the isolation of *Candida* spp., but not *Klebsiella* spp., *Staphylococcus* spp., *Acinetobacter* spp., or *Pseudomonas* spp. in the broncho-alveolar lavage was associated with an increased risk of death and invasive ventilation. Besides, the patients co-infected with *Candida* spp. showed the significant changes of immunity and inflammatory markers, including lymphocyte numbers, $CD3^+$, $CD4^+$, $CD8^+$ T cells, and the ratio of CD4/CD8.

Our study has several limitations. First, our study was retrospectively designed and the effects of confounding factors might be underestimated, therefore, more prospective studies are required. Second, it's should be considered that the analysis cannot meet the requirements of the event per cariable due to the small sample size. However, considering the rarity of such a population and the

interpretability of the results, they are still shown. Therefore, studies with a larger sample size should be carried out to validate our conclusions. Finally, our analyses were limited to the available data extracted from the electronic medical records. However, data from our study contributes to a better understanding of co-infections in patients with COVID-19 in ICU patients with the SARS-CoV-2 omicron variant.

In conclusion, our results showed that co-infection is common in ICU patients with Omicron SARS-CoV-2 infection, which displayed worse clinical features and outcomes. Importantly, *Candida app*. in the broncho-alveolar lavage was significantly associated with an increased risk of death in ICU COVID-19 patients. Collectively, our data suggested that if a ICU patient with Omicron SARS-CoV-2 infection shows strong evidence of a fungal co-infection, this possibility should not be ignored.

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 studies involving humans were approved by the Ethical Committee of the Shanghai Tenth People's 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 because this is a retrospective study and the demographic information, clinical details, and 28-day mortality were obtained through the electronic patient records.

Author contributions

D-JL: Conceptualization, Resources, Writing – original draft. C-CZ: Data curation, Writing – original draft. FH: Data curation, Writing – review & editing. F-MS: Conceptualization, Writing – review & editing. Y-CL: Conceptualization, Investigation, 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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Prevalence and factors associated with maternal and neonatal sepsis in sub-Saharan Africa: a systematic review and meta-analysis

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Objectives: This study aimed to determine the prevalence and factors associated with maternal and neonatal sepsis in sub-Saharan Africa.

Methods: This systematic review and meta-analysis used the PRISMA guideline on sepsis data in sub-Saharan Africa. The bibliographic search was carried out on the following databases: Medline/PubMed, Cochrane Library, African Index Medicus, and Google Scholar. Additionally, the reference lists of the included studies were screened for potentially relevant studies. The last search was conducted on 15 October 2022. The Joanna Briggs Institute quality assessment checklist was applied for critical appraisal. Estimates of the prevalence of maternal and neonatal sepsis were pooled using a random-effects meta-analysis model. Heterogeneity between studies was estimated using the Q statistic and the I2 statistic. The funnel plot and Egger's regression test were used to assess the publication bias.

Results: A total of 39 studies were included in our review: 32 studies on neonatal sepsis and 7 studies on maternal sepsis. The overall pooled prevalence of maternal and neonatal sepsis in Sub-Saharan Africa was 19.21% (95% CI, 11.46–26.97) and 36.02% (CI: 26.68–45.36), respectively. The meta-analyses revealed that Apgar score < 7 (OR: 2.4, 95% CI: 1.6–3.5), meconium in the amniotic fluid (OR: 2.9, 95% CI: 1.8–4.5), prolonged rupture of membranes >12 h (OR: 2.8, 95% CI: 1.9–4.1), male sex (OR: 1.2, 95% CI: 1.1–1.4), intrapartum fever (OR: 2.4, 95% CI: 1.5–3.7), and history of urinary tract infection in the mother (OR: 2.7, 95% CI: 1.4–5.2) are factors associated with neonatal sepsis. Rural residence (OR: 2.3, 95% CI: 1.01–10.9), parity (OR: 0.5, 95% CI: 0.3–0.7), prolonged labor (OR: 3.4, 95% CI: 1.6–6.9), and multiple digital vaginal examinations (OR: 4.4, 95% CI: 1.3–14.3) were significantly associated with maternal sepsis.

Conclusion: The prevalence of maternal and neonatal sepsis was high in sub-Saharan Africa. Multiple factors associated with neonatal and maternal sepsis were identified. These factors could help in the prevention and development of strategies to combat maternal and neonatal sepsis. Given the high risk of bias and high heterogeneity, further high-quality research is needed in the sub-Saharan African context, including a meta-analysis of individual data.

Systematic review registration: PROSPERO (ID: CRD42022382050).

KEYWORDS

maternal sepsis, neonatal sepsis, prevalence, associated factors, sub-Saharan Africa

Introduction

Maternal and neonatal sepsis is a generalized inflammatory response with systemic manifestations caused by one or more infectious agents (1), which occurs during pregnancy, childbirth, after abortion, or during the postpartum period (42 days) in women or in the first 28 days of life in newborns (2–4). Sepsis is a major cause of maternal and neonatal morbidity and mortality (2, 3, 5–9). It is the third leading cause of death in women and accounts for a quarter of neonatal deaths (1, 10–14). It is an obstacle to achieving the third Sustainable Development Goal (SDG), which aims to reduce maternal and neonatal mortality and morbidity (15). Low- and middle-income countries (LICs) are particularly affected by maternal and neonatal sepsis (11, 16, 17). In sub-Saharan Africa (SSA), it is estimated to be responsible for 130,000 maternal deaths and 300,000 neonatal deaths per year, although this may be an underestimation (2, 18). These deaths reflect a number of challenges, including policy, poverty, health inequalities, and the health system (19). However, few data are available on the prevalence and factors associated with sepsis across the continuum from pregnancy to postpartum or post-abortion, making it difficult to make a real estimation of maternal and neonatal sepsis in these countries (2, 10, 11). In the literature, the factors associated with maternal and neonatal sepsis are diverse, including prolonged labor, failure to perform antenatal consultation (ANC), prolonged rupture of membranes, history of infection in the mother, repeated vaginal examinations, intrapartum fever, gestational age, parity, type of delivery, prematurity, chorioamnionitis, meconium-stained amniotic fluid, Apgar score < 7, low birth weight < 2.5 kg, resuscitation of the newborn, age of the newborn <7 days, and male sex (5–7, 20–23).

Prevention, early recognition of signs, and rapid and appropriate management of cases are the main factors associated with a reduction in the morbidity and mortality associated with maternal and neonatal sepsis (5, 11, 24–26). A recent meta-analysis carried out in SSA in 2022 identified several risk factors for neonatal sepsis (27) but did not explore the magnitude of neonatal and/or maternal sepsis nor the factors associated with maternal sepsis. Other studies have shown that the prevalence of maternal sepsis was 39% in Ethiopia (28), 12.20% in Keyna (29), and 20% in Tanzania (30); for neonatal sepsis, it was 77.9 in Ethiopia (31), 20.5% in South Africa (32), 49.8 in Tanzania (33), 37.6 in Nigeria (34), and 17.5 in Ghana (35). Although these single studies reported data on the prevalence and factors associated with maternal and neonatal sepsis, there are no regionally representative pooled data on the magnitude and factors associated with maternal and neonatal sepsis in SSA. However, a better understanding of the burden and a synthesis of the evidence on the factors associated with maternal and neonatal sepsis are needed to optimize prevention strategies and management guidelines against this scourge. The aim of this systematic review with meta-analysis was to estimate the prevalence and factors associated with maternal and neonatal sepsis in sub-Saharan Africa. To the best of our knowledge, this is the first systematic review and meta-analysis to examine both the prevalence of and factors associated with maternal and neonatal sepsis in SSA. It aimed to answer the following questions:

What is the prevalence of maternal and neonatal sepsis in sub-Saharan Africa?

What are the associated factors with maternal and neonatal sepsis in sub-Saharan Africa?

Materials and methods

This systematic review was reported in accordance with PRISMA (Preferred Reporting Item for Systematic Review and Meta-analysis) guidelines (36). The protocol for this review was developed and registered in the "International prospective register of systematic reviews PROSPERO" (ID: CRD42022382050).

Search strategies

To identify eligible studies, Medline/PubMed, Cochrane Library, African Index Medicus, and Google Scholar databases were searched. We also conducted manual searches of the bibliographic references of included studies and meta-analyses. The adapted PECO format was used for this systematic review. This PECO included population (P), exposure (E), comparison (C), and outcome (O), as shown in Table 1. It consisted of using all the identified keywords and indexing terms to search different databases. All the search terms used and the MeSH terms for the search were added, as well as the Boolean operators, to guarantee the exhaustiveness of the search process. Key terms defining the same concept were introduced using the "OR" operator, and the "AND" operator was used to introduce different concepts.

TABLE 1 PECOT framework for the review objective.

Components	Characteristics
Population	Pregnant women, women in labor, postpartum women, newborns,
Exposure	Associated factors: prolonged labor, failure to perform ANC, prolonged rupture of membranes, history of infection in the mother, repeated vaginal examinations, intrapartum fever, gestational age, parity, cesarean delivery, prematurity, meconium amniotic fluid, Apgar score < 7, birth weight < 2.5 kg, age of newborn <7 days, prematurity.
Comparison	Absence of exposure
Results	Maternal sepsis and associated factors

Selection of studies/eligibility criteria

The result of bibliographic searches carried out on the various search tools was exported to Zotero, where duplicates were identified and removed using the Duplicates command and manually also removed during the screening. The study selection process followed two evaluation stages (37). The first evaluation was based on an examination of the titles and abstracts of the articles. The titles and abstracts that were outside the scope of the study were excluded. The second assessment consisted of examining the full text of eligible studies. The entire process was carried out by two reviewers (FBT and NHD). They independently performed abstract screening and full-text study selection, where both authors had to approve the inclusion of the study in the systematic review. The reference lists of the included studies were screened for potentially relevant studies (38).

Studies reporting on the prevalence and/or at least one factor associated with maternal and/or neonatal sepsis in SSA in pregnant women from 28 weeks of amenorrhea (SA), postpartum women up to 42 days after delivery, post-abortion women, and newborns within 28 days of birth were included. Cross-sectional, cohort, and case–control studies on the prevalence, frequency, and factors associated with maternal and neonatal sepsis in SSA published in French and English between January 2012 and October 2022 were included. Qualitative studies, systematic reviews, and case series were excluded from the analysis, but the reference lists of these were screened.

Assessment of study quality and risk of bias

The quality of the included studies was assessed by two authors (FBT and NHD) using the Joanna Briggs Institute (JBI) quality assessment checklist (39). For cross-sectional studies, the following criteria were used: (1) conformity between target population and source population; (2) appropriate sampling technique; (3) representativeness of the sample; (4) description of the subject and context of the study; (5) data analysis with sufficient sample coverage; (6) valid methods for identifying the condition; (7) standard and reliable way of measuring the condition for all participants; (8) appropriate statistical test; and (9) adequate response rate. When items received a score ≥ 6 out of 9, they were considered to be of high quality. The following were used to assess cohort studies: (1) similarity of groups, (2) similarity of exposure measurement, (3) validity and reliability of measurement, (4) identification of confounders, (5) strategies for dealing with confounders, (6) adequacy of groups/participants at study entry, (7) validity and reliability of measured outcomes, (8) sufficient duration of follow-up, (9) completeness of follow-up or description of reasons for loss to follow-up, (10) strategies for dealing with incomplete follow-up, and (11) adequacy of statistical analysis. The criteria used to evaluate case–control studies are (1) comparable groups, (2) appropriateness of cases and controls, (3) criteria for identifying cases and controls, (4) standard exposure measurement, (5) similarity of exposure measurement for cases and controls, (6) treatment of confounding factors, (7) strategies for treating confounding factors, (8) standard evaluation of results, (9) appropriateness of duration of exposure, and (10) appropriateness of statistical analysis (see Table 2).

Data extraction

Two authors (FBT and NHD) independently extracted data using a tested form on Microsoft Excel. If discrepancies between data extractors continued, a third reviewer (EMD) was involved. Data extracted included author name and year of publication, country, study period, study setting, study design, study population, sample size, type of sepsis (maternal or neonatal), prevalence of neonatal sepsis, prevalence of maternal sepsis, and risk bias.

For the associated factors, data were extracted on the age of the newborn, Apgar score, gestational age, birth weight, resuscitation of the newborn at birth, sex of the newborn, antenatal consultation (ANC), presence of prolonged rupture of membranes, repeated vaginal examinations, intrapartum fever, gestational age, parity, type of delivery, prematurity, meconium amniotic fluid, history of infection in the mother, maternal fever, type of delivery, and prolonged labor.

Statistical analysis

Data were entered into Microsoft Excel and then exported into Stata version 17 software. We pooled maternal and neonatal sepsis prevalence estimates using a random-effects meta-analysis model because it accounts for variability between studies. We examined the heterogeneity of effect size using the Q statistic and the I^2 statistic (40). An I^2 value ≥ 50% was considered strong heterogeneity, and a random-effects model was used (41, 42). The random-effect RELM method was mainly used. The funnel plot and Eggers's regression test were used to check for publication bias (43). To complete the tests for publication bias, we added the Begg and Thompson tests on R software. Forest plots were used to display the results graphically. A subgroup analysis was performed according to study design (prospective or retrospective), sepsis diagnostic criteria (clinic or clinic and biology), risk of bias (low or moderate), and different regions of SSA (East, West, or South Africa).

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TABLE 2 Quality assessment results of included studies in sub-Saharan Africa from January 2002–October 2022 using the Joanna Briggs Institute (JBI) quality appraisal checklist.

Author	Quality assessment questions												
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Yes Total	Quality status
Cross-sectional studies													
Tsehaynesh G/eyesus	N	Y	Y	UC	Y	Y	UC	Y	UC			5/9	Medium risk
Kumera Bekele	Y	Y	Y	UC	Y	Y	Y	Y	UC			7/9	Low risk
Abebe Sorsa	Y	UC	Y	Y	UC	Y	Y	UC	Y			6/9	Low risk
Abimbola Ellen Akindolire	Y	Y	Y	UC	Y	Y	Y	Y	Y			8/9	Low risk
Fortress Yayra Aku	UC	UC	Y	N	Y	Y	Y	Y	UC			5/9	Medium risk
Alemnew Wale	UC	Y	Y	UC	Y	UC	Y	Y	N			5/9	Medium risk
Bua John, 2015	N	Y	N	Y	Y	UC	Y	Y	Y			6/9	Low risk
Debora C. Kajegukaa, 2020	UC	Y	Y	N	UC	Y	Y	Y	UC			5/9	Medium risk
Aytenew Getabelew, 2018	Y	Y	Y	N	N	N	Y	Y	UC			5/9	Medium risk
Tchouambou SN Clotilde, 2022	UC	N	Y	UC	N	Y	Y	Y	Y			5/9	Medium risk
Abdulhakeem Abayomi Olorukooba, 2020	Y	Y	Y	Y	Y	N	UC	Y	Y			7/9	Low risk
Mekitrida L. Kiwone, 2020	UC	N	Y	Y	UC	Y	Y	Y	UC			5/9	Medium risk
Tilahun Tewabe, 2017	Y	Y	Y	UC	Y	Y	Y	UC	Y			7/9	Low risk
Endalk Birrie, 2020	Y	Y	Y	Y	Y	Y	Y	Y	Y			9/9	Low risk
Abdurahman Kedir Roble, 2022	UC	Y	Y	Y	UC	Y	Y	Y	UC			7/9	Low risk
Zelalem Agnche, 2020	Y	Y	Y	Y	Y	UC	Y	Y	Y			8/9	Low risk
Daniel Atlaw, 2019	Y	Y	Y	UC	Y	Y	Y	Y	Y			8/9	Low risk
Alemale Admas, 2020	Y	Y	Y	U	UV	Y	Y	Y	Y			8/9	Low risk
Yenew Engida Yismaw, 2019	Y	Y	Y	UC	Y	UC	Y	UC	Y			6/9	Low risk
Tinuade A Ogunlesi, 2010	Y	Y	Y	Y	UC	Y	Y	Y	Y			8/9	Low risk
Agricola Joachim, 2009	Y	Y	Y	UC	Y	Y	Y	Y	Y			8/9	Low risk
Neema Kayange, 2010	Y	Y	Y	UC	Y	UC	UC	Y	Y			6/9	Low risk
Ogundare Ezra Olatunde, 2015	Y	Y	Y	UC	Y	Y	Y	Y	Y			8/9	Low risk

(Continued)

TABLE 2 (Continued)

Author	Quality assessment questions												
	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8	Q9	Q10	Q11	Yes Total	Quality status
BA West, 2014	Y	Y	UC	Y	UC	N	Y	Y	Y			6/9	Low risk
Cohort studies													
Violet Okaba Kayom	Y	Y	N	Y	UC	Y	Y	Y	Y	N	Y	8/11	Low risk
Shatry N. A., 2022	Y	Y	Y	Y	UC	Y	Y	N	UC	Y	Y	8/11	Low risk
Case–control study													
Mulunesh Alemu	Y	Y	Y	UC	Y	Y	Y	Y	UC	Y		8/10	Low risk
Kalkidan Béjituel	Y	Y	Y	UC	Y	Y	Y	Y	Y	Y		9/10	Low risk
Getu Alemu Demisse	Y	Y	Y	UC	Y	Y	Y	Y	N	Y		8/10	Low risk
Dejene Edosa Dirirsa	Y	Y	Y	UC	Y	Y	UC	Y	Y	Y		8/10	Low risk
Gujo Teshome	Y	Y	Y	Y	Y	Y	N	Y	UN	Y		8/10	Low risk
Peter Adatara, 2019	Y	Y	Y	UC	Y	N	UC	Y	Y	Y		7/10	Medium risk
Peter Adatara, 2018	Y	Y	Y	N	UN	Y	Y	UC	UC	Y		6/10	Medium risk
Destaalem Gebremedhin	Y	Y	Y	Y	Y	UC	UC	Y	Y	Y		8/10	Low risk
Pendo P. Masanja	Y	Y	Y	Y	Y	N	N	Y	UC	Y		7/10	Low risk
Atkuregn Alemayehu, 2020	Y	N	Y	Y	Y	UC	UC	Y	Y	Y		7/10	Low risk
Tadesse Yirga AkaluID, 2020	Y	Y	Y	U	UC	UC	Y	Y	Y	Y		8/10	Low risk
Soressa Gemechu Kitessa, 2021	Y	Y	Y	Y	Y	UC	UC	Y	Y	Y		8/10	Low risk
Mate Siakwa, 2014	Y	Y	Y	UC	UC	N	UC	Y	Y	Y		6/10	Medium risk

Q, Question; Y, yes; N, no; UC, unclear.

Results

A total of 2,638 titles were screened (2,033 from PubMed, 58 from the African Index of Medicus, 41 from Cochrane, and 506 from Google Scholar). After removing duplicates, 2,145 studies remained. Evaluation of titles and abstracts led to the exclusion of 2,019 studies. Nine studies were excluded because the full text was not available. The full-text review involved 126 studies. The number of articles retained for inclusion was 39. The reasons for the exclusion of 87 studies were the lack of availability of the variable of interest and the difference in the target population (Figure 1).

Characteristics of the studies included in the meta-analysis

A total of 39 studies were included in our review: 32 studies of neonatal sepsis and 7 studies of maternal sepsis. There were 24 cross-sectional studies (22, 30–34, 44–60), 13 case–control studies (28, 35, 61–69), and 2 cohorts (29, 70).

Twenty-one studies were from Ethiopia (28, 31, 44–46, 49, 51–56, 60–64, 66, 68, 69, 71), five from Tanzania (22, 30, 33, 67, 72), five from Nigeria (34, 47, 59, 73, 74), four from Ghana (35, 48, 65, 75), two from Uganda (50, 70), one from Kenya (29), and one

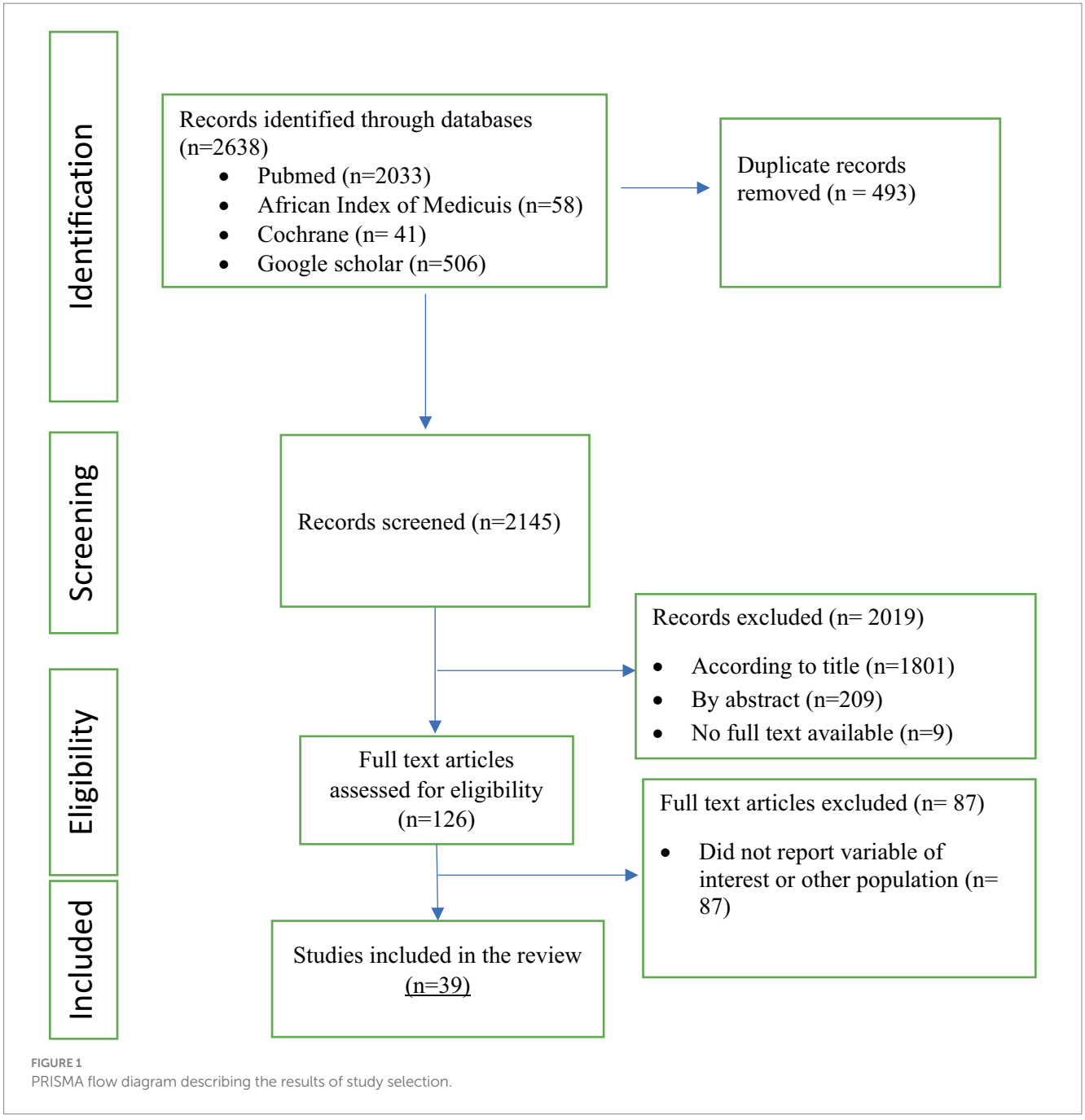

FIGURE 1
PRISMA flow diagram describing the results of study selection.

from South Africa (32). The total study population was 12,777, including 10,494 newborns and 2,283 women. After quality assessment, 30 studies had a low risk of bias and 9 had a medium risk of bias (see Table 3).

Prevalence of maternal sepsis

The pooled prevalence of maternal sepsis in SSA was 19.21% (95% CI, 11.46–26.97). Significant heterogeneity was observed between studies ($I^2 = 93.26\%$, $p < 0.000$). A random effects model was used to measure pooled prevalence. The highest prevalence was reported by Alemale et al. (54) (33.7%) and the lowest by Debora et al. (22) (11.5%); Figure 2 shows the details.

Subgroup analysis of the prevalence of maternal sepsis

A subgroup analysis of the prevalence of maternal sepsis was carried out according to the diagnostic criteria for sepsis (clinical or clinical and biological). It was 25.33 (9.28–41.38) for sepsis based on clinical signs and 15.43 (8.28–22.58) for sepsis based on clinical signs and biology (22, 29, 30). See Table 4 for details.

Prevalence of neonatal sepsis

The pooled prevalence of neonatal sepsis in SSA was 36.02% (95% CI, 26.68–49.36). A significant heterogeneity between the included

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TABLE 3 Characteristics of included studies.

N	First author, publication year	Country	Study period	Setting	Study design	Study population	Sample size	Type of sepsis	Prevalence	Risk of bias
1	Tsehaynesh G/eyesus, 2017	Ethiopia	September 2015 to May 2016	Hospital	Prospective Cross-sectional	Newborn	251	Neonatal	46.61%	Medium risk
2	Kumera Bekele, 2022	Ethiopia	January 2021 to March 2021	Hospitals	Transversale prospective	Newborn	378	Neonatal	52.27%.	Low risk
3	Mulunesh Alemu, 2019	Ethiopia	1 February to 30 March 2018	Hospitals	Case-control	Newborn	246	Neonatal	-	Low risk
4	Kalkidan Béjituel, 2022	Ethiopia	1 August to 30 September 2020	Hospitals	Case-control	Newborn	331	Neonatal	-	Low risk
5	Getu Alemu Demisse, 2019	Ethiopia	1 February to 30 April 2018	Hospitals	Case-control	Mother	280	Maternal	-	Low risk
6	Dejene Edosa Dirirsa, 2021	Ethiopia	May 2018 to August 2018	Hospitals	Case-control	Newborn	220	Neonatal		Low risk
7	Abebe Sorsa, 2019	Ethiopia	April 2016 to May 2017	Hospital	Prospective Cross-sectional	Newborn	303	Neonatal	34%	Low risk
8	Abimbola Ellen Akindolire, 2016	Nigeria	November 2013 and February 2014	Hospitals	Prospective Cross-sectional	Newborn	202	Neonatal	12.37	Low risk
9	Fortress Yayra Aku, 2020	Ghana	January and May 2016	Hospitals	Prospective Cross-sectional	Newborn	150	Neonatal	17.3%	Medium risk
10	Alemnew Wale, 2021	Ethiopia	May to November 2019	Hospital	Prospective Cross-sectional	Newborn	193	Neonatal	26.1%	Medium risk
11	Gujo Teshome, 2022	Ethiopia	1 October to 10 November 2021	Hospitals	Case-control	Newborn	293	Néonatal	-	Low risk
12	Peter Adatara, 2019	Ghana	January and December 2017	Hospital	Case-control	Newborn	900	Neonatal	-	Low risk
13	Peter Adatara, 2018	Ghana	4 weeks	Hospital	Case-control	Newborn	383	Neonatal	17.50%	Medium risk
14	Destaalem Gebremedhin, 2016	Ethiopia	December 2014 to June 2015	Hospitals	Case-control	Newborn	234	Neonatal	-	Low risk
15	Bua John, 2015	Uganda	January and August 2013	Health Center	Prospective Cross-sectional	Mother and newborn	174	Neonatal	21.80%	Low risk
16	Debora C. Kajegukaa, 2020	Tanzania	January 2015 to December 2015	Hospital	Retrospective Cross-sectional	Mother	183	Maternal	11.5%	Medium risk
17	Violet Okaba Kayom, 2018	Uganda	March to May 2012	Community	Prospective Cohort	Mother and newborn	335	Neonatal		Low risk
18	Pendo P. Masanja, 2019	Tanzania	May to July 2017	Hospitals	Case–control	Mother and newborn	322	Neonatal		Low risk

(Continued)

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TABLE 3 (Continued)

N	First author, publication year	Country	Study period	Setting	Study design	Study population	Sample size	Type of sepsis	Prevalence	Risk of bias
19	Atkuregn Alemayehu, 2020	Ethiopia	April to July 2019	Hospitals	Case–control	Newborn	385	Neonatal		Low risk
20	Tadesse Yirga AkaluID, 2020	Ethiopia	March 2018 to April 2018	Hospitals	Case–control	Newborn	231	Neonatal		Low risk
21	Aytenew Getabelew, 2018	Ethiopia	1 February 2016 to 1 February 2017	Hospitals	Retrospective Cross-sectional	Newborn	224	Neonatal	77.9%	Medium risk
22	Tchouambou SN Clotilde, 2022	South Africa	1 January and 30 June 2018	Hospitals	Prospective Cross-sectional	Newborn	210	Neonatal	20.5%	Medium risk
23	Abdulhakeem Abayomi Olorukooba, 2020	Nigeria	May 2017 to May 2018	Hospital	Retrospective Cross-sectional	Newborn	409	Neonatal	37.6%.	Low risk
24	Mekitrida L. Kiwone, 2020	Tanzania	August to October 2018	Hospital	Retrospective Cross-sectional	Newborn	263	Neonatal	49.8%	Medium risk
25	Tilahun Tewabe, 2017	Ethiopia	30 April to 30 May 2016	Hospital	Retrospective Cross-sectional	Newborn	225	Neonatal		Low risk
26	Endalk Birrie, 2020	Ethiopia	1 January to 30 July 2021	Hospital	Prospective Cross-sectional	Newborn	344	Neonatal	79.4%	Low risk
27	Abdurahman Kedir Roble, 2022	Ethiopia	1 January 2019 to 31 December 2019	Hospitals	Prospective Cross-sectional	Newborn	361	Neonatal	45.80%	Low risk
28	Zelalem Agnche, 2020	Ethiopia	March to April 2019	Hospitals	Prospective Cross-sectional	Mother and newborn	352	Neonatal	64.8%	Low risk
29	Soressa Gemechu Kitessa, 2021	Ethiopia	May to October 2020	Hospitals	Case–control	Mother	428	Maternal	−39%	Low risk
30	Shatry N. A., 2022	Kenya	March to November 2015	Hospital	Prospective Cohort	Mother	566	Maternal	12.20%	Low risk
31	Daniel Atlaw, 2019	Ethiopia	1 September to 30 December 2017	Hospital	Prospective Cross-sectional	Mother	219	Maternal	17.2%,	Low risk
32	Alemale Admas, 2020	Ethiopia	January to May 2017	Hospital	Prospective Cross-sectional	Mother	166	Maternal	33.70%	Low risk
33	Ayenew Engida Yismaw, 2019	Ethiopia	1st September to 30th November 2017	Hospital	Prospective Cross-sectional	Newborn	423	Neonatal	11.70%	Low risk
34	Tinuade A Ogunlesi, 2010	Nigeria	January 2006 to December 2008	Hospital	Retrospective Cross-sectional	Newborn	1,050	Neonatal	16.5	Low risk

(Continued)

TABLE 3 (Continued)

N	First author, publication year	Country	Study period	Setting	Study design	Study population	Sample size	Type of sepsis	Prevalence	Risk of bias
35	Agricola Joachim, 2009	Tanzania	October 2008 to March 2009	Hospital	Prospective Cross-sectional	Mother	300	Maternal	20%	Low risk
36	Neema Kayange, 2010	Tanzania	March to November 2009	Hospital	Prospective Cross-sectional	Newborn	770	Neonatal	39%	Low risk
37	Mate Siakwa, 2014	Ghana	January 2011 and December 2013	Hospital	Case-control	Newborn	196	Neonatal		Medium risk
38	Ogundare Ezra Olatunde, 2015	Nigeria	September 2008 to March 2009	Hospital	Prospective Cross-sectional	Newborn	360	Neonatal	16%	Low risk
39	BA West, 2012	Nigeria	July to December 2007	Hospital	Prospective Cross-sectional	Newborn	406	Neonatal	41.6%	Low risk

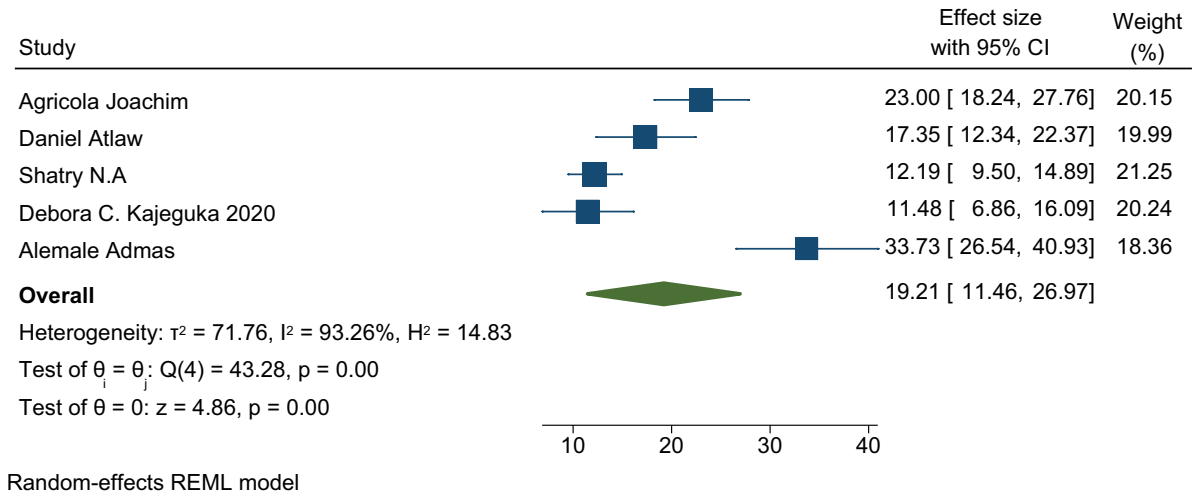

FIGURE 2
Pooled prevalence of maternal sepsis in SSA.

studies was observed (I2 = 99.1%, p < 0.000). Therefore, a random effects model was used to estimate the pooled prevalence. The prevalence ranged from 11.1 (56) up to 77.9% (31) (see Figure 3).

Subgroup analysis of the prevalence of neonatal sepsis

A subgroup analysis of prevalence was performed according to the design (prospective and retrospective), subdivision of SSA (East, West, or South Africa), definition of sepsis (clinical when diagnostic based on clinical signs or clinical and biological when diagnostic based on clinical signs and confirmed by biological tests), and risk of bias (low or moderate).

Depending on the design, the prevalence of neonatal sepsis was 32.88 (22.35–43.41) for prospective studies and 45.46 (25.97–64.94) for retrospective studies. According to the diagnostic criteria for sepsis, it was 44.11 (27.33–60.90) for sepsis based on clinical signs and 31.25 (22.24–40.26) for sepsis based on clinical signs and biology. According to the subdivision, the prevalence of neonatal sepsis was 42.96 (30.75–55.16) in East Africa, 23.59 (15.53–33.65) in West Africa, and 20.48 (15.0–25.93) in South Africa. The prevalence of neonatal sepsis was 34.45 (23.93–44.98) for studies with a low risk of bias and 39.69 (21.58–57.80) for studies with a medium risk of bias (see Table 5).

TABLE 4 Sub-group analysis of the prevalence of maternal sepsis in sub-Saharan Africa.

Variable	Characteristics	Pooled prevalence (95% CI)	I^2 (value of *p*)
Diagnostic criteria	Clinical	25.33 (9.28–41.38)	92.54% (<0.000)
	Clinical and biological	15.43 (8.28–22.58)	89.72% (<0.000)

Publication bias

We assessed publication bias using the funnel plot and Egger's regression test (76). Compared with the maternal sepsis studies, a visual asymmetrical distribution was observed, and Egger's regression test (value of $p = 0.01$) indicated the presence of a publication bias (see Figure 4). We did not use the Begg and Thompson tests due to the number of studies less than 10.

For the study concerning neonatal sepsis, we assessed publication bias using the funnel plot, Egger's regression test, Begg's test, and Thompson test. The diagrams showed asymmetry, and the result of Egger's test showed the presence of bias ($p = 0.04$; see Figure 5). However, Begg's test (value of $p = 0.06$) and the Thompson test (value of $p = 0.14$) did not reject the city Ho. Therefore, the asymmetry on the funnel plot is reflected much more by a small study effect than by a publication bias.

Meta-analysis of factors associated with maternal sepsis

A total of eight risk factors were included in the meta-analysis: place of residence for 3 studies (22, 28, 63), parity for 3 studies (22, 30, 54), mode of delivery for 5 studies (22, 28, 54, 60, 63), prolonged labor for 5 studies (22, 28, 29, 54, 63), multiple vaginal examinations for 3 studies (28, 29, 63), performance of ANC for 4 studies (28, 54, 60, 63), history of urinary tract infection for 3 studies (22, 29, 30), and level of education for 4 studies (28, 29, 60, 63). Table 6 shows the details.

Meta-analysis of factors associated with neonatal sepsis

A meta-analysis was carried out for 15 risk factors classified into maternal and neonatal factors. The maternal factors were history of urinary tract infection in the mother (13 studies) (31, 45, 53, 55, 56, 61, 62, 65, 66, 68–71), parity (10 studies) (31, 45, 48, 53, 56, 61, 62, 65, 67, 68), prolonged labor (5 studies) (31, 53, 62, 69, 73), intrapartum fever (13 studies) (31, 45, 48, 52, 55, 56, 61, 64, 66, 68, 70, 72, 73), multiple vaginal examinations (7 studies) (45, 53, 61, 64, 66–68), performance of ANC (13 studies) (45, 48, 50, 55, 61, 64, 65, 67–71), prolonged rupture of membranes (18 studies) (31, 33, 45, 46, 52, 53, 56, 61, 62, 65–73), and mode of delivery (14 studies) (31, 32, 35, 44, 46, 48, 52, 61, 62, 64, 66–68, 72). The neonatal factors reported were Apgar (18 studies) (31, 33–35, 44–46, 48, 52, 56, 61, 62, 64–66, 68, 69, 71), prematurity (17 studies) (31, 33–35, 44–46, 50, 52, 53, 61, 62, 64–67, 71), meconium amniotic fluid (12 studies) (31, 45, 46, 52, 61, 64–69, 72), birth weight (17 studies) (31–35, 44–48, 52, 53, 61, 62, 64–69, 72), neonatal resuscitation (09 studies) (33–35, 52, 53, 62, 65, 66, 71), neonatal age < 7 days (11 studies) (36, 38, 48, 53, 57, 65, 66, 69, 72, 75), and neonatal sex (14 studies) (32, 34, 35, 45, 46, 48, 53, 55, 61, 62, 65, 66, 72, 73) (see Table 7).

Associated factors with maternal and neonatal sepsis

Factors associated with maternal sepsis

Rural residence (OR: 2.32, IC 95%: 1.01–10.9, I2 = 90.3%), parity (OR: 0.5, IC95%: 0.3–0.7, $I^2 = 0\%$), prolonged labor (OR: 3.4, IC95%: 1.6–6.9, I2 = 81.5%), and multiple vaginal examinations (OR: 4.4, IC95%: 1.3–14.3, I2 = 90.4%) were independently associated with maternal sepsis.

Factors associated with neonatal sepsis

The meta-analysis identified 6 factors as being significantly associated with neonatal sepsis, including Apgar score < 7 (OR: 2.4, CI95%: 1.6–3.5, I2 = 88.8%), the presence of meconium in the amniotic fluid (OR: 2.9, CI95%: 1.8–4.5, I2 = 78.6%), prolonged rupture of membranes >12 h (OR: 2.8, IC95%: 1.9–4.1, I2 = 82.9%), male sex (OR: 1.2, IC95%: 1.1–1.4, I2 = 0%), intrapartum fever (OR: 2.4, IC95%: 1.5–3.7, I2 = 84.2%), and history of urinary tract infection in the mother (OR: 2.76, IC95%: 1.4–5.2, I2 = 89.5%).

Discussion

Knowledge of the burden and risk factors of maternal and neonatal sepsis is crucial for developing preventive measures and reducing maternal and neonatal mortality. This systematic review with meta-analysis filled the gaps in the literature on the prevalence of maternal and neonatal sepsis and associated factors in SSA. Based on 39 studies included in the final analysis, we found a pooled prevalence of 19.21% for maternal sepsis and 36.02% for neonatal sepsis. However, considerable heterogeneity was observed.

Factors such as rural residence, parity, prolonged labor, and multiple vaginal examinations significantly increased the risk of maternal sepsis in our study. The factors associated with neonatal sepsis in this review are classified into maternal and neonatal factors. Maternal factors such as prolonged rupture of membranes > 12 h, intrapartum fever, and history of maternal urinary tract infection and neonatal factors such as Apgar score < 7, presence of meconium in amniotic fluid, and male sex were significantly associated with neonatal sepsis. Our estimate confirms that maternal and neonatal sepsis is a major public health problem in SSA. The pooled prevalence of maternal sepsis in our review is in line with other reviews conducted in 2009 (77) and 2021 (20). This high prevalence can be explained by various factors, such as the coverage of childbirth in health facilities, the asepsis and hygiene of surfaces and materials used, the personal hygiene of pregnant women, and certain harmful practices. Our study

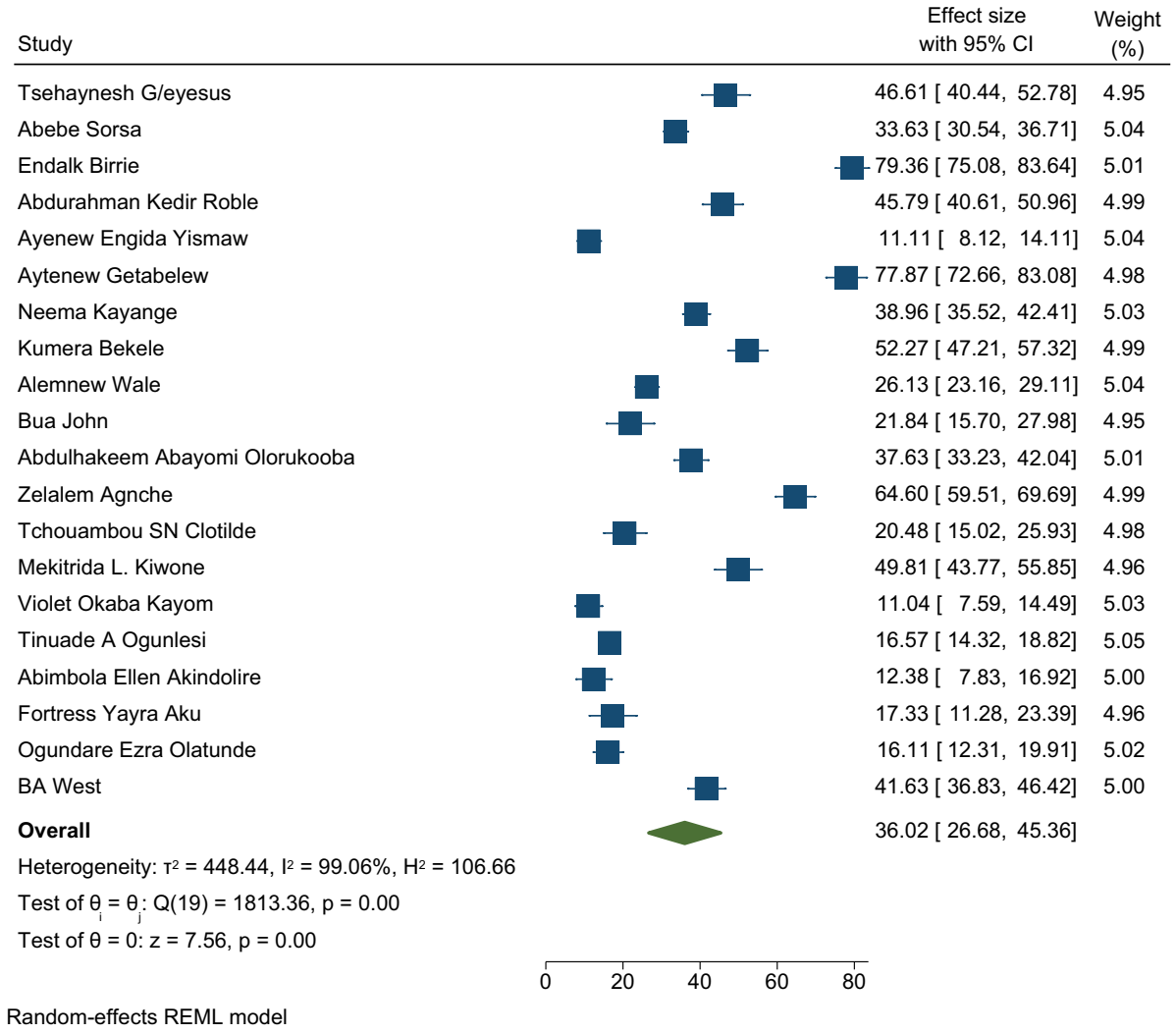

FIGURE 3
Pooled prevalence of neonatal sepsis in SSA.

is the first to examine both the extent and the factors associated with maternal and neonatal sepsis, which sets it apart from other reviews. It identified other factors, such as rural residence and parity, which were not identified in the 2022 review or the WHO guidelines (78). Our results could serve as a broader database for the development of interventions for the prevention and management of maternal and neonatal sepsis.

All the maternal sepsis studies included were from East Africa. The subgroup analysis of the prevalence of maternal sepsis ranged from 15.43% based on clinical signs and biology to 25.33% based on clinical signs.

The subgroup analysis of the prevalence of neonatal sepsis varied from one subdivision to another. East Africa recorded the highest prevalence, 42.96%, which was higher than the pooled prevalence. This could be explained by the weakness of the health system, the quality of services offered, and socio-cultural factors. The under-analysis of sepsis in other parts of Africa, due to the small number of studies carried out there, masks a probable high prevalence in these countries, as areas of high prevalence in these countries have probably not been covered by the small number of studies published. The prevalence of neonatal sepsis was 44.11% for the diagnostic criterion based on clinical signs and 31.25% for clinical signs and biology. Despite the various subgroup analyses, they did not make it possible to explore the source of the heterogeneity. This could be explained by the heterogeneous definition of sepsis in the different studies. The clinical signs differed from one study to another, as did the laboratory tests, which is why a meta-analysis of the individual data was necessary to identify a single algorithm.

The factors associated with maternal sepsis in our review are partly in accordance with a review of the literature in other systematic reviews in 2009 and 2022 (27, 77), where the risk factors identified were intrapartum maternal fever, multiple vaginal examinations, foul-smelling vaginal discharge, prematurity, prolonged rupture of membranes, prolonged labor, and multiple vaginal touches.

Living in a rural area was significantly associated with maternal sepsis. Women living in rural areas were 2.3 times more likely to develop sepsis than those living in urban areas. The possible explanation could be poor hygiene levels, lack of water sources, poor cord care, overcrowding in homes, and low education level of mothers in rural areas (73, 75, 79). Another

TABLE 5 Sub-group analysis of the prevalence of neonatal sepsis in sub-Saharan Africa.

Variables	Characteristics	Pooled prevalence (95% CI)	I^2 (value of *p*)
Countries	East Africa	42.96 (30.75–55.16)	97.41% (<0.000)
	West Africa	23.59 (15.53–33.65)	98.98% (<0.000)
	South Africa	20.48 (15.0–25.93)	0%
Study design	Foresight	32.88 (22.35–43.41)	98.98% (<0.000)
	Retrospective	45.46 (25.97–64.94)	99.05% (<0.000)
Diagnostic criteria	Clinical	44.11 (27.33–60.90)	99.37% (<0.000)
	Clinical and biological	31.25 (22.24–40.26)	97.64% (<0.000)
Risk of bias	Low	34.45 (23.93–44.98)	99.06% (<0.000)
	Medium	39.69 (21.58–57.80)	98.95% (<0.000)

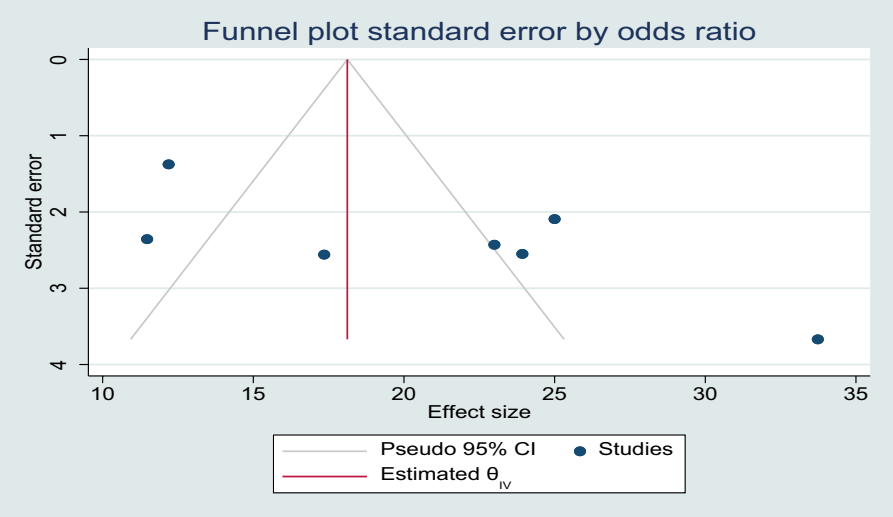

FIGURE 4
Funnel plot showing publication bias in maternal sepsis studies.

possible explanation could be the fact that mothers living in urban areas are close to health facilities and have various means of transport to get to these facilities, and the availability of qualified staff and adequate technical platforms in urban areas. A vaginal examination ≥ 5 times was significantly associated with maternal sepsis. The probability of maternal sepsis was 4.4 times higher in women who had undergone a vaginal examination ≥ 5 times compared with those who had not. During vaginal examinations, there is a high likelihood of microorganisms ascending from the lower to the upper genital tract, which can lead to sepsis. Simple interventions accepted to reduce the incidence of maternal sepsis are using sterile and aseptic technical equipment by providers (hand washing, sterile drapes and instruments, and sterile gloves).

The factors associated with neonatal sepsis in our review are in line with other reviews in Pakistan (78), East Africa (21), SSA (27), and India (80), which reported maternal factors such as intrapartum maternal fever, prolonged rupture of water membranes, gestational age < 37 weeks, prolonged labor, multiple vaginal touches, history of maternal urinary tract infection and low socioeconomic status, neonatal factors such as resuscitation at birth, low birth weight < 2.5 kg, Apgar score < 7, absence of crying immediately after birth, meconium-stained amniotic fluid, and male sex.

Intrapartum fever was significantly associated with maternal sepsis. The probability of maternal sepsis was 2.4 times higher in women with a fever during pregnancy than in those without a fever. Intrapartum fever is suggestive of a maternal infection that is transmitted to the baby *in utero* or during passage through the genital tract, leading to sepsis.

Membrane rupture > 12 h significantly increased the risk of neonatal sepsis, and a history of urinary tract infection in the mother was significantly associated with neonatal sepsis. The water membrane protects the upper genital tract; when it is ruptured, bacteria can proliferate through the dilated cervix into the upper internal genital tract, causing infection. These pathogens also colonize the birth canal, which could contaminate the newborn during passage through the canal. Intrapartum antibiotic prophylaxis has been recommended as an effective practice for at-risk mothers to reduce sepsis worldwide (81).

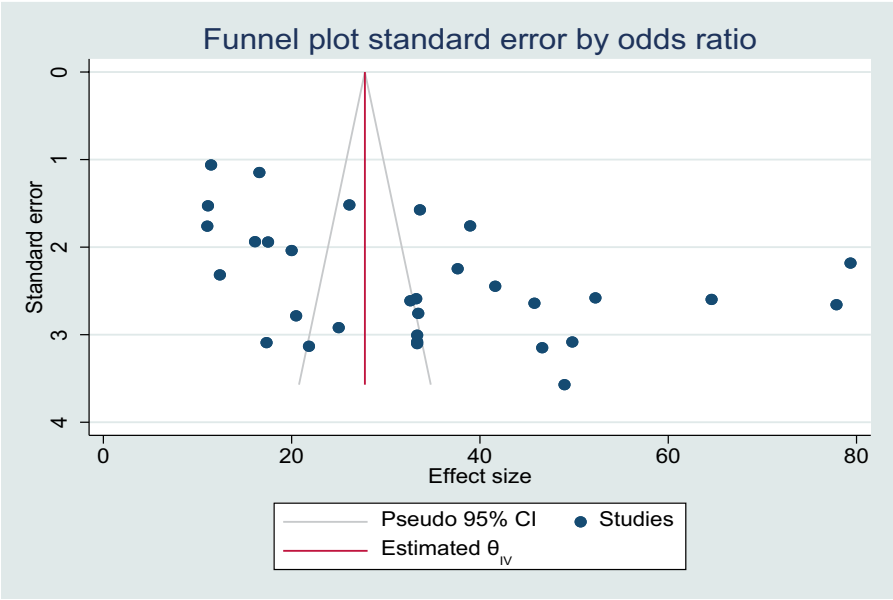

FIGURE 5
Funnel plot showing publication bias d in neonatal sepsis studies.

TABLE 6 Risk factors included in the meta-analysis for maternal sepsis.

Result	Comparison	Number studies	Effect size	Pooled estimate	Q	Heterogeneity I^2, value of *p*
Residence	Rural, urban	3	Odds ratio	3.32 (1.1–10.96)	20.64	90%, 0.048
Parity	Primiparous, multiparous	3	Odds ratio	0.50 (0.32–0.78)	0.42	0.0%, 0.003
Delivery method	CS/ VD	5	Odds ratio	1.47 (0.54–4.01)	35.70	88.8%, 0.448
Prolonged labor	>12 h,<12 h	5	Odds ratio	3.37 (1.64–6.95)	21.60	81.5%, 0.001
Multiple vaginal examinations	>5, <5	3	Odds ratio	4.33 (1.31–14.36)	20.73	90.4%, 0.016
ANC	<4, ≥4	4	Odds ratio	1.849 (0.77–4.42)	15.65	80.8%, 0.168
History of urinary tract infections	Yes or No	3	Odds ratio	1.672 (0.71–3.90)	5.02	60.2%, 0.234
Level of education	No education, education	4	Odds ratio	1.32 (0.93–1.86)	0.99	0.0%, 0.112

CS, Cesarean section; VD, Vaginal delivery.

Having an Apgar score < 7 significantly increased the risk of neonatal sepsis. As a result, the probability of neonatal sepsis was 2.4 times higher in neonates with an Apgar score < 7 compared with those with an Apgar score > 7. This finding is consistent with studies from Iraq (82) and Indonesia (83). Neonates with low Apgar scores tend to have a poor adaptation to extrauterine life due to the stress experienced during labor and therefore are more prone to infection. In addition, resuscitation procedures following birth asphyxia tend to expose newborns to pathogenic microbes. In our study, however, we did not assess birth asphyxia as a risk factor for neonatal sepsis.

Male sex has been identified as a factor associated with neonatal sepsis, and this finding has been made in the journals in India and SSA. However, we suggest further research into this factor to give a more rational explanation.

Strengths and limitations

Our review used an appropriate search strategy, with the combination of global and regional databases reducing the risk of missing relevant regional studies. Duplicate screening and data extraction, as well as rigorous quality assessment of included data and subgroup analysis, was also performed. The relatively high number of included studies for neonatal sepsis is a strength. The number of articles included was small for maternal sepsis (7), which may limit the generalizability of the results. Most of the studies were cross-sectional, which could be a limitation. The majority of studies included in this systematic review were from East Africa, which may affect the generalizability of our results to sub-Saharan Africa. There were also publication biases between studies and a high degree of heterogeneity. We believe that this is

TABLE 7 Risk factors included in the meta-analysis for neonatal sepsis.

Results	Comparison	Number studies	Effect size	Pooled estimate	Q	Heterogeneity I^2, value of p
Apgar	<7 or >7	18	Odds ratio	2.38 (1.61–3.53)	151.21	88.8%, 0.000
Preterm	<37 or >37	17	Odds ratio	1.36 (0.81–2.26)	221.03	92.8%, 0.235
Birth weight	<2.5 kg >2.5 kg	17	Odds ratio	1.28 (0.85–1.93)	210.79	90.5%, 0.228
Mode of delivery	CS/VD	14	Odds ratio	1.05 (0.67–1.65)	93.07	86.0%, 0.807
Amios	Yes or No	12	Odds ratio	2.9 (1.83–4.58)	51.37	78.6%, 0.000
PROM	>12H, <12H	18	Odds ratio	2.8 (1.96–4.18)	99.27	82.9%, 0.000
Sex	Male/Female	14	Odds ratio	1.2 (1.1–1.42)	11.92	0.0%, 0.001
CPN	<4, ≥4	13	Odds ratio	1.4 (0.82–2.18)	67.69	82.3%, 0.170
Vaginal examination	>5, <5	7	Odds ratio	1.2 (0.51–3.09)	126.14	95.2%, 0.616
Intrapartum fever	Yes or No	13	Odds ratio	2.4 (1.52–3.75)	76.14	84.2%, 0.000
Age of newborn	<7 days, >7 days	11	Odds ratio	0.9 (0.52–1.89)	108.72	90.8%, 0.995
Newborn resuscitation	Yes, No	9	Odds ratio	1,7 (0.94–3.30)	94.67	91.5%, 0.076
Parity	Primiparous, multiparous	10	Odds ratio	1.2 (0.83–1.69)	36.48	75.3%, 0.324
History of maternal UTI	Yes, No	13	Odds ratio	2.76 (1.38–5.21)	114.73	89.5%, 0.003
Prolonged labor	>12H, <12H	5	Odds ratio	1.8 (1.47–1.90)	40.15	90.0%, 0.128

an important subject that has not been sufficiently explored and that a meta-analysis of individual data will be necessary.

Conclusion

In our review, the prevalence of maternal and neonatal sepsis was high. Several factors were significantly associated with this prevalence, which could help prevent maternal and neonatal sepsis by developing appropriate standard infection prevention techniques, reducing certain harmful practices, and reducing susceptibility to infection by improving maternal health through nutritional supplementation and treating infections during pregnancy. However, there is a need for evidence on other important risk factors for maternal and neonatal sepsis, including in the community. Given the high risk of bias and high heterogeneity, further high-quality research is needed in the sub-Saharan African context.

Data availability statement

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

Author contributions

FT: Conceptualization, Formal analysis, Investigation, Methodology, Software, Supervision, Writing – original draft, Writing – review & editing. CS: Conceptualization, Methodology, Writing – review & editing. ED: Conceptualization, Writing – review & editing, Investigation. BC: Investigation, Methodology, Writing – review & editing. SS: Software, Writing – review & editing, Formal analysis. ADi: Formal analysis, Validation, Writing – review & editing. ND: Conceptualization, Investigation, Writing – review & editing. BL: Methodology, Writing – review & editing. MA: Conceptualization, Writing – review & editing. KK: Formal analysis, Software, Supervision, Writing – review & editing. AT: Supervision, Writing – review & editing. AC: Writing – review & editing. ADe: Supervision, Validation, Writing – review & editing. HS: Supervision, Validation, Writing – review & editing. IT: Formal analysis, 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. The authors declared no conflicts of interest.

Publisher's note

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Vaccination against emerging and reemerging infectious diseases in places of detention: a global multistage scoping review

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Background: Despite the elevated risks of infection transmission, people in prisons frequently encounter significant barriers in accessing essential healthcare services in many countries. The present scoping review aimed to evaluate the state of availability and model of delivery of vaccination services within correctional facilities across the globe.

Methods: Following the methodological framework for scoping reviews and adhering to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) extension for scoping reviews criteria, we conducted a systematic search across four peer-reviewed literature databases (Medline via PubMed, Web of Science, the Cochrane Library, Science Direct, and EBSCO), as well as 14 sources of grey literature. Two researchers meticulously examined the identified papers independently to extract pertinent data published between 2012 and 2022. The quality of the selected publications was assessed using established quality assessment tools.

Results: Of the 11,281 identified papers 52 met the inclusion criteria. With the exception of one, all the included publications presented data from high-income countries, predominantly originating from the United States. Across the world, the most prevalent vaccines available in prison settings were COVID-19 and HBV vaccines, typically distributed in response to health crises such as pandemics, epidemics, and local outbreaks. Vaccine coverage and uptake rates within correctional facilities displayed noteworthy disparities among various countries and regions. Besides, individual and organizational barriers and facilitating factors of vaccination in prison settings emerged and discussed in the text.

Discussion: The lack of vaccination services combined with low rates of vaccination coverage and uptake among people living and working in correctional facilities represents a cause for concern. Prisons are not isolated from the broader community, therefore, efforts to increase vaccine uptake among people who live and work in prisons will yield broader public health benefits.

KEYWORDS

infectious diseases, vaccination, immunization, primary prevention, prisons

Introduction

Globally, over 11.5 million people are living in prisons and other places of detention on any day (1). People living in prisons (PLP) often lack access to adequate healthcare services in many countries (2). This situation not only represents a violation of their right to health but also contradicts international agreements such as the "the United Nations Standard Minimum Rules for the Treatment of Prisoners" commonly known as "the Nelson Mandela Rules" (3). The Nelson Mandela Rules clearly stipulate that "The provision of health care for prisoners is a State responsibility. Prisoners should enjoy the same standards of health care that are available in the community, and should have access to necessary health-care services free of charge without discrimination on the grounds of their legal status" (3). Yet, the lack of availability of healthcare services coupled with individual risk factors render PLP susceptible to various infectious diseases. This vulnerability is substantiated by the alarmingly elevated prevalence of infectious diseases among PLP, worldwide (4).

The recent COVID-19 pandemic has brought to light the sluggish and inadequate responses to controlling infection transmission in many prisons across the globe. Multiple past influenza outbreaks within prison facilities have resulted in numerous fatalities, underscoring the susceptibility of PLP to airborne diseases (5, 6). Moreover, since the outset of the COVID-19 pandemic, various stakeholders, including international organizations, prison healthcare professionals, scientists, and activists, had cautioned prison systems about the looming COVID-19 crisis on a global scale (7, 8). Nonetheless, the alarmingly elevated number of COVID-19 cases in prisons (9) serves as a glaring indicator of the inadequate response to the disease in numerous countries.

Although previous reviews have occasionally addressed vaccination in prison settings (10–13), there are still numerous aspects of vaccination in prisons that remain largely under-researched. This review is a part of the "Reaching the hard to reach: Increasing access and vaccine uptake among prison populations in Europe (RISE-Vac)" project co-funded by the European Union, aimed at enhancing the health status of people in Europe by increasing vaccine uptake among people who live and work in prisons in this region. Aligned with the aims and objectives of the RISE-Vac, the present review was conducted to map the following: (a) the availability, accessibility, and coverage of vaccination services, (b) models of vaccine delivery, and (c) to explore the perceived barriers and determinants of vaccine uptake and refusal in prisons.

Methods

Methodology of the current review is published elsewhere extensively (14). Co-funded by the European Commission, the research initiative RISE-Vac aims to increase the rates of vaccine uptake within European prisons. Its objectives consist of identifying gaps in vaccine coverage, improving vaccine knowledge among PLPs and prison staff, and facilitating the transferability of the project's health models and knowledge. Nine European partners from six countries participate in the RISE-Vac consortium: Germany, France, Italy, Moldova, Cyprus, and the United Kingdom. Detailed information about the project is available on the project's website.[1]

Data identification

This review adhered to the methodological framework for scoping reviews (15) and the PRISMA extension for scoping reviews (16). The data collection process comprised three key phases: first, a comprehensive literature search was conducted to explore both peer-reviewed and gray literature sources. Secondly, a public call for data was announced and disseminated through various platforms, including the Worldwide Prison Health Research and Engagement Network's (WEPHREN) website, as well as social media channels, e.g., X (Twitter) and LinkedIn to access potential information not publicly available. Lastly, a system outreach was distributed via email among the international network of the authors as well as members of the RISE-Vac advisory board. The RISE-Vac advisory board comprises experienced researchers, prison health policymakers, healthcare providers, stakeholders affiliated with national, regional, and global organizations engaged in prison health, as well as experts who have personal experience of incarceration.

Search strategy

Our search strategy was methodically executed across five distinct databases to identify peer-reviewed publications: Medline via PubMed, Web of Science, the Cochrane Library, Science Direct, and EBSCO. The goal was to procure insights into interventions geared toward increasing vaccine uptake within correctional facilities. In the pursuit of the optimal search query, a comprehensive exploration of Medical Subject Headings (MeSH), Entry terms, and non-MeSH keywords was undertaken. Subsequently, we settled upon the following search combination for our PubMed inquiry: ((Prison* OR Inmate OR Inmates OR Penitentiaries OR Penitentiary OR Jail OR Jails OR Detention Center OR incarcerat*) AND (Vaccin* OR Immunization)). The search terms were adapted for each database, given their unique search algorithms.

We expanded our search to scrutinize 14 gray literature sources, including WHO, CDC, ECDC, UNODC, WEPHREN, ResearchGate, Google, and Google Scholar. Tailored search terms were employed for each website to maximize precision. Particularly in Google, a wide array of terms, including vaccine-preventable diseases, were methodically combined with incarceration-related terms, with distinct combinations for each search.

Inclusion/exclusion criteria and quality assessment

Although the initial database searches were conducted in English, publications in other languages were also identified and scrutinized. Inclusion criteria encompassed papers published in peer-reviewed scientific journals or gray literature between January 1, 2012, and December 31, 2022, reporting information on vaccination services for people who live and work in prisons. Conversely, papers published prior to 2012, those focused on pre-or post-incarceration periods, and those with no pertinent information were excluded. Our review imposed no restrictions regarding the age of the study participants, correctional setting types, or locations. Third reviewers (EP and LT) were consulted when discrepancies arose during the assessment. Additionally, for quality assessment we employed the National

1 https://wephren.tghn.org/rise-vac/

Institute of Health's tools for quantitative research and the Critical Appraisal Skills Program checklist for qualitative research to evaluate the quality of the included papers.

Data classification and analysis

We systematically extracted, categorized, and presented the key variables including publication year, location (country/region), scope, total prison population, sample size of study, publication type, type of setting, target population characteristics, model of delivery of the vaccination services (services are offered by who, where, and when), target diseases, and challenges encountered during implementation of the vaccination services in prisons. In this review, 'coverage' is used to denote the percentage of individuals who have received at least one dose of vaccine before their incarceration, while 'uptake' is used to indicate the percentage of individuals who have received at least one dose of vaccine while in detention centers.

Results

General characteristics of the included studies

Of the 11,281 reviewed publications, 52 studies published between 2012 and 2022 met the inclusion criteria (Figure 1). The majority of the included publications were peer-reviewed in forms of original articles (35/52), followed by brief reports (10/52), research letters (4/52), opinion paper (1/52), case report (1/52), and research abstract (1/52). Most of the included publications came from high-income countries including the US (27/52), the UK (6/52), Canada (4/52), Italy (4/52), Australia (2/52), France (2/52), Switzerland (2/52), Spain (1/52), and Sweden (1/52). Aside from the high-income countries, we found data from only one middle income country: Thailand (1/52). Two included publications reported data at regional levels, both from Europe (one publication reporting data from seven countries, namely Spain, Northern Ireland, Ireland, Poland, Finland, Sweden, and the

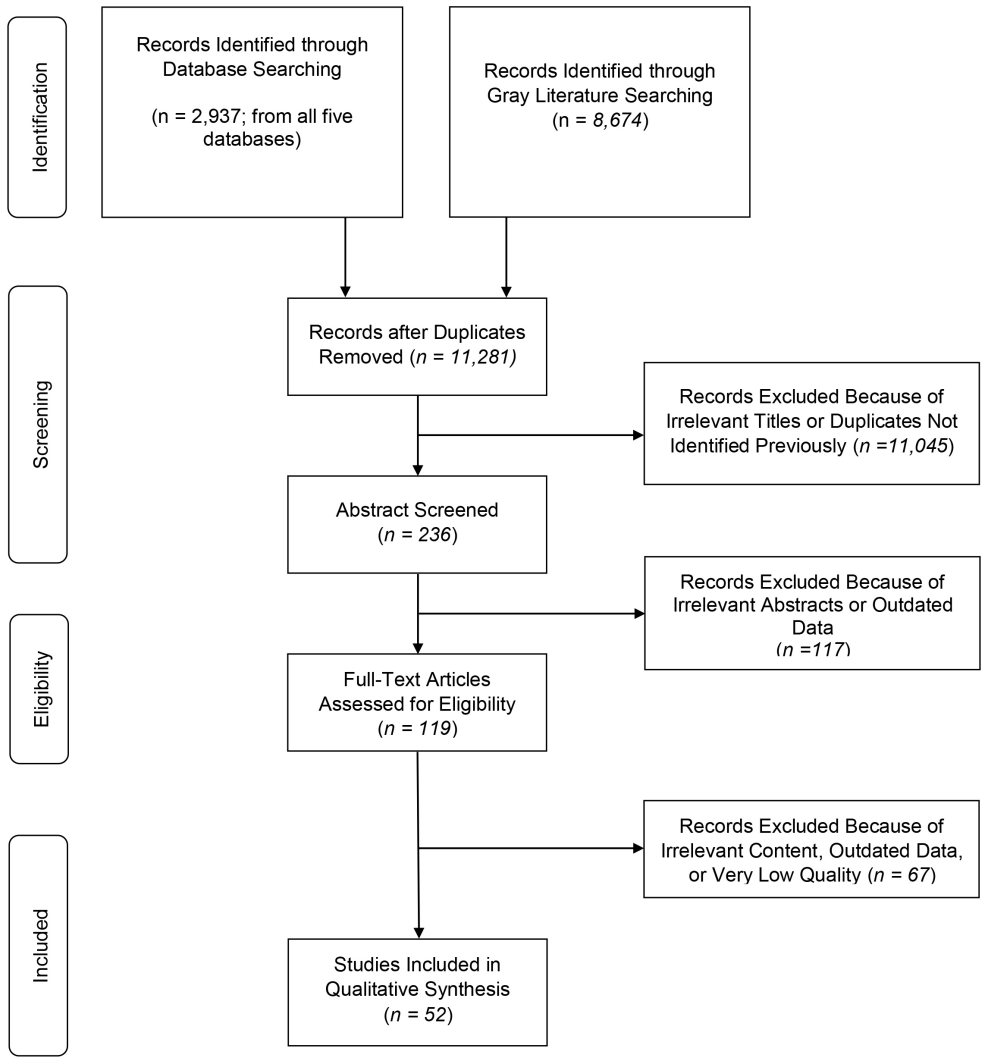

FIGURE 1
PRISMA chart of the included studies.

other from the EU/EEA countries). General characteristics of the included studies are presented in Table 1.

Quality of the included studies

Based on our assessment, none of the included studies were reporting high-quality evidence. Level of evidence was moderate-to-high in 3 publications, moderate in majority of the included publications (32/52), and low in 17 publications.

Settings and samples

Sample size of the included studies varied widely from 46 to 164,283 participants. The included studies reported data from various settings including prisons (25/52), jails (10/52), various facilities combined (3/52), and other facilities, e.g., juvenile detention centers (14/52). Type of setting was not reported in one study. In the majority of the included publications, adult PLP were the main target population of the vaccination programs (36/52), followed by PLP and staff members combined (10/52), juvenile (3/52), and staff only (2/52). Target population was not reported in one publication. In 20 out of 52 publications, the gender of the target population was reported. Among these, the majority included both males and females (13 out of 20), while six publications focused solely on males, and one publication exclusively on females.

Availability of vaccination services by country

Included articles reported on vaccination programs in prisons covering various diseases including COVID-19 (17/52), HBV (10/52), HPV (6/52), influenza (5/52), measles (3/52), varicella (3/52), HAV (2/52), pneumonia (1/52), diphtheria (1/52), or two or more diseases combined (4/52). The countries implementing vaccination programs in prisons comprise Australia (measles, HBV), Canada (influenza, COVID-19, varicella), France (HBV), Italy (measles, COVID-19, HBV), Spain (HAV, HBV, Tdap, pneumonia, influenza), Sweden (HPV), Switzerland (HAV, Tdap, polio, MMR, HBV, HPV), Thailand (Tdap), the UK (HBV, influenza, measles), and the US (HPV, COVID-19, HAV, HBV, Tdap, MCV4, varicella, influenza, pneumonia). One of the papers reporting data at regional level reported that COVID-19 vaccines were available and offered in Spain, Northern Ireland, Poland, Finland, Ireland, and Sweden. According to the other regional publication, except in Bulgaria, Latvia, Lithuania and Romania, HBV vaccines were available in all EU/EEA countries. Figure 2 shows the availability of vaccines in prisons by country, region, and type of vaccine.

Model of delivery of vaccination services in prisons

According to the included publications, in two European countries, Czech Republic and Sweden, HBV vaccines are offered only to at-risk populations (e.g., men who have sex with men (MSM)). In the Netherlands, HBV vaccines are offered only upon request by physicians. In Germany, nine out of the 16 states offered HBV vaccines to all eligible people, while five states offered the vaccines only to at-risk populations. HBV vaccines are available on an opt-in basis in one state in Germany.

Despite the lack of data, vaccines have reportedly been delivered in prisons by internal or external providers including clinical and non-clinical prison staff members, community healthcare workers, e.g., nurses and attending physicians, and researchers (in case the vaccination program was part of a research project). No study reported data on the time (e.g., immediately after admission or during incarceration) and location of delivery of vaccines in prisons.

In 25/52 included publications, the program was implemented as a response to existing health crises such as pandemics, epidemics, or local outbreaks. In one of the implemented interventions at the time of outbreak of influenza, only PLP in affected living units received the vaccines. In addition, evidence shows that in 6/52 settings offering vaccines there was no routine vaccination programs in place and vaccines were offered only for research purposes.

Rates of vaccination coverage and uptake in prisons

Data on vaccination coverage among people who live and work in prisons were reported in 14 publications. Very low levels of coverage (0–25%) were reported from Spain, the UK, and the US; low levels (26–50%) from Finland, Ireland, Switzerland, the UK, and the US; moderate levels (51–75%) from Canada, France, Poland, Spain, Sweden, Switzerland, the UK, and the US; and high levels (76–100%) from Estonia, Northern Ireland, Spain, and the US (Table 2).

Data on vaccine uptake among people who live and work in prisons were reported in 28 included publications. Very low levels of uptake (0–25%) were reported from Italy, Spain, Sweden, the UK, and the US; low levels (26–50%) from Australia, Sweden, the UK, and the US; moderate levels (51–75%) from France, Italy, the UK, and the US; and high levels (76–100%) from Canada, Italy, the UK, and the US (Table 3).

Factors facilitating vaccine delivery and uptake in prisons

At an individual level, higher levels of education, knowledge of vaccine and disease, vaccination being offered free of charge, recommendation from trusted individuals, history of vaccination, history of contracting infectious diseases, gender, living in shared housing, the offer of incentives to get vaccinated, immunization status of family members and friends, race, and cues to action were reported to be the facilitators of vaccine uptake among PLP. For staff members, older age, race (white and black), belief in safety and efficacy of vaccines, and protection of the community were found to be the facilitators of vaccine uptake. At an organizational level, availability of vaccines and type of facility (living in a prison rather than other facilities) were reportedly facilitators of vaccine uptake.

TABLE 1 Worldwide availability and model of vaccine delivery and uptake in prisons from 2012 to 2022.

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Allison et al., 2018 (17)	2016–17	US	3 Facilities in Kansas	Jail	PLP Adults B	HPV	NA	NA	NA	NA	Vaccines being offered at no cost	NR	Survey of intention
Allison et al., 2019 (18)	2017–18	US	1 Facility in Kansas	Jail	PLP Juvenile B 10–18 years	HPV	1	NR	NR	NR	Knowledge of vaccine and disease	Self-reported reasons to refuse: Side effects, confidentiality, pain from needle	Survey of intention plus vaccine offer
Beck et al., 2012 (19)	2003–10	England and Wales	147 Facilities (National)	Prison	PLP	HBV	NR	NR	22 (5–49%)	36% (16–59%)	NR	NR	
Berk et al., 2021(20)	2020–21	US	1 Facility	Prison with various facilities	PLP/STF	COVID-19	2	Yes (2 doses: booster after 4 months)	NR	First dose: 76.4% PLP 68.4% STF Booster: 77.7% PLP 69.6% STF	NR	NR	Response to an existing pandemic
Besney et al., 2017 (21)	2013	Canada	1 Facility	Remand facility	PLP from affected living units	Influenza	NR	NR	NR	PLP 95.5% (138/144)	NR	NR	Response to an existing outbreak; Only PLP on affected living units were offered vaccine
Biondi et al., 2022 (22)	2020–2021	US	National	All types	PLP	COVID-19	NR	Yes, in some institutions with a wide variation	NR	NR	Vaccine availability, preferences of PLP	Distrust in prison staff	Response to an existing outbreak
Borthwick et al., 2021 (23)	2017	UK	1 Facility	A high secure forensic mental health facility	Patients PLP	Influenza	1	Yes	NR	PLP 77.2%	Determinants of intention: past behavior, vaccine knowledge, cues to action Determinants of behavior: cues to action	NR	Vaccination For research purposes; Study of intention and behavior
Brinkley-Rubinstein et al., 2022 (24)	2021	US	6 Facilities	A jail-like intake facility	PLP/STF B	COVID-19	At least 1	NR	NR	NR	NR	NR	Response to an existing pandemic
Chatterji et al., 2014 (25)	2013	Australia	1 Facility	Correctional facility (no more detail)	PLP/STF B	Measles	NR	NR	NA	All except one PLP and two STF	NR	NR	Mass vaccination as a response to an outbreak
Chin 2021 (26)	2021	US	1 Facility	low-to-medium security prison	PLP	COVID-19	NR	Partly	56.6%	NR	NR	NR	Response to an existing pandemic
Costumbrado et al., 2012 (27)	2007–10	US	1 Facility	Jail	Self-defined MSM PLP	HAV/HBV	Up to four	Partly	NR	PLP: 1650 (42%) first doses; 1,215 (31%) second doses; 891 (23%) third doses; and 175 (4%) 12-month booster doses	those who had tested positive for any STI were more likely to start the immunization series	NR	MSM samples
Couper et al., 2013 (28)	2010–11	UK	1 Facility	Prison	PLP/STF	Influenza	NR	Partly	NR	STF: 20% PLP: NR	NR	Lack of audit of vaccine uptake due to the high turnover	Response to an existing outbreak

(Continued)

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TABLE 1 (Continued)

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Da Costa et al., 2021 (29)	2021	Europe	Regional	Prison	PLP/STF	COVID-19	NR	Partly	Spain: Healthcare STF: 100% PLP: 97% Northern Ireland: PLP: 87.3% Poland: PLP: 74% Finland: PLP: 34.4% Ireland: PLP: 43.7% Sweden: PLP: 59.1%	NR	NR	NR	Response to an existing pandemic
Emerson et al., 2020 (30)	2016–17	US	1 Facility	Jail	Juveniles PLP (aged 9–18) and young adults (aged 19–26)	HPV	1	No	NR	No adults; 2 juveniles	Facilitator: A shared commitment to offering HPV vaccination services by leaders and staff in the two agencies	Barriers against collaboration between HD and jail: constrained resources and divergent organizational cultures and priorities Barriers to offer the vaccine: parental consent and the unpredictable, often brief duration of juvenile detent Ions; Potential barrier: criminal background check required by prison for "volunteers" (or non-employees) entering the jail	Study to find barriers and facilitators of collaboration between HD and jail to implement HPV vaccination; Vaccination offered for research purposes
Emerson et al., 2021 (31)	2017–18	US	4 states with 192 jails	Jail	NR	HPV	NR	NR	NR	1 jail has HPV program in place	Determinant of cooperation between HD and jail: Existing any vaccination program in jail	NR	Study to find determinants of cooperation between HD and jail to implement HPV vaccination
Fussilo et al., 2018 (32)	2017	Italy	1 Facility	Prison	PLP B	Measles	NR	NR	NR	First dose: 90 prisoners (74 males and 16 females); Second dose: 17 PLP	NR	NR	Vaccination for research purposes; After a month the program continued to vaccinate all PLP at entry

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TABLE 1 (Continued)

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Gahrton et al., 2019 (33)	2017	Sweden	9 Facilities (1 county)	Prison	PLP B	HBV	1–3	Partly	NR	Full vaccine: 40.6% Susceptible to HBV and not received 3 doses of vaccine in prison combined with negative anti-HBs and negative anti-HBc: 18.6% Potentially susceptible to HBV and not received 3 doses in prison and not tested: 31%	NR	NR	
Gaskin et al., 2015 (34)	2011–12	US	1 Facility	Juvenile detention facility	PLP B	Tdap, MCV4, hepatitis A (HepA; two-shot series), varicella zoster virus (VZV; two-shot series), and HPV (Gardasil; three-shot series; offered routinely to boys and girls at the juvenile detention facility since 2009)	Various based on the type	Partly	Before prison vs. after prison: All 9 vaccines: 3% vs. 27%; Tdap: 63 vs. 91%; HAV 1st dose (76% vs. 92%); HAV 2nd dose: (58% vs. 79%); VZV 1st: 84 vs. 89% VZV 2nd: 47 vs. 65%; MCV4 1st dose: 51 vs. 85%; HPV 1st dose boys: 8% vs. 81%; HPV 1st dose girls: 38 vs. 85%; HPV 3rd dose boys (completed): 1 vs. 35%; HPV 3rd dose girls (completed): 18 vs. 45%	NR	NR	NR	Evaluating vaccination reports; Routine vaccination program exists in this facility

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TABLE 1 (Continued)

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Getaz et al., 2016 (35)	2009	Switzerland	1 Facility	Pre-trial prison	PLP M serology-negative	HAV	NR	NR	NR	NR	NR	NR	Vaccines offered for research purposes
Di Giuseppe et al., 2022 (36)	2021	Italy	3 Facilities	Prison	PLP	COVID-19	NA	NA	NA	NA	Predictor: older age; Self-reported reasons to receive vaccine among those willing to uptake: safety, reduction of risk of infection, and effectiveness	Self-reported concerns among those unwilling to uptake: safety of vaccine, effectiveness of vaccine, is not recommended by physicians	KAP study on COVID-19 vaccine; Response to an existing pandemic
Goldman et al., 2022 (37)	2021	US	1 Facility	Juvenile detention center	PLP M (youths aged 10–21 years)	COVID-19	In total 50 doses	NR	97% unvaccinated 3% partially vaccinated	94% at least 1 dose 2% discharged	NR	Barriers to uptake: limited parental involvement to help access vaccination, feeling unlikely to be infected with COVID-19 or unlikely to become significantly ill, mistrust of the vaccines, and influence by adults who express mistrust, and misinformation about vaccine safety. Barriers to provide vaccine: lack of transportation, distance, and a need to provide advanced notice to probation officers	Response to an existing pandemic
Hagan et al., 2021 (38)	2020–21	US	National	Multiple settings under the coverage of the Federal Bureau of Prisons	PLP/STF B	COVID-19	1–2 doses (Janssen brand)	Partly	PLP 44.8% at least 1 dose 29.9% fully vaccinated	PLP: 0.3% were fully vaccinated before prison; Full uptake: 29.8%; STF: 50.2% at least 1 dose; 47.2% Full vaccination	Predictors of vaccine uptake: being male, being previously infected with COVID-19, higher age, and number of medical conditions associated with severe COVID-19	Predictors of vaccine refusal: being female, non-Hispanic black, Asian	Data from the Federal Bureau of Prisons in the US; Electronic registration system exists; Response to an existing pandemic
Hyatt et al., 2021 (39)	2021	US	National	Multiple settings	STF	COVID-19	NA	NA	NA	NA	Self-reported reasons for vaccine uptake: safety of the respondent and community, efficacy; Older, white and black participants reported being more likely to be vaccinated	Self-reported intention to refuse: 15.7–48.7%; Self-reported reasons of refusal: Being unsafe, no need when you are healthy; Young people and Ethno-racial groups including Hispanic, and American-Indian or Alaska Natives reported to be more likely to refuse vaccine	Study evaluating beliefs and self-reported reasons for vaccine refusal; Response to an existing pandemic
Jacomet et al., 2015 (40)	2012–13	France	1 Facility	Prison	PLP	HBV	NR	NR	NR	54.4%	NR	54.4% accepted and 12.1% refused to uptake. Due to the long delay for receiving serological test results and early release of PLP without consultation it was impossible to offer vaccines to the other PLP.	Vaccines were offered for research purposes.

(Continued)

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TABLE 1 (Continued)

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Jeannot et al., 2016 (41)	2009–11	Switzerland	1 Facility	Juvenile correctional facility	PLP	Tdap Polio MMR HBV HPV	NR	NR	Tdap: 36.2% Polio 47.4% MMR 61.2% HBV 37% HPV 52.2% (Only females)	NR	NR	NR	Study on coverage of VPDs
Junghans et al., 2018 (42)	2016	UK	1 Facility	Prison	PLP	Measles	NR	Partly	NR	30%	NR	Barriers: delay in vaccine supply from the manufacturer, lack of staff, lack of protocols, rapid turnover; Reasons for refusal: Low trust in authorities, distrust of vaccine or vaccinator, and lack of knowledge	Mass vaccination at the time of outbreak
Khorasani et al., 2021 (43)	2013–20	US	1 State (14 facilities)	Jail	PLP	Influenza	1	partly	NR	1.9–11.8%	NR	Vaccine hesitancy, lack of a linkage system between the society and prison, and lack of a universal approach to influenza vaccination in the state	
Khorasani et al., 2021 (44)	2020–21	US	1 Facility	Jail	PLP/STF	COVID-19	NR	NR	NR	NR	NR	PLP: Determinants: Being black Self-reported reasons: Mistrust in vaccine Safety of vaccine Rushed timeline Effectiveness of vaccine STF: Predictor: Being health staff Self-reported: Concerns of safety efficacy Mistrust in vaccine Rushed timeline	Study of willingness Response to an existing crisis
CDC 2012 (45)	2010–11	US	1 Facility	Residential facility for children and youths	PLP with neurologic and neuro developmental conditions	Influenza	1 dose	Yes	NR	At least 10% (all 13 samples)	NR		Study at the time of crisis

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TABLE 1 (Continued)

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Lessard et al., 2022 (46)	2021	Canada	3 Facilities	Prison	PLP B	COVID-19	NR	NR	NR	Self-reported desire to receive vaccine: 73%	Self-reported facilitators: environmental context and resources, social influences, beliefs about consequences, knowledge (reassurance about vaccine outcomes), and emotions (having experienced COVID-19-related stress)	Self-reported barriers: social influences (receiving strict recommendations, believing in conspiracies to harm), beliefs about consequences (infection control measures will not be fully lifted, concerns with vaccine-related side effects), and knowledge (lack of vaccine-specific information)	Qualitative Study of barriers and facilitators Study at the time of a pandemic
Leung et al., 2014 (47)	2020–11	US	1 Facility	Prison	PLP	Varicella	2 doses	Yes	NR	10 PLP out of 1,000 exposed	NR	NR	Vaccination at the time of outbreak
Li et al., 2020 (48)	2005–14	Australia	34 Facilities (National)	Prison	PLP (lifetime IDUs)	HBV	3 doses	Partly	NR	30%	NR	NR	HBV vaccines are available and offered to PWIDs in prisons in Australia
Liu et al., 2022 (49)	2021	US	1 State	Jail	PLP	COVID 19	At least 1	NR	NR	At least 1 dose: 56.2%	Older age, being woman, being vaccinated for influenza, living in shared housing	Concerns of side effects and efficacy, costs, need for an annual booster, mistrust of staff	Lower vaccine acceptance was observed in PLP than the general population; Study at the time of a pandemic
Moore et al., 2019 (50)	2016–17	US	1 Facility	NR	PLP F	HPV	NA	NA	NA	NA	NR	Self-reported barriers: Uncertainty about source of information, concerns about adverse reaction, mistrust of staff, and being gay or lesbian	A study of attitude in a facility that offers no vaccination
Moreau et al., 2016 (51)	2013	Canada	1 Facility	Youth offender correctional center	PLP M	Varicella	NA	NA	70% (single dose)	NA	NA	NA	Vaccination at the time of outbreak
Murphy et al., 2018 (52)	2016–17	US	3 Facilities	Prison	PLP	Varicella	1 or 2	Partly	NR	Prison 1: 48/384 (12.5%); Prison 2: 5/46 (10.9%); Prison 3: 7/97 (7.2%)	NR	NR	Vaccination at the time of outbreak
Nakitanda et al., 2021 (53)	2016–17	EU/EEA	Regional	prison	PLP	HBV	NR	NR	Coverage data from two countries: Estonia: 96 PLP Sweden: 66%	HBV vaccines available in 21/26 countries (80.8%); In 10 countries vaccines are offered to all eligible PLP; Czech Republic: offers vaccines for at risk groups; Sweden: only MSM; Netherlands: only upon request by physicians; Germany offers opt-out vaccine to all eligible PLP in 16 states, 5 to high-risk groups, opt-in (upon request) in 1 state	NR	NR	Regional data

(Continued)

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TABLE 1 (Continued)

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Ortiz-Predes et al., 2022 (54)	2021	Canada	3 Facilities	Prison	PLP M	COVID-19	NR	NR	NR	NR	Self-reported reasons for acceptance: Education, incentives, receiving the vaccine from a trustworthy provider, vaccination of family and friends	Self-reported reasons for refusal: Low risk perception, universal distrust, STF attitudes and relationships, perceived unimportance of vaccines, negative past experience with vaccines, subjective norm, social pressure and social responsibility, role of media and communication, lack of info and accurate knowledge, religious and moral convictions, healthcare delivery, strict public health measures, and lack of incentives	Qualitative study of intention Study and vaccination at the time of pandemic
Parsons et al., 2021 (55)	2021	US	1 State	Prison	PLP	COVID-19	NR	No	NR	40% (still ongoing at the time of study)	NR	NR	Vaccination/study at the time of pandemic
Perret et al., 2013 (56)	2013	UK Wales	National	Prison	PLP/STF	HBV	NR	NR	NR	NR	NR	NR	Only mentioning the availability of HBV vaccination and interventions to increase access
Perret et al. 2019 (57)	2013–17	UK (Wales)	National	Prison	PLP M	HBV	1–3	Partly	1st dose from 2013 to 2017: 41.6% 50.3% 56.8% 56.8% 55.1% Full coverage: 28.7% 36.1% 37.8% 41% 39.6%	NR	NR	NR	
Perrodeau 2016 (58)	2013–14	France	1 Facility	Prison	PLP B	HBV	3	Partly	63% coverage of 2 doses for PLP who needed initial vaccination	NR	NR	NR	
Prince et al., 2022 (59)	2020–21	US	1 State	Prison	STF B	COVID-19	1–3	Partly	NR	First 2 months: 26% vs. 52% custodial vs. health STF By June 2021: 39% vs. 63% custodial vs. health STF	NR	Younger age, prior COVID-19 infection, residing in a community with relatively low rates of vaccination, sharing shifts with coworkers who had relatively low rates of vaccination	Study and vaccination at the time of pandemic

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TABLE 1 (Continued)

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Ramaswamy et al., 2020 (60)	2017–18	US	4 States	Jail	PLP	HPV	NR	NR	2% of local health departments had HPV vaccine or planned to implement soon	NR	Parameters associated with interest in implementation: employees Perception of importance of vaccines, already providing a vaccine	Self-reported barriers to implement: Costs, PLP's short length of stay, availability of medical STF	Survey of intention to implement vaccination
Ryckman et al., 2021 (61)	2020–21	US	1 State	Prison	PLP/STF	COVID-19	NR	Partly	36–76% PLP; 40% STF	NR	NR	NR	Vaccination and study at the time of pandemic; A modeling study
Sanchez et al., 2021 (62)	2018	US	1 Facility	Prison	PLP STF	Pneumonia	1	Partly	NR	78% PLP; 63% medical STF; 86% non-medical STF	NR	NR	Study and vaccination at the time of outbreak
Stasi et al., 2019 (63)	2016–17	Italy	1 Province (15/17 facilities)	Prison	PLP	HBV	1–3	Partly	NR	92.4% 1st dose; 83% 3rd dose	NR	Foreigners were significantly less likely to get vaccinated in prison	
Stasi et al., 2022 (64)	2016–17	Italy	1 Province	Prison	PLP	HBV	1–3	NR	NR	85.2% residents; 72% recently arrived	NR	NR	
Stern et al., 2021 (65)	2020	US	4 States	Prison and Jail	PLP	COVID-19	NA	NA	NA	NA	Predictors of willingness: Higher age, being in a prison rather than jail, being Hispanic/Latino (Hispanic) and American Indian/ Alaska Native	NR	Study of willingness
Tiamkao et al., 2019 (66)	2014	Thailand	1 Facility	Prison	PLP	Diphtheria	NR	NR	NR	NR	NR	NR	Response to an existing outbreak
Vincente-Alcalde et al., 2020 (67)	2008–18	Spain	3 Facilities	Prison	PLP B	HAV HBV TD Pneumonia Influenza	NR	Partly	HBV: 52.3% vaccinated (75.7% completed schedule); HAV: 1.8% vaccinated (11.1% completed schedule); TD: 71.9% vaccinated (58.4% completed schedule); Pneumonia: 08% vaccinated/ completed	Influenza: up to 16.2% between 2010 and 2013	Age was found to be a predictor	Problems: low quality of the records, poor and incomplete digitalization	Random selection of samples; Influenza vaccine was distributed during the study (no routine program)

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TABLE 1 (Continued)

Source/ year of publication/ Reference	Year of study	Country/ Region	Scope	Type of setting(s)	Target population	Target disease/ Vaccine	Doses delivered	Schedule completed	Coverage*	Uptake**	Determinants of/self-reported reasons for uptake	Determinants of refusal/ Barriers to uptake	Comments
Zellmer et al., 2021 (68)	2019	US	1 Facility	Jail	PLP B	HAV	NR	NR	NR	7.1% (that showed a significant increase from 0.6% after changing the protocols)	NR	NR	Response to an existing outbreak

PL, Peer-Reviewed Literature; GL, Grey Literature; M, Male; F, Female; B, Both Genders; NA, Not Available/Applicable; NR, Not Reported; RA, Review Article; BR, Brief Report; Abs, Abstract; LE, Letter to the Editor; STF, Staff; OA, Original Article; PLP, People Living in Prison and other closed settings; MM, Mixed-Methods; MSM, Men who have Sex with Men; PLWUD, People who Use Drugs; HD, Health Department; IDU, Injecting Drug User; TD, Tetanus-Diphtheria. *Of all subjects eligible for vaccination. **% of those eligible people receiving vaccine/booster doses in prison (if applicable).

Barriers toward vaccine delivery and uptake in prisons

Barriers toward vaccine uptake in adult PLP at an individual level included cost of vaccines, concerns of safety and efficacy, concerns of confidentiality, pain from needle, distrust in prison staff members, recommendation from people other than physicians, female gender, race (non-Hispanic black, Asian, American-Indian, and Alaska native), social pressure and social responsibility, religious and moral convictions, being foreign national, and lack of incentive. For juvenile PLP, limited parental involvement to increase vaccine uptake, distrust in prison staff members, being influenced by adults who express mistrust, need to secure parental consent, low perceived risk, and often brief duration of detention were reportedly the main barriers to vaccine uptake. Among staff members, younger age, history of infection, living in a community with low rates of vaccination, sharing shifts with coworkers with low rates of vaccination, being a healthcare worker, concerns of safety and efficacy of vaccine, and rushed timelines were found to be the barriers toward vaccine uptake.

At an organizational level, high turnover of PLP, long delay in receiving serological results and release of PLP, delay in vaccine supply by the manufacturer, strict public health measures, shortage of staff members, lack of protocols, lack of transportation, distance, and necessity to provide advanced notice to probation officers were reportedly the barriers toward vaccination offer and uptake in prisons, worldwide.

Discussion

Our review revealed that evidence regarding the availability of vaccination services in prisons primarily originates from high-income countries. However, data on the accessibility, acceptability, quality, and the delivery models of vaccination services in prison settings remain limited. Vaccine coverage and uptake rates within prisons exhibit significant variations across different countries and regions. COVID-19 vaccination stood out as the most frequently reported vaccine in prisons, underscoring the lack of attention given to other vaccine-preventable diseases within correctional facilities worldwide. Due to the paucity of data, the coverage and uptake of vaccines in prisons exhibit substantial disparities depending on the country and the specific type of vaccine. Notably, many of the included publications indicated that vaccination services were typically implemented during times of crises such as pandemics, epidemics, and local disease outbreaks. This highlights the absence of routine vaccination programs within the prison systems across the globe. Moreover, our investigation identified various individual and organizational barriers that hinder the provision and uptake of vaccines within prison settings worldwide.

The findings of this review align with previous studies (10, 11) and underscore the limited availability of vaccines in prisons and the low vaccination coverage among PLP. Our review has highlighted that certain countries primarily offer vaccination services during crises such as epidemics and pandemics. Furthermore, it is important to note that in some cases, not all people living in a prison receive vaccines, but rather, only those in affected living units (21). Vaccines are recognized as one of the most effective preventive measures for reducing the incidence, morbidity, hospitalization, and mortality associated with infectious

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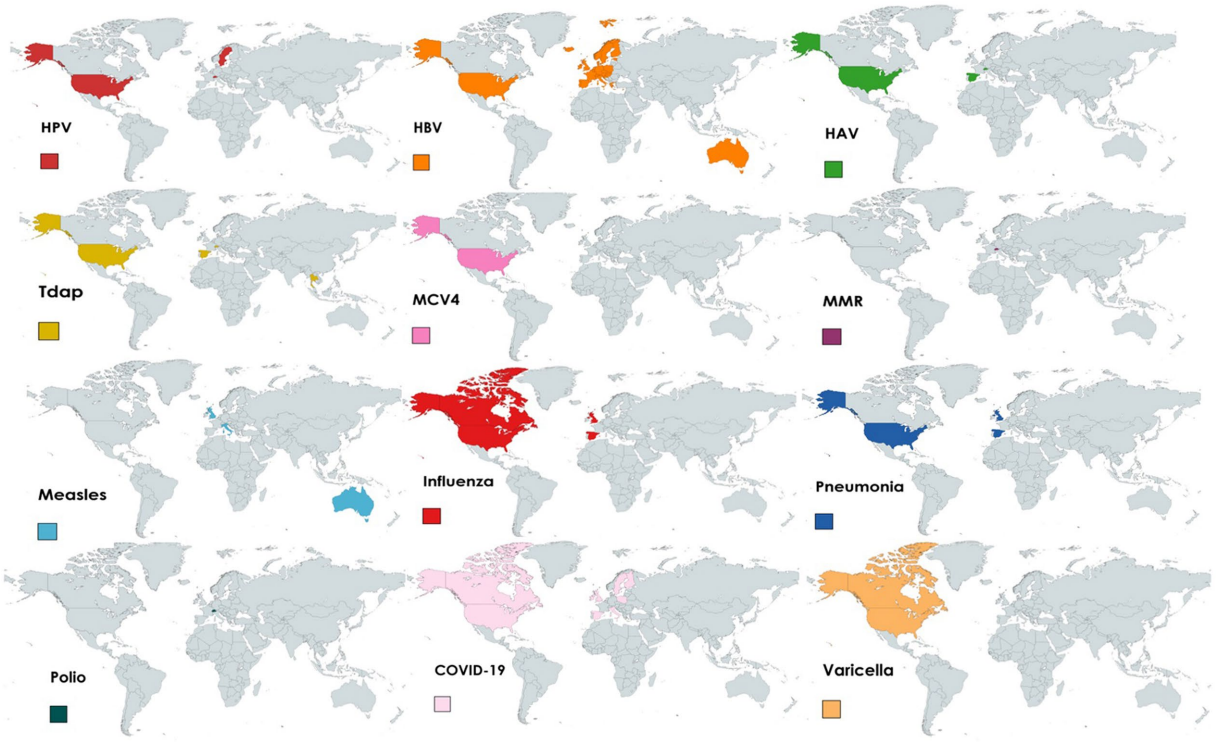

FIGURE 2
Availability of vaccines in prison settings by country, region, and type of vaccine from 2012 to 2022.

TABLE 2 Rates of vaccination coverage among PLP from 2012 to 2022[§].

	COVID-19	HBV	HAV	HPV	VZV	MCV4	Tdap	MMR	Varicella	Polio	Pneumonia
US*	3-76		79	45	65	85	91				
Canada*									70		
Switzerland*		37		52.2			36.2	61.2		47.4	
Spain	97**	75.7*	11.1*				58.4*				8*
France*		63									
England-Wales***		22-41									
Northern Ireland**	87.3										
Ireland**	43.7										
Poland**	74										
Finland**	34.4										
Sweden**	59.1										
Estonia**		66									

[§]Data reported are expressed in percentages among PLP; data on HAV and HBV refer to the complete course of vaccination. *Data reported from one to three facilities.
**Regional data.
***National data.

diseases within correctional facilities (69). However, relying solely on vaccination as a responsive strategy during crises could undermine its overall effectiveness in mitigating the health and financial burdens of infectious diseases in prison settings.

Having knowledge about vaccines and infectious diseases was identified as a significant facilitator for vaccine uptake among incarcerated people. On the other side, misinformation and disinformation are among the major factors hindering vaccine uptake, as observed widely during the COVID-19 pandemic across the world. In this regard numerous studies have shown the negative association between vaccine hesitancy and level of knowledge as well as between vaccine hesitancy and behavioral intention (70, 71). As social contacts are one of the most common sources of health information among PLP (72), the risk of dissemination of misleading information about

TABLE 3 Rates of vaccination uptake among PLP from 2012 to 2022[§].

	COVID-19	Influenza	HBV	HAV	MMR	Varicella	Pneumonia
US*	29.8–94	1.9–11.8	23	7.1–23		7.2–12.5	78
Canada*		95.5					
Spain*		16.2					
France*			54.4				
England-Wales		20–77.2*	36***		30*		
Italy			83–85.2**		3.9*		
Sweden**			40.6				
Australia***			30				

[§]Data reported are expressed in percentages among PLP; data on HAV and HBV refer to the complete course of vaccination. *Data reported from one to three facilities. **Regional data. ***National data.

healthcare services, e.g., vaccination is high. Some community-based recommendations, e.g., active participation of healthcare professionals to address misleading information (73) can be adapted and implemented in prison settings as well. This should be taken into consideration that immediate response plays a crucial role in tackling infodemics (74).

Vaccine hesitancy, however, is multifaceted and goes beyond misleading information alone. Various multicomponent dialog-based interventions have been recommended to address vaccine hesitancy in the community (75). These recommendations include but not limited to targeting specific populations, e.g., unvaccinated or under-vaccinated; increasing knowledge and awareness on vaccines; enhancing access and convenience of vaccination services; to engage influencing people in the program; embedding vaccination services in routine healthcare practices and procedures; and addressing mistrust in healthcare providers and institutions through engagement and dialog (75). While these recommendations are primarily designed for the general population, they can also be applied in correctional settings to enhance vaccine uptake among people who live and work within prisons.

Various interventions have been implemented to address vaccine hesitancy and to increase vaccine uptake in prison settings around the world. These interventions are mostly focused on information dissemination through educational interventions including courses with or without panel discussions, posters, factsheets, pamphlets, etc. (14). In addition to the educational interventions, some countries have implemented organizational interventions including implementing the vaccination programs by external healthcare providers, applying accelerated vaccination schedules for hepatitis B, modifying the vaccination protocols to offer vaccines at the time of entrance, and prioritizing PLP for vaccination implemented by the governments. As evidence on the effectiveness of the aforementioned interventions is scarce (14), these interventions should be implemented cautiously.

Overcrowding stands as one of the most pervasive issues and a significant contributor to substandard prison conditions on a global scale, which, in turn, significantly compromises the quality of healthcare services within correctional facilities. Evidence shows that prisons in 118 countries currently exceed their maximum occupancy limits (76). In the United States, for instance, overcrowding has resulted in inmates having to sleep in gyms, hallways, and even triple-and quadruple-bunked in their cells (77). Numerous strategies, as recommended in the literature, can be employed to mitigate overcrowding within prison settings. These strategies include diverting minor cases away from the criminal justice system; enhancing access to justice and improving case management during pre-trial detention; fostering the development and implementation of constructive non-custodial measures and sentences; reducing sentence lengths while ensuring a consistent approach to sentencing; and establishing avenues for parole or other forms of early release, along with comprehensive post-release support to deter recidivism (78). Applying these measures can effectively alleviate overcrowding and, consequently, enhance the quality of healthcare services, including the administration of vaccinations, in prisons worldwide.

The recently-published WHO framework for assessing prison health system performance has been developed to assist countries in enhancing their prison health systems (79). To achieve this objective, the framework outlines five key priorities: strengthening prison information systems to improve surveillance and response capacity; monitoring health service provision within correctional facilities; tracking and evaluating system performance; acquiring valid and reliable measures of the health status of incarcerated individuals; and engaging in intersectoral collaboration to enhance overall performance and outcomes. Incorporating these components into the design and implementation of healthcare services, including vaccination programs, will significantly enhance the quality and sustainability of these services.

People who work in prisons can play a significant role in bringing the infectious diseases, in specific airborne diseases, from the community to prisons and vice versa. Yet, the health assessment of people who work in prisons has historically been overlooked. During the COVID-19 pandemic, for example, low vaccination rates among prison staff members were reported from many countries (59, 80). Evidence also shows that in many countries implementing COVID-19 vaccination programs prison staff members were not among the priority groups to receive the vaccine (81). Besides that, in our review we found 10/52 publications including both people who live and work in prisons and only 2/52 publications targeting prison staff alone. The lack of publication is another factor highlighting the lack of attention to prison staff members as a key population in prisons. It should be considered that prison-based vaccination plans excluding people who work in prisons would be incomplete and suboptimal.

Task shifting entails the purposeful redistribution of tasks to healthcare providers with fewer qualifications, extending beyond their traditional scope of work (82). When supported by strong evidence and executed efficiently, task shifting can significantly enhance health

outcomes and contribute to the long-term sustainability of healthcare systems (83). In particular, task shifting has demonstrated its effectiveness and viability in managing infectious diseases (84, 85). Given that a shortage of human resources is a primary obstacle to delivering optimal healthcare services within correctional facilities, the adoption of task shifting, involving non-medical staff members in healthcare service delivery, is anticipated to offer a solution for enhancing the quality and long-term sustainability of healthcare services including vaccination in prison settings.

Strengths and limitations of the review

This review is, to our knowledge, the first of its kind classifying and reporting on the characteristics of the existing vaccination programs in prisons in the world. However, results of the present review should be seen in light of some limitations. Lack of published data on various aspects of vaccination in prisons is one of the main limitations of the current review and, on a broader scale, one of the most important barriers toward taking evidence-based decisions on prison health globally. The neglected aspects of vaccination in prisons comprise gender and racial disparities in vaccine uptake and hesitancy; subpopulations of PLP, e.g., the LGBTQ+, older adults, and those living with chronic conditions; determinants of vaccine uptake and refusal; and strategies to increase vaccine uptake in places of correction. Lack of quality was another main limitation of this review, as none of the included studies were found to be reporting high-quality evidence. In our review we addressed these limitations using a multistage search strategy, applying a wide range of inclusion/exclusion criteria, and taking advantage of the established quality assessment tools.

Recommendations and future directions

We propose the following recommendations are made to enhance the quality of vaccination services in prison settings, to address vaccine hesitancy, and to increase the rates of vaccine uptake among people who live and work in prisons:

- To establish more valid and reliable data that can inform prison policy-makers and enhance the effectiveness and quality of the vaccination services in prison settings, funding organizations should expand their support on prison health research, and prison policy-makers should facilitate data collection in prison settings.
- To address mistrust and distrust of vaccination among prison staff members, health providers should take forward the implementation of vaccination services and related interventions, e.g., knowledge dissemination.
- To fight against infodemics, policymakers should capitalize the knowledge of lived experience to provide PLP with reliable and updated information through peer-led educational activities and ensure they are able to make an informed decision on vaccination.
- Implementing mandatory interventions including vaccination, or putting sanctions/restrictions for not using services, is violation of the rights of PLP as human beings; therefore, use of the entire healthcare services in prison settings must be voluntarily with no obligation. At the same time, evidence-based strategies should be in place to facilitate access to services and increase service uptake.
- Policymakers should undertake needs assessment to identify the needs of populations and subpopulations of PLP before implementing vaccination services. All interventions should be tailored based on the needs of the target populations considering their age, gender, race, sexual orientation, and cultural diversity.
- Monitoring and evaluation should be in place to track the effectiveness of the implemented vaccination interventions in prisons. Routine monitoring and evaluation will help prison policymakers and healthcare providers identify the gaps and find solutions to address the possible problems.
- High turnover and short duration of stay is one of the contributing factors preventing PLP from finishing vaccination schedules of vaccines requiring more than one dose. Therefore, there is a need for a referral system to ensure completion of vaccine schedules among people with unfinished vaccination schedules after release.
- The immunization status of those who work in prisons should be checked before and during their employment on a regular basis.

Conclusion

In this review we evaluated over 11,000 publications and found that very few countries, worldwide, offer vaccines to people who live and work in prisons. The most frequently-offered vaccine in prison settings was COVID-19, underlining the lack of attention to the other vaccine-preventable diseases in prisons during the past decade. Similarly, the vast majority of the included publications came from high-income countries and regions, highlighting the abandonment of prison health in low-and middle-income settings. It should be considered that over 90% of PLP will eventually return to their communities. On the other side, prison staff members commute daily between prisons and communities. Therefore, providing accessible, acceptable, affordable and high-quality vaccination services for people who live and work in prisons is a public health investment. Apart from their public health aspects, provision of healthcare services in prison settings is an effort toward reaching international targets such as the UN sustainable development goal 3 of ensuring healthy lives and promoting well-being for all.

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

BM: Conceptualization, Investigation, Methodology, Software, Supervision, Visualization, Writing – original draft, Writing – review

& editing. NI: Conceptualization, Investigation, Methodology, Writing – review & editing. NA: Conceptualization, Investigation, Methodology, Writing – review & editing. SM: Conceptualization, Investigation, Methodology, Writing – review & editing. DP: Conceptualization, Investigation, Visualization, Writing – review & editing. AA: Conceptualization, Investigation, Validation, Visualization, Writing – review & editing. JD'A: Conceptualization, Investigation, Methodology, Validation, Writing – review & editing. EP: Conceptualization, Investigation, Methodology, Supervision, Validation, Writing – review & editing. LT: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Supervision, Writing – review & editing. HS: Conceptualization, Investigation, Methodology, Project administration, 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.

Publisher's note

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Description of the COVID-19 epidemiology in Malaysia

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Introduction: Since the COVID-19 pandemic began, it has spread rapidly across the world and has resulted in recurrent outbreaks. This study aims to describe the COVID-19 epidemiology in terms of COVID-19 cases, deaths, ICU admissions, ventilator requirements, testing, incidence rate, death rate, case fatality rate (CFR) and test positivity rate for each outbreak from the beginning of the pandemic in 2020 till endemicity of COVID-19 in 2022 in Malaysia.

Methods: Data was sourced from the GitHub repository and the Ministry of Health's official COVID-19 website. The study period was from the beginning of the outbreak in Malaysia, which began during Epidemiological Week (Ep Wk) 4 in 2020, to the last Ep Wk 18 in 2022. Data were aggregated by Ep Wk and analyzed in terms of COVID-19 cases, deaths, ICU admissions, ventilator requirements, testing, incidence rate, death rate, case fatality rate (CFR) and test positivity rate by years (2020 and 2022) and for each outbreak of COVID-19.

Results: A total of 4,456,736 cases, 35,579 deaths and 58,906,954 COVID-19 tests were reported for the period from 2020 to 2022. The COVID-19 incidence rate, death rate, CFR and test positivity rate were reported at 1.085 and 0.009 per 1,000 populations, 0.80 and 7.57%, respectively, for the period from 2020 to 2022. Higher cases, deaths, testing, incidence/death rate, CFR and test positivity rates were reported in 2021 and during the Delta outbreak. This is evident by the highest number of COVID-19 cases, ICU admissions, ventilatory requirements and deaths observed during the Delta outbreak.

Conclusion: The Delta outbreak was the most severe compared to other outbreaks in Malaysia's study period. In addition, this study provides evidence that outbreaks of COVID-19, which are caused by highly virulent and transmissible variants, tend to be more severe and devastating if these outbreaks are not controlled early on. Therefore, close monitoring of key epidemiological indicators, as reported in this study, is essential in the control and management of future COVID-19 outbreaks in Malaysia.

KEYWORDS

COVID-19, epidemiology, outbreak, variant, Cases

Introduction

The emergence of the COVID-19 infection caused by the SARS-CoV-2 virus was first reported in Wuhan, China, in late December 2019 (1). Within a short period since its discovery, it spread rapidly across many countries globally. The World Health Organization (WHO) subsequently declared COVID-19 a Public Health Emergency of International Concern (PHEIC) on 30 January 2020 (2). The COVID-19 public health emergency lasted for over 3 years till WHO declared it over on 5 May 2023. During this time, COVID-19 had infected almost 766 million individuals, which resulted in 6.9 million deaths globally (3).

In Malaysia, the first case of COVID-19 was reported on 25 January 2020, which marked the beginning of the pandemic, which lasted from 25 January 2020 till the disease was declared endemic in Malaysia on 1 April 2022. There was a total of 4 outbreaks caused by various COVID-19 variants over the 2-year period. These outbreaks were caused by the following 4 COVID-19 variants: Wuhan, Beta, Delta, and Omicron. Subsequently, each outbreak was then named corresponding to the circulating variants. The first outbreak by the Wuhan variants lasted for 29 weeks, from January 2020 to August 2020 [Epidemiological Week (Ep Wk)9/2020 to 37/2020] (4). This outbreak was characterized by a high case fatality rate and was when the Public Health Social Measure (PHSM), which included movement restrictions, was initiated.

The second Beta variant outbreak occurred from September 2020 to March 2021 (Ep Wk 37/2020 to 13/2021), which lasted for a duration of 30 weeks (5). This was followed by the highly virulent Delta outbreak from April 2021 to January 2022, which lasted for 43 weeks from (Ep Wk 13/2021 to 3/2022) (6). The Delta outbreak was predominantly a severe outbreak with high case burden and mortality. In addition, it was during this outbreak that the introduction of the COVID-19 vaccine commenced. The fourth outbreak, which was caused by the Omicron variant, marked the point this disease was declared endemic in Malaysia (7). The Omicron outbreak occurred from January 2022 till the disease was declared endemic in April 2022 (Ep Wk 3/2022 to 18/2022).

As the disease evolved, the demographic characteristics changed due to the various evolving variants requiring specific interventions and control measures. Hence, it is essential to examine each outbreak's demographic, epidemiological characteristics, and trends to understand the pandemic, which would subsequently assist in improving the management and control of the disease. In addition, a detailed description of each outbreak variant during the pandemic would enable us to understand better the progression, evolution, and severity of the various COVID-19 outbreak variants (8–10). Furthermore, by systematically analyzing these epidemiological indicators, evidence of the effectiveness of pharmaceutical and non-pharmaceutical outbreak-based control measures would assist the surveillance system in monitoring the pandemic. Hence, the knowledge of the disease evolution is important in the process of initiating and adjusting PHSM and vaccination (11–13).

To date, limited published studies have described epidemiological indicators and their trends during each COVID-19 outbreak during the pandemic in Malaysia (14). Therefore, the main aim of this study is to describe and compare the epidemiological indicators during the Wuhan, Beta, Delta, and Omicron outbreak from 2020 (Ep Wk 4/2020 to 18/2022) in terms of their disease demographics (case incidences and mortality rate), hospital admissions [intensive care unit (ICU) admissions, ventilated cases] and diagnostic testing (testing capacity, test positivity rates). This paper will provide a comprehensive analysis and understanding of the various epidemiological indicators and their progression during the COVID-19 pandemic, which would provide a more comprehensive knowledge of the disease evolution and comparison of distinct characteristics during each outbreak in Malaysia (15).

Materials and methods

Data source

Data on COVID-19 cases, deaths, ICU admissions, ventilator requirements and tests was sourced from the GitHub repository (MoH-Malaysia/covid19-public) and the Ministry of Health official COVID-19 website[1,2] (16, 17). The study period was from the beginning of the COVID-19 outbreak in Malaysia, which began during Ep Wk 4/2020, to when it was declared endemic, corresponding to the end of the Omicron outbreak in Ep Wk 18/2022 (18). Daily COVID-19 data were aggregated based on epidemiological week (which starts on Sunday) for each outbreak (i.e., Wuhan, Beta, Delta and Omicron) at the national level (19).

Data analysis

Data was analyzed using the Statistical Package for the Social Sciences (SPSS) version 26.0 (20). Table 1 shows percentages and frequencies of cases, deaths, average ICU admissions, average ventilator requirements, laboratory-confirmed test incidence rate, death rate, CFR, and test positivity rate. Average weekly incidence and death rate was estimated to allow for comparison between outbreaks. In addition, the age-gender-specific case, death, incidence rate, death rate and CFR were estimated. A 10-year interval was used to represent the age distribution as follows: 0–9 years, 10–19 years, 20–29 years, 30–39 years, 40–49 years, 50–59 years, 60–69 years, 70–79 years and more than 80 years. The analysis was done for the overall duration of the COVID-19 outbreak in Malaysia from Ep Wk 4/2020 to Ep Wk 18/2022, and for each outbreak of COVID-19, namely the Wuhan (Ep Wk 9/2020 to 37/2020), Beta (Ep Wk 37/2020 to 13/2021), Delta (Ep Wk 13/2021 to 3/2022), and Omicron (Ep Wk 3/2022 to 18/2022), and presented in tabular and time series plots.

Results

Cases and incidence rate

Overall

There was a total of 4,456,736 cases reported for the overall study period in which the highest and lowest weekly COVID-19 cases were

1 https://github.com/MoH-Malaysia/covid19-public

2 https://covid-19.moh.gov.my/

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reported in Ep Wk 10/2022 ($n = 205,864$) and Ep Wk 8/2020 ($n = 0$), respectively. The overall weekly average COVID-19 incidence rate was 108.51 per 100,000 population, as shown in Table 2.

By outbreaks

During the Wuhan outbreak, a total of 9,375 (0.19%) cases were reported, with the highest and lowest weekly COVID-19 cases being reported in Ep Wk 14/2020 ($n = 1,163$) and Ep Wk 8/2020 ($n = 0$), respectively. On the other hand, during the Beta outbreak, a total of 333,488 (7.48%) cases were reported, with the highest and lowest weekly COVID-19 cases being reported in Ep Wk 4/2021 ($n = 29,206$) and Ep Wk 38/2020 ($n = 299$) respectively. For the Delta outbreak, a total of 2,467,804 (55.37%) cases were reported, with the highest and lowest weekly COVID-19 cases being reported in Ep Wk 33/2021 ($n = 150,933$) and Ep Wk 13/2021 ($n = 8,968$) respectively.

As for the Omicron outbreak, a total of 1,646,047 (36.93%) cases were reported, with the highest and lowest weekly COVID-19 cases being reported on Ep Wk 10/2022 ($n = 205,864$) and Ep Wk 18/2022 ($n = 8,732$) respectively. Overall, the highest number of cumulative cases was reported during the Delta ($n = 2,467,804$, 55.37%) outbreak, followed by the Omicron ($n = 1,646,047$, 36.93%), Beta (333,488, 7.48%) and Wuhan (8,493, 0.19%) outbreak, respectively. The average weekly incidence rate for the Wuhan, Beta, Delta and Omicron outbreaks was 1.48, 32.91,169.89 and 304.53 per 100,000 populations, respectively.

Trends

During the Wuhan outbreak, the epidemiological curve represented a slow increase in which the number of cases started to increase from Ep Wk 10/2020 ($n = 68$) and peaked in Ep Wk 14/2020 ($n = 1,163$). Following this, during the Beta outbreak, the epidemiological curve peaked on Ep Wk 4/2021 ($n = 29,206$). This was followed by the Delta outbreak, where the epidemiological curve peaked at Ep Wk 33/2021 ($n = 150,933$). Subsequently, during the Omicron outbreak, the epidemiological curve rose rapidly and peaked at Ep Wk 10/2022 ($n = 205,864$), corresponding to the highest number of weekly cases reported through the study duration in Malaysia (Figure 1).

ICU admission and ventilator requirements

ICU admission trends

During the Wuhan outbreak, the average number of cases requiring ICU admission started to increase from Ep Wk 13/2020 ($n = 369$) and peaked in Ep Wk 15/2020 ($n = 549$). Subsequently, the average ICU case trends decreased from Ep Wk16/2020 ($n = 374$) to Ep Wk36/2020 ($n = 35$). Following this, the Beta outbreak began where the average ICU case trends started to increase from Ep Wk37/2020 ($n = 53$) to Ep Wk 4/2021 ($n = 2,902$), corresponding to the longest increasing average ICU case trends of 21 weeks. A downward trend of the average ICU cases was observed from Ep Wk

TABLE 1 Epidemiological indicators.

No.	Epidemiological indicator	Formula
1	Incidence rate*	$\frac{\text{Number of COVID}-19\,\text{cases}}{\text{Total Population}} \times 100{,}000$
2	Case Fatality rate (CFR)	$\frac{\text{Number of COVID}-19\,\text{deaths}}{\text{Total COVID}-19\,\text{cases}} \times 100$
3	Test positivity rate	$\frac{\text{Number of positive case}}{\text{Total tests}\left(\text{RT}-\text{PCR and RTK}-\text{Ag}\right)} \times 100$
4	Death rate*	$\frac{\text{Number of COVID}-19\,\text{deaths}}{\text{Total Population}} \times 100{,}000$

*Rates were calculated based on weekly averages; Gender-Age specific incidence and death rates were calculated per 1,000 population.

TABLE 2 COVID-19 cases, deaths, incidence rate, death rate, CFR, testing capacity and test positivity rate by COVID-19 variants outbreaks in Malaysia.

	COVID-19 Waves COVID				Total (Ep Wk 4/2020 to Ep Wk 18/2022)
	Wuhan (Ep Wk 9/2020 to Ep Wk 37/2020)	Beta (Ep Wk 37/2020 to Ep Wk 13/2021)	Delta (Ep Wk 13/2021 to Ep Wk 3/2022)	Omicron (Ep Wk 3/2020 to Ep Wk 18/2022)	
Cases, N (%)	9,375 (0.19)	333,488 (7.48)	2,467,804 (55.37)	1,646,047 (36.93)	4,456,736
Deaths, N (%)	128 (0.34)	1,132 (3.18)	30,549 (85.86)	3,770 (10.60)	35,579
Average weekly incidence rate*	1.48	32.91	169.89	304.53	108.51
Average weekly death rate*	0.02	0.11	2.10	0.70	0.87
CFR (%)	1.42	0.34	1.24	0.23	0.80
Total test, N (%)	853,379 (1.45)	8,781,144 (14.91)	33,603,291 (57.04)	15,157,523 (25.73)	58,906,954
Test positivity rate (%)	1.00	3.80	7.34	10.86	7.57

*Weekly incidences /death rates were estimated and averaged over the number of Epidemiological Weeks for each outbreak.

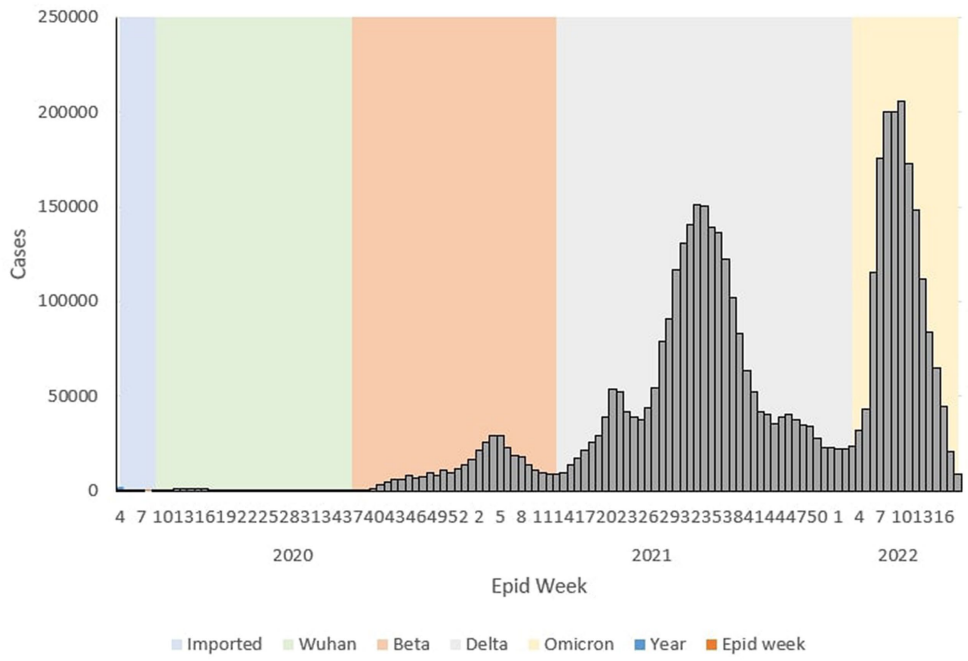

FIGURE 1
Weekly COVID-19 cases by outbreaks in Malaysia, 2020 to 2022*.

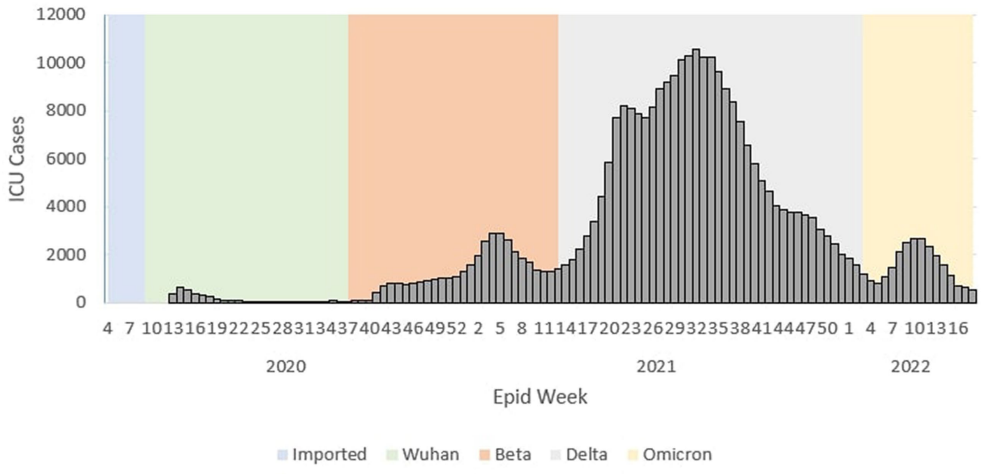

FIGURE 2
Weekly COVID-19 average ICU cases by outbreaks in Malaysia, 2020 to 2022.

5/2021 ($n = 2,873$) to Ep Wk 15/2021 ($n = 1,775$). This was followed by the Delta outbreak, where in the ICU case trend started to increase at Ep Wk 16/2021 ($n = 2,024$) to Ep Wk 32/2021 ($n = 10,586$) and subsequently decreased from Ep Wk 35/2021 ($n = 9,637$) until Ep Wk 5/2022 ($n = 810$) which corresponded to the longest decreasing average ICU case trends of 23 weeks. Following this, the Omicron outbreak began where the average ICU case trend started to increase at Ep Wk 6/2022 ($n = 1,047$) to Ep Wk 15/2022 ($n = 1,102$) and subsequently decreased at Ep Wk 16/2022 ($n = 702$) till Ep Wk 18/2022 ($n = 506$) as shown in Figure 2.

Ventilation requirement trend

During the Wuhan outbreak, the average number of ICU cases requiring ventilation increased from Ep Wk 13/2020 ($n = 252$) and peaked in Ep Wk 14/2020 ($n = 381$). Subsequently, the average ventilated case trends decreased from Ep Wk 15/2020 ($n = 279$) to Ep Wk 33/2020 19 ($n = 4$). Following this, the Beta outbreak began where the average ventilated case trends started to once again increase from Ep Wk 34/2020 ($n = 22$) until Ep Wk 5/2021 ($n = 1,482$), and this corresponded to the longest increasing average ventilated case trends of 25 weeks. A downward trend of average ventilated cases was

observed from Ep Wk 6/2021 ($n = 1,270$) until Ep Wk 13/2021 ($n = 750$).

This was followed by the Delta outbreak, wherein the average number of ICU cases requiring ventilation increased from Ep Wk 14/2021 ($n = 811$) and peaked in Ep Wk 31/2021 ($n = 6,388$). The average ventilated case trends decreased from Ep Wk 32/2021 ($n = 6,217$) until Ep Wk 5/2022 ($n = 420$), corresponding to the longest decreasing average ventilated case trend of 26 weeks. Following this, the Omicron outbreak began when the average ventilated cases started to increase Ep Wk 6/2022 ($n = 558$) till Ep Wk 11/2022 ($n = 1,558$) and subsequently decreased from Ep Wk 12/2022 ($n = 1,377$) till Ep Wk 18/2022 ($n = 297$) as shown in Figure 3.

Deaths, death rate, and CFR

Overall

There was a total of 35,579 deaths reported for the overall study period, in which the highest weekly COVID-19 death was reported in Ep Wk 37/2021 ($n = 2,647$), and no deaths were reported for several epidemiological weeks (17 weeks), respectively. The overall weekly average COVID-19 death rate and CFR were 0.87 per 100,000 population and 0.80%, respectively, as shown in Table 2.

By outbreak

During the Wuhan outbreak, a total of 128 (0.34%) deaths were reported, with the highest and lowest weekly COVID-19 deaths being reported in Ep Wk 13/2020 ($n = 27$) and several Epidemiological Weeks (6 weeks) with no deaths, respectively. While during the Beta outbreak, a total of 1,132 (3.18%) deaths were reported, with the highest and lowest weekly COVID-19 deaths being reported in week Ep Wk 5/2021 ($n = 111$) and Ep Wk 38 and 39/2020 ($n = 3$), respectively. For the Delta outbreak, a total of 30,549 (85.86%) deaths were reported, with the highest and lowest weekly COVID-19 deaths being reported in Ep Wk 37/2021 ($n = 2,647$) and Ep Wk 14/2021 ($n = 35$), respectively.

As for the Omicron outbreak, a total of 3,770 (10.6%) deaths were reported, with the highest and lowest weekly COVID-19 deaths being reported in Ep Wk 11/2022 ($n = 609$) and Ep Wk 18/2022 ($n = 32$), respectively. Overall, the highest number of cumulative deaths was reported during the Delta outbreak ($n = 30,549$, 85.86%), followed by the Omicron ($n = 3,770$, 10.60%), Beta ($n = 1,132$, 3.18%) and Wuhan ($n = 128$, 0.34%) outbreak, respectively. The CFR for the Wuhan, Beta, Delta and Omicron outbreaks was 1.42, 0.34, 1.24, and 0.23%, respectively, as shown in Table 2.

Trends

The epidemiological curve represents a sharp increase during the Wuhan outbreak, peaking at Ep Wk 14/2020 ($n = 26$). Following this, during the Beta outbreak, the epidemiological curve peaked at Ep Wk 5/2021 ($n = 111$). This was followed by the Delta outbreak, where the epidemiological curve peaked at Ep Wk 37/2021 ($n = 2,647$). Subsequently, during the Omicron outbreak, the epidemiological curve rose rapidly and peaked at Ep Wk 11/2022 ($n = 609$), as shown in Figure 4.

Gender distribution of cases

Overall

Of the total 4,456,736 cases reported during the study period, 2,379,375 (53.39%) and 2,077,361 (46.61%) were males and females, respectively. The male-to-female cases ratio was 1.2. The COVID-19 gender-specific incidence rates were 141.87 and 130.78 per 1,000 males and females, respectively (Table 3).

Outbreaks

Of the 8,493 total cases reported during the Wuhan outbreak, 6,183 (72.80%) and 2,310 (27.20%) were males and females, respectively. The male-to-female cases ratio was 2.7. The COVID-19 gender-specific incidence rates per 1,000 populations for the Wuhan

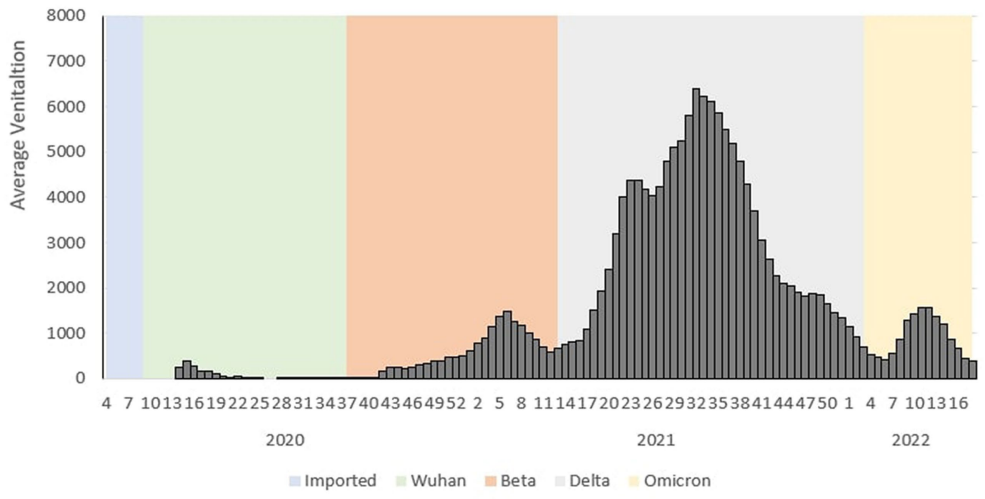

FIGURE 3
Weekly COVID-19 average ventilated cases by outbreaks in Malaysia, 2020 to 2022.

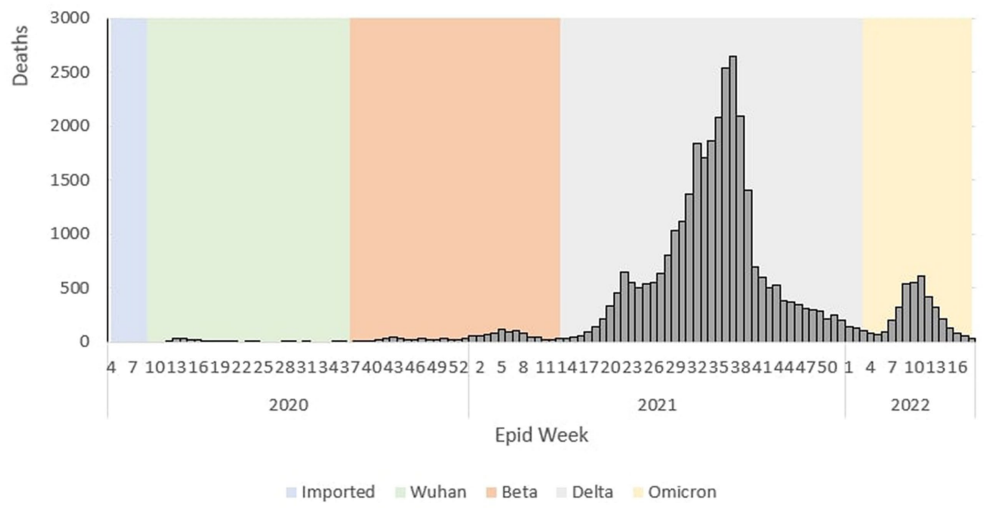

FIGURE 4
Weekly COVID-19 deaths by outbreaks in Malaysia, 2020 to 2022.

outbreak were 0.37 and 0.10 in males and females, respectively (Table 3; Figures 5, 6). During Beta outbreak, 333,488 cases were reported, with 224,086 (67.19%) and 109,402 (32.81%) males and females, respectively. The male-to-female cases ratio was 2.1. The COVID-19 gender-specific incidence rates per 1,000 populations for the Beta outbreak were 13.37 and 6.91 in males and females, respectively (Table 3; Figures 5, 6). Of the 2,467,804 total cases reported for the Delta outbreak, 1,352,873 (54.82%) and 1,114,931 (45.18%) were males and females, respectively. The male-to-female cases ratio was 1.2.

The COVID-19 gender-specific incidence rates per 1,000 populations for the Delta outbreak were 80.67 and 70.19 in males and females, respectively (Table 3; Figures 5, 6). As for the Omicron outbreak, 1,646,047 cases were reported, with 795,645 (48.34%) and 850,402 (51.66%) males and females, respectively. The ratio of male to female cases was 0.9. The COVID-19 gender-specific incidence rates per 1,000 populations for the Omicron outbreak were 47.44 and 53.5 in males and females, respectively (Table 3; Figures 5, 6). A higher number of COVID-19 cases and incidence rates were observed among males across all the outbreaks except for the Omicron outbreak, which reported higher numbers of COVID-19 cases and incidence rates among females (Figure 5). The male-to-female cases ratio reduced from the Wuhan outbreak to the Omicron outbreak (Figure 6).

Gender distribution of deaths

Overall

Of the 35,579 total deaths reported during the study period, 20,434 (57.43%) and 15,145 (42.57%) were males and females, respectively. The male-to-female deaths ratio was 1.4. The COVID-19 gender-specific death rates per 1,000 populations during this study period were 1.22 and 0.95 in males and females, respectively. The COVID-19 gender-specific CFR rates during this study period were 0.86 and 0.73% in males and females, respectively (Table 3).

Outbreaks

Of the 121 total deaths reported during the Wuhan outbreak, 88 (72.73%) and 33 (27.27%) were males and females, respectively. The male-to-female deaths ratio was 2.7. The COVID-19 gender-specific death rates per 1,000 populations for the Wuhan outbreak were 0.01 and 0.002 in males and females, respectively. The COVID-19 gender-specific CFR rates for the Wuhan outbreak were 1.42 and 1.18% in males and females, respectively (Table 3). During the Beta outbreak, 1,132 total deaths were reported, with 732 (64.66%) and 400 (35.34%) being males and females, respectively. The ratio of male to female deaths was 1.8. The COVID-19 gender-specific death rates per 1,000 populations for the Beta outbreak were 0.04 and 0.03 in males and females, respectively. The COVID-19 gender-specific CFR rates for the Beta outbreak were 0.33 and 0.37% in males and females, respectively (Table 3). Of the 30,549 total deaths reported in the Delta outbreak, 17,441(57.09%) and 13,108 (42.91%) were males and females, respectively. The ratio of male to female deaths was 1.3.

The COVID-19 gender-specific death rates per 1,000 populations for the Delta outbreak were 1.04 and 0.83 in males and females, respectively. The COVID-19 gender-specific CFR rates for the Delta outbreak were 1.29 and 1.18% in males and females, respectively (Table 3). As for the Omicron outbreak, 3,770 deaths were reported, with 2,168 (57.51%) and 1,602 (42.49%) males and females, respectively. The male-to-female deaths ratio was 1.4. The COVID-19 gender-specific death rates per 1,000 populations for the Omicron outbreak were 0.13 and 0.10 in males and females, respectively. The COVID-19 gender-specific CFR rates for the Omicron outbreak were 0.27 and 0.19% in males and females, respectively (Table 3; Figure 7). A higher number of COVID-19 deaths, death rate and CFR were

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TABLE 3 Gender distribution of COVID-19 cases and deaths by COVID-19 variants outbreaks in Malaysia.

		Wuhan			Beta			Delta			Omicron			Overall (Ep Wk 4/2020 to Ep Wk 18/2022)		
		Male	Female	Ratio	Male	Female	Ratio	Male	Female	Ratio	Male	Female	Ratio	Male	Female	Ratio
Cases	N	6,183	2,310	2.68	224,086	109,402	2.05	1,352,873	1,114,931	1.21	795,645	850,402	0.94	2,379,375	2,077,361	1.15
	%	72.80	27.20		67.19	32.81		54.82	45.18		48.34	51.66		53.39	46.61	
	IR	0.37	0.15		13.37	6.91		80.67	70.19		47.44	53.54		141.87	130.78	
Death	N	88	33	2.67	732	400	1.83	17,441	13,108	1.33	2,168	1,602	1.35	20,434	15,145	1.35
	%	72.73	27.27		64.66	35.34		57.09	42.91		57.51	42.49		57.43	42.57	
	DR	0.01	0.002		0.04	0.03		1.04	0.83		0.13	0.10		1.22	0.95	
	CFR	1.42	1.18		0.33	0.37		1.29	1.18		0.27	0.19		0.86	0.73	

observed among males across all the outbreaks except for the Beta outbreak, where a higher CFR was observed among females.

Age distribution of cases

Overall

From the total of 4,456,736 cases reported during the study period, the age group with the highest and lowest cases were reported for individuals aged 20–29 years ($n = 1,027,286$) and 80+ years ($n = 39,852$), respectively. The proportion of COVID-19 cases by 10-year age during the study period are as follows: 0–9 years (11.26%), 10–19 years (11.73%), 20–29 years (23.50%), 30–39 years (22.19%), 40–49 years (13.20%), 50–59 years (8.98%), 60–69 years (5.78%), 70–79 years (2.46%) and more than 80 years (0.91%) as shown in Table 4. The COVID-19 age-specific incidence rates per 1,000 populations by the 10-year age groups for the study period were highest and lowest for individuals ages 30–39 years (incidence rates 177.21) and 10–19 years (incidence rates 97.7), respectively (Figure 8).

Outbreaks

Of the 8,493 cases reported during the Wuhan outbreak, the highest and lowest cases were in the age group 20–29 years ($n = 2,138$) and 80+ years ($n = 84$), respectively. The proportion of COVID-19 cases by 10-year age group for the Wuhan outbreak are as follows: 0–9 years (3.35%), 10–19 years (10.93%), 20–29 years (27.69%), 30–39 years (19.73%), 40–49 years (12.21%), 50–59 years (13.0%), 60–69 years (9.26%), 70–79 years (2.73%), and 80+ years (1.09%). The COVID-19 age-specific incidence rates per 1,000 populations in 10-year age groups during the Wuhan outbreak were highest for individuals ages 20–29 years and 60–69 years (incidence rates 0.34) and lowest for individuals ages 0–9 years (incidence rates 0.05) respectively (Table 4; Figures 9, 10).

During the Beta outbreak, 333,488 cases were reported, with the highest and lowest cases in the age group 20–29 years ($n = 96,346$) and 80+ years ($n = 1,708$), respectively. The proportion of COVID-19 cases by 10-year age group for the Beta outbreak are as follows: 0–9 years (6.23%), 10–19 years (7.84%), 20–29 years (30.59%), 30–39 years (27.31%), 40–49 years (13.6%), 50–59 years (8.07%), 60–69 years (4.31%), 70–79 years (1.53%) and 80+ years (0.54%). The COVID-19 age-specific incidence rates per 1,000 populations in 10-year age groups during the Beta outbreak were highest for individuals ages 30–39 years (incidence rates 15.84) and lowest for individuals ages 0–9 years (incidence rates 3.86) respectively (Table 4; Figures 9, 10).

During the Delta outbreak, 2,467,804 cases were reported, with the highest and lowest cases in the age group 20–29 years ($n = 548,620$) and 80+ years ($n = 2,251$), respectively. The proportion of COVID-19 cases by 10-year age group for the Delta outbreak are as follows: 0–9 years (12.12%), 10–19 years (12.48%), 20–29 years (22.74%), 30–39 years (20.87%), 40–49 years (12.95%), 50–59 years (9.25%), 60–69 years (6.12%), 70–79 years (2.56%) and 80+ years (0.91%). The COVID-19 age-specific incidence rates per 1,000 populations in 10-year age groups during the Delta outbreak were highest for individuals ages 30–39 years (incidence rates 91.97) and lowest for individuals ages 80+ years (incidence rates 55.54) respectively (Table 4; Figures 9, 10).

Of the 1,646,047 cases reported during the Omicron outbreak, the age group with the highest and lowest cases were 20–29 years

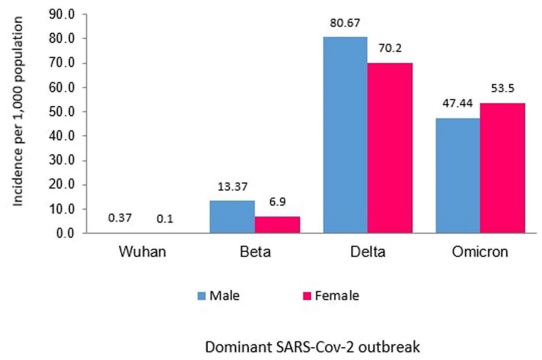

FIGURE 5
Male and female COVID-19 incidence rates (per 1,000 population) in Malaysia.

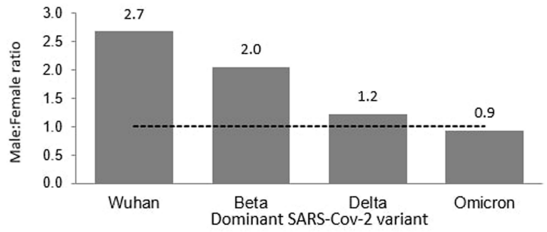

FIGURE 6
Male to female ratio of COVID-19 cases in Malaysia.

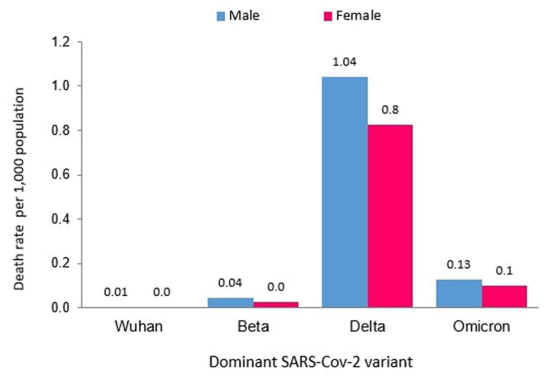

FIGURE 7
Male and female COVID-19 death rates (per 1,000 population) in Malaysia.

($n = 379,988$) and 80+ years ($n = 15,994$), respectively. The proportion of COVID-19 cases by 10-year age group for the Omicron outbreak are as follows: 0–9 years (10.99%), 10–19 years (11.39%), 20–29 years (23.23%), 30–39 years (23.16%), 40–49 years (13.49%), 50–59 years (8.74%), 60–69 years (5.54%), 70–79 years (2.48%) and 80+ years (0.98%). The COVID-19 age-specific incidence rates per 1,000 populations in 10-year age groups during the Omicron outbreak were highest for individuals ages 30–39 years (incidence rates 69.21) and lowest for individuals ages 10–19 years (incidence rates 35.49), respectively (Table 4; Figures 11, 12).

Across all the outbreaks, the highest and lowest number of COVID-19 cases by age was reported for individuals aged 20–29 and over 80 years, respectively. The number of COVID-19 cases by age starts to reduce among individuals ages 30 and above across all the outbreaks (Figure 8). For all the outbreaks, the highest COVID-19 age-specific incidence rate was reported among individuals aged 30–39 years, except for the Wuhan outbreak, which had the highest incidence rate among individuals aged 20–29 years and 60–69 years, respectively. The lowest COVID-19 age-specific incidence rate was reported among individuals in the age groups 0–9 years and 10–19 years for all outbreaks except for the Delta outbreak, which reported the lowest COVID-19 age-specific incidence rate among individuals in the age group more than 80 years (Figure 10).

Age distribution of death

Overall

From the total of 35,579 deaths reported during the study period, the highest and lowest deaths were reported in the age group 60–69 years ($n = 8,258$) and 10–19 years ($n = 106$), respectively. The proportion of COVID-19 deaths by 10-year age group during the study period is as follows: 0–9 years (0.3%), 10–19 years (0.3%), 20–29 years (2.12%), 30–39 years (6.85%), 40–49 years (12.97%), 50–59 years (18.33%), 60–69 years (23.21%), 70–79 years (19.90%) and 80+ years (16.03%). The COVID-19 age-specific death rates per 1,000 populations in 10-year age groups during the study period were highest for individuals ages 80+ years (death rates 14.36) and lowest for individuals ages 0–9 years and 10–19 years (death rates 0.02), respectively. The COVID-19 age-specific CFR rates in 10-year age groups during the study period were highest for individuals ages 80+ years (CFR 14.3%) and lowest for individuals ages 0–9 years and 10–19 years (CFR 0%) respectively (Table 4).

Outbreaks

From the total of 121 deaths reported during the Wuhan outbreak, the highest and lowest deaths were reported in the age group 60–69 years ($n = 38$) and the group 0–9 years and 10–19 years ($n = 0$), respectively. The proportion of COVID-19 deaths by 10-year age group for the Wuhan outbreak is as follows: 0–9 years (0.0%), 10–19 years (0.0%), 20–29 years (2.48%), 30–39 years (7.44%), 40–49 years (7.44%), 50–59 years (15.7%), 60–69 years (31.4%), 70–79 years (20.66%) and 80+ years (14.88%). The COVID-19 age-specific death rates per 1,000 populations in 10-year age groups for the Wuhan outbreak were highest for individuals ages 80+ years (death rates 0.05) and lowest for the age group between 20 and 29 years (death rates 0.0005), respectively. The COVID-19 age-specific CFR rates in 10-year age groups for the Wuhan outbreak were highest for individuals ages 80+ years (CFR 21.4%) and lowest for individuals ages 20–29 years (CFR 0.1%), respectively (Table 4; Figure 11).

During the Beta outbreak, a total of 1,132 deaths were reported, with the highest and lowest deaths noted in the age group 60–69 years ($n = 327$) and 10–19 years ($n = 5$), respectively. The proportion of COVID-19 deaths by 10-year age group for the Beta outbreak are as follows: 0–9 years (0.62%), 10–19 years (0.44%), 20–29 years (1.50%), 30–39 years (3.53%), 40–49 years (8.22%), 50–59 years (16.87%), 60–69 years (28.89%), 70–79 years (22.61%) and 80+ years (17.31%). The COVID-19 age-specific death rates per 1,000 populations in 10-year age groups for the Beta outbreak were highest and lowest for individuals ages 80+ years (death rates 0.52) and 0–9 years and

TABLE 4 Age distribution of COVID-19 cases and deaths by COVID-19 variants outbreaks in Malaysia.

Age group	Wuhan					Beta					Delta					Omicron					Overall (Ep Wk 4/2020 to Ep Wk 18/2022)				
	Cases	*IR	Death	**DR	CFR	Cases	*IR	Death	**DR	CFR	Cases	*IR	Death	**DR	CFR	Cases	*IR	Death	**DR	CFR	Cases	*IR	Death	**DR	CFR
0-9	259 (3.35)	0.05	0 (0.00)	0.00	-	19,613 (6.23)	3.86	7 (0.62)	0.00	0.0	292,456 (12.12)	58.08	64 (0.21)	0.01	0.0	179,844 (10.99)	35.71	36 (0.95)	0.01	0.0	492,225 (11.26)	97.75	107 (0.30)	0.02	0.0
10–19	844 (10.93)	0.16	0 (0.00)	0.00	-	24,687 (7.84)	4.63	5 (0.44)	0.00	0.0	300,988 (12.48)	57.34	75 (0.25)	0.01	0.0	186,291 (11.39)	35.49	26 (0.69)	0.00	0.0	512,881 (11.73)	97.70	106 (0.30)	0.02	0.0
20–29	2,138 (27.69)	0.34	3 (2.48)	0.00	0.1	96,346 (30.59)	15.16	17 (1.50)	0.00	0.0	548,620 (22.74)	87.31	686 (2.25)	0.11	0.1	379,988 (23.23)	60.47	47 (1.25)	0.01	0.0	1,027,286 (23.50)	163.48	753 (2.12)	0.12	0.1
30–39	1,523 (19.73)	0.28	9 (7.44)	0.00	0.6	86,013 (27.31)	15.84	40 (3.53)	0.01	0.0	503,471 (20.87)	91.97	2,260 (7.40)	0.41	0.4	378,908 (23.16)	69.21	129 (3.42)	0.02	0.0	970,141 (22.19)	177.21	2,438 (6.85)	0.45	0.3
40–49	943 (12.21)	0.25	9 (7.44)	0.00	1.0	42,833 (13.60)	11.32	93 (8.22)	0.02	0.2	312,325 (12.95)	81.29	4,244 (13.89)	1.10	1.4	220,714 (13.49)	57.45	269 (7.14)	0.07	0.1	576,963 (13.20)	150.18	4,615 (12.97)	1.20	0.8
50–59	1,004 (13.00)	0.32	19 (15.70)	0.01	1.9	25,406 (8.07)	8.20	191 (16.87)	0.06	0.8	223,100 (9.25)	71.47	5,884 (19.26)	1.88	2.6	143,069 (8.74)	45.83	427 (11.33)	0.14	0.3	392,673 (8.98)	125.79	6,521 (18.33)	2.09	1.7
60–69	715 (9.26)	0.34	38 (31.40)	0.02	5.3	13,585 (4.31)	6.44	327 (28.89)	0.16	2.4	147,598 (6.12)	67.69	7,153 (23.41)	3.28	4.8	90,698 (5.54)	41.60	737 (19.55)	0.34	0.8	252,654 (5.78)	115.88	8,258 (23.21)	3.79	3.3
70–79	211 (2.73)	0.21	25 (20.66)	0.02	11.8	4,815 (1.53)	4.75	256 (22.61)	0.25	5.3	61,818 (2.56)	57.74	5,842 (19.12)	5.46	9.5	40,528 (2.48)	37.85	952 (25.25)	0.89	2.3	107,401 (2.46)	100.31	7,079 (19.90)	6.61	6.6
80+	84 (1.09)	0.22	18 (14.88)	0.05	21.4	1,708 (0.54)	4.51	196 (17.31)	0.52	11.5	22,051 (0.91)	55.54	4,341 (14.21)	10.93	19.7	15,994 (0.98)	40.29	1,147 (30.42)	2.89	7.2	39,852 (0.91)	100.38	5,702 (16.03)	14.36	14.3
Total	7,721	2.17	121	0.10		315,006	74.71	1,132	1.02		2,412,427	628.42	30,549	23.21		1,636,034	423.90	3,770	4.37		4,372,076	1,128.67	35,579	28.66	

IR = age-specific incidence rate per 1,000 populations. DR = age-specific deaths per 1,000 populations. Denominators for cases and deaths population estimates sourced from Department of Statistics Malaysia website (http://pqi.stats.gov.my). *Incidence of COVID-19 in 2020–2021 per 1,000 population [(Total 2020 and 2021 cases)/(Total populations of 2020 and 2021)*1,000]. **DR = Deaths of COVID-19 in 2020–2021 per 1,000 population. Values for cases and death are number and %. Values for CFR are %. Missing age data for cases and deaths in Wuhan strain, $n = 772$. Missing age data for cases and deaths in Beta strain, $n = 18,482$. Missing age data for cases and deaths in Delta strain, $n = 55,377$. Missing age data for cases and deaths in Omicron strain, $n = 10,013$. Missing age data for cases and deaths in Overall cases, $n = 84,660$.

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10–19 years (death rates 0.001), respectively. The COVID-19 age-specific CFR rates in 10-year age groups for Beta outbreak were highest for individuals ages 80+ years (CFR 11.5%) and lowest for individuals ages 0–39 years (CFR 0.1%), respectively (Table 4; Figure 11).

During the Delta outbreak, 30,549 deaths were reported, with the highest and lowest deaths in the age group of 60–69 years ($n = 7,153$) and 0–9 years ($n = 64$), respectively. The proportion of COVID-19 deaths by 10-year age group for the Delta outbreak are as follows: 0–9 years (0.21%), 10–19 years (0.25%), 20–29 years (2.25%), 30–39 years (7.40%), 40–49 years (13.89%), 50–59 years (19.26%), 60–69 years (23.41%), 70–79 years (19.12%) and 80+ years (14.21%). The COVID-19 age-specific death rates per 1,000 populations in 10-year age groups for the Delta outbreak were highest for individuals ages 80 + years (death rates 10.93) and lowest for individuals aged 0–9 years and 10–19 years (death rates 0.01), respectively. The COVID-19 age-specific CFR rates in 10-year age groups for the Delta outbreak were highest for individuals ages 80+ years (CFR 19.7%) and lowest for individuals ages 0–9 and 10–19 years (CFR 0.0%) respectively (Table 4; Figure 11).

Of the 3,770 deaths reported during the Omicron outbreak, the age group with the highest number of deaths was 80+ years ($n = 1,147$), and the lowest was 10–19 years ($n = 26$), respectively. The proportion of COVID-19 deaths by 10-year age group for the Omicron outbreak are as follows: 0–9 years (0.95%), 10–19 years (0.69%), 20–29 years (1.25%), 30–39 years (3.42%), 40–49 years (7.14%), 50–59 years (11.33%), 60–69 years (19.55%), 70–79 years (25.25%) and 80+ years (30.42%). The COVID-19 age-specific death rates per 1,000 populations in 10-year age groups for the Omicron outbreak were highest for individuals aged 80+ years (death rates 2.89) and lowest for individuals aged 10–19 years (death rates 0.0), respectively. The COVID-19 age-specific CFR rates in 10-year age groups for Omicron outbreak were highest for individuals ages 80+ years (CFR 7.2%) and lowest for individuals ages 0–39 years (CFR 0.0%), respectively (Table 4; Figure 11).

Across all the outbreaks, the highest and lowest number of COVID-19 deaths by age was reported among individuals in the age groups of 60–69 years and 0–19 years, respectively, except for the Omicron outbreak, which reported the highest number of COVID-19 death by age among individual age group 80 and above. The number of COVID-19 deaths by age starts to reduce among individuals ages 70 and above across all the outbreaks (Figure 9) except during the Omicron outbreak, in which COVID-19 deaths tend to increase with advancing age. Across all the outbreaks, the highest COVID-19 age-specific death rate was reported among individuals in the age groups of 80 and above years for all the outbreaks. The lowest COVID-19 age-specific death rate was reported among individuals in the age groups 10–19 years for all outbreaks except for the Wuhan outbreak, which reported the lowest COVID-19 age-specific death rate among individuals in the age group of 20–29 years (Figure 9). Similarly, the highest age-specific CFR was reported among individuals aged 80 years and above across all the outbreaks.

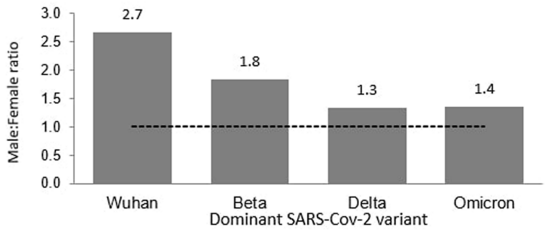

FIGURE 8
Male and female ratio of COVID-19 death Malaysia.

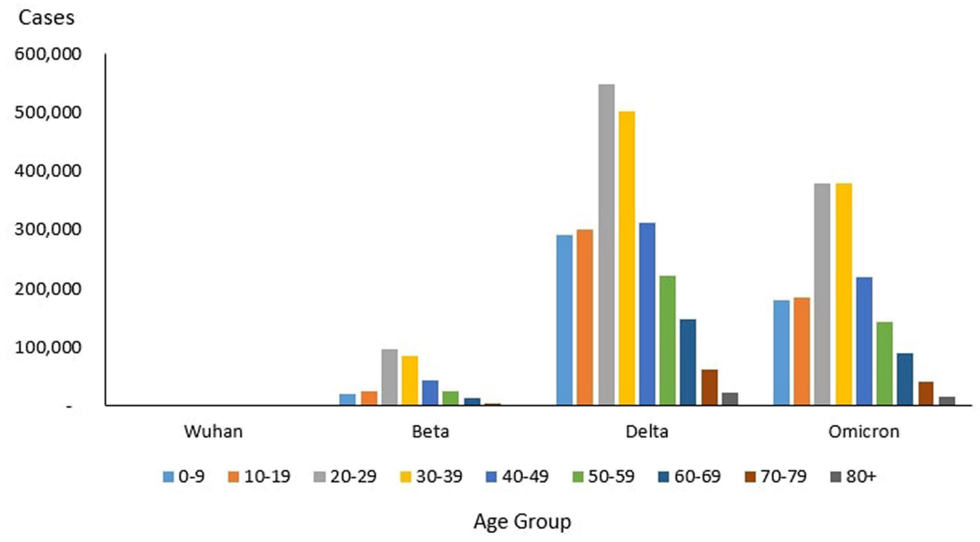

FIGURE 9
Number of COVID-19 cases by age group, Malaysia 2020-2022.

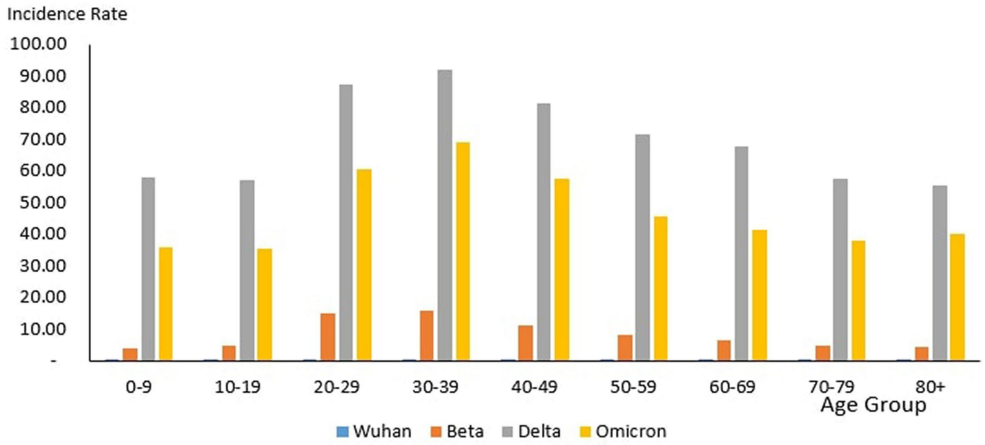

FIGURE 10
Age-specific COVID-19 incidence rate (per 1,000 population), Malaysia 2020-2022.

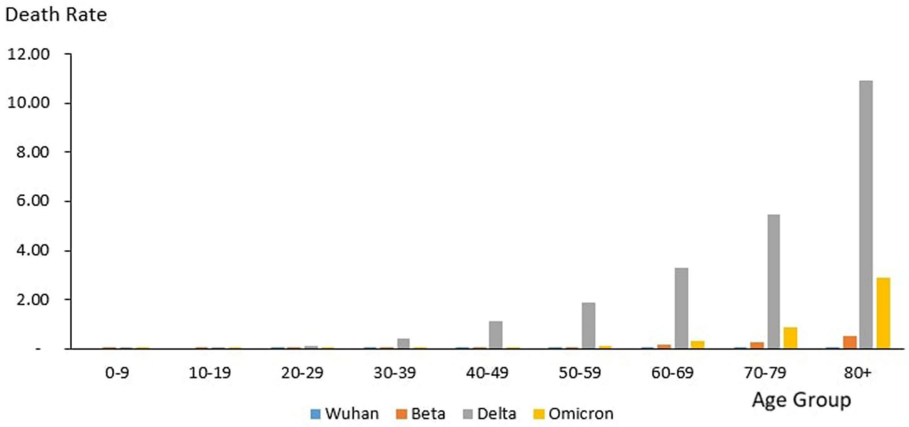

FIGURE 11
Age-specific COVID-19 death rate (per 1,000 population), Malaysia 2020-2022.

Testing and test positivity rate

Overall

A total of 58,906,954 COVID-19 tests were conducted during the study period in which the highest weekly COVID-19 total test (RT-PCR and RTK-AG) and the total test positivity rate (RT PCR and RTK Ag) was reported at 1,788,410 tests (Ep Wk7/2022) and 7.57%, respectively, (Table 2).

Outbreaks

During the Wuhan outbreak, a total of 853,379 (1.5%) tests were done, wherein the highest ($n = 86,018$) and lowest ($n = 586$) weekly COVID-19 total test was reported in Ep Wk18/2020 and Ep Wk 9, respectively. The test positivity rate was reported at 1.0% during this period. As for the Beta outbreak, a total of 8,781,144 (14.91%) tests were done where the highest ($n = 629,225$) and lowest ($n = 51,673$) weekly COVID-19 total test were reported in Ep Wk 3/2021 and Ep Wk 37/2020, respectively. The test positivity rate was reported at 3.8% during this period.

In the time of the Delta outbreak, a total of 33,603,291(57.04%) tests were done where the highest ($n = 1,119,105$) and lowest ($n = 231,648$) weekly COVID-19 total tests were reported in Ep Wk 33/2021 and Ep Wk 3/2022, respectively. The test positivity rate was reported at 7.34% during this period. During the Omicron outbreak, a total of 15,157,523 (25.73%) tests were done where the highest ($n = 1,788,410$) and lowest ($n = 221,636$) weekly COVID-19 total tests were reported in Ep Wk 7/2022 and Ep Wk 18/2022, respectively. The test positivity rate was reported at 10.86%, as shown in Table 2. The Wuhan, Beta, Delta, and Omicron outbreak test positivity rates were 0.995, 3.798, 7.344, and 10.860, respectively.

Trends

During the Wuhan outbreak, the number of tests increased from Ep Wk 10 ($n = 3,904$) and peaked in Ep Wk 18 ($n = 86,018$). Subsequently, the number of tests decreased from Ep Wk 19

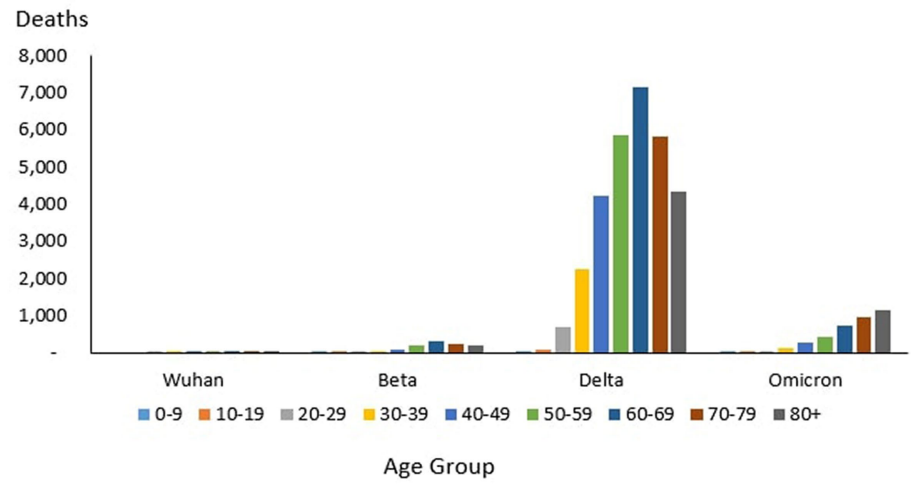

FIGURE 12
Age-specific COVID-19 death, Malaysia 2020-2022.

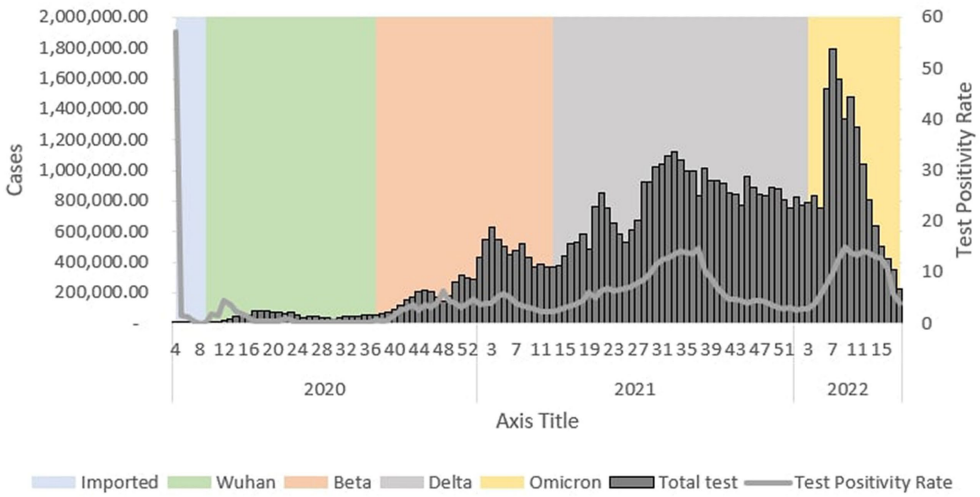

FIGURE 13
Weekly total COVID-19 tests and test positivity rate by outbreaks in Malaysia, 2020-2022.

($n = 82,072$) to Ep Wk 25 ($n = 40,609$). Following this, the Beta outbreak began, where testing trends started to increase from Ep Wk 38/2020 ($n = 66,922$) and peaked in Ep Wk 3/2021 ($n = 629,225$). Subsequently, a downward testing trend has been observed from Ep Wk 4/2021 ($n = 544,745$) till Ep Wk 13/2021 ($n = 369,701$). This was followed by the Delta outbreak, wherein the testing trends started to increase from Ep Wk 14/2021 ($n = 382,171$) and peaked in Ep Wk 33/2021 ($n = 1,119,015$), which corresponded to the longest increase of testing trends of 33 weeks. A downward trend of testing has been observed from Ep Wk 34/2021 ($n = 1,071,056$) till Ep Wk 3/2022 ($n = 776,217$), corresponding to the longest decrease in the testing trend of 23 weeks. Following this, the Omicron outbreak began where a testing trend started to increase from Ep Wk 4/2022 ($n = 831,806$) and peaked at Ep Wk 7/2022 ($n = 1,788,410$), which was followed by a downward trend of testing from Ep Wk 8/2022 ($n = 1,596,093$) to Ep Wk 18/2022 ($n = 221,636$) as shown in Figure 13.

Discussion

This study described the COVID-19 case demographics, hospital admissions and testing capacities for the four outbreaks, namely the Wuhan, Beta, Delta and Omicron, from the beginning of the outbreak in January 2020 till it was declared endemic in April 2022. A total of 4,456,736 cases was analyzed in this study, which showed that the Delta and Omicron outbreaks reported the highest numbers of COVID-19 cases, which was also found in studies in India and Bangladesh (21–25). These findings can be explained by the increased transmissibility in the Delta and Omicron variants of the COVID-19 virus. Moreover, studies have reported that the Delta variant is 50% more transmissible than the Alpha, Beta or Wuhan variants (26). In addition, a higher number of infections that occurred during the Delta and Omicron outbreaks can be attributed to the longer outbreak duration of these variants. Furthermore, the easing of movement

control measures resulted in increased travel and population mobility during the Delta and Omicron outbreaks, which allowed for further spread of the disease. Finally, increased testing capacity during the Delta and Omicron outbreaks led to higher case detection, resulting in increased case numbers. This higher transmissibility, longer outbreak period and increased population mobility and testing contributed to conditions that caused the massive spread of the disease, causing extreme suffering of the populations as well as overwhelming the healthcare systems (27). The presence of highly transmissible variants would require an urgent need for the health systems to closely monitor the outbreak and promptly institute outbreak control measures (28).

This study also found higher COVID-19 incidence among males aged between 20 and 39. This finding can be explained by individuals in this age group being more socially active, therefore having an increased risk of being infected. In addition, differences in behavior and immunological factors may also explain the increased prevalence of the disease in this population (29). This could be attributed to high-risk behaviors such as smoking and alcohol consumption among males, resulting in more severe forms of COVID-19 disease (30). Studies conducted by the Center for Disease Control (CDC) in the United States also showed similar findings affecting males in this age group to have higher infection rates and deaths (30–32).

This study showed that the highest number of patients requiring ICU admission, ventilator support, and deaths occurred during the Delta outbreak as compared to the other outbreaks. The increased morbidity and mortality of the Delta variant can be attributed to higher virulence, resulting in more severe forms of the disease (33). In addition, the longer duration of the Delta outbreak, causing a prolonged, sustained combination of high case numbers with more severe forms of the disease, would overwhelm the healthcare system, limiting the availability of the healthcare system to provide patient care, which would ultimately increase case mortality (33–35). The increased morbidity and mortality of the Delta variant were also observed in a meta-analysis involving 16 countries worldwide, including studies in the US, UK and India, where this finding was attributed to the variant's higher disease severity and transmissibility (36–38).

The CFR during the Delta outbreak was reported at 1.24, which corresponded to the second-highest CFR during the study period. While the highest CFR was reported during the Wuhan outbreak at 1.42, the high CFR during the Wuhan outbreak is an effect of limited testing with a low detection rate, which biased the high observed CFR during this period. Interestingly, a reduction in trends of COVID-19 patients requiring ICU admission, ventilator support and mortality was observed as the Delta outbreak progressed. This can be attributed to the vaccination programs that had resulted in 70% of the population completing two doses of COVID-19 vaccination as of mid-2022 in Malaysia (39, 40).

A cumulative of 58,906,954 COVID-19 tests were conducted during the pandemic period, and it was observed that the test positivity rate has been increasing since the beginning of the outbreak in Malaysia. The test positivity rate for the different outbreaks was reported for the Wuhan (1.0), Beta (3.8), Delta (7.34), and Omicron (10.86) outbreaks, respectively. In addition, this study found that the highest number of COVID-19 tests was reported during the Delta outbreak in Malaysia. The reason could be due to the longer duration of the Delta outbreak ($n = 43$ weeks) compared to other outbreaks and the increased transmissibility of the virus. The longer outbreak duration allows more testing to be conducted due to increased testing capacity during the Delta outbreak (41–44) Furthermore, there was an increased requirement for COVID-19 testing for certain socio-economic activities such as return to work, travel and pre-operative procedures during the Delta outbreak and with the introduction of simple self-testing via the rapid antigen test made accessible to the public (45–47). The weekly COVID-19 test requirement since August 2021 by the health authorities for back-to-work employees to prevent the spread of COVID-19 has also greatly contributed to the increase in testing done nationally (45–47).

Conclusion

The Delta outbreak was the most severe compared to other outbreaks during the study period in Malaysia. This is evident by the highest number of COVID-19 cases, ICU admissions, ventilatory requirements and deaths observed during the Delta outbreak. In addition, this study provides evidence that outbreaks of COVID-19, which are caused by highly virulent and transmissible variants, tend to be more severe and devastating if they are not controlled early on. This was the first attempt to describe the COVID-19 outbreak in detail. Hence, close monitoring of key epidemiological indicators, as reported in this study, is essential in the control and management of future COVID-19 outbreaks in Malaysia.

Data availability statement

The raw data that supports the findings of this study are available on request from the corresponding author.

Ethics statement

The study was registered with the National Medical Research Register (NMRR ID-22-00940-PF6).

Author contributions

MM: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization. AM: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. SS: Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Data curation, Conceptualization. MS: Writing – review & editing, Project administration, Methodology, Investigation, Formal analysis, Conceptualization. CL: Writing – review & editing, Visualization, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation. CT: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis, Data curation. TA: Writing – review & editing, Visualization,

Supervision, Project administration. HM: Writing – review & editing, Validation, Supervision, Methodology, Investigation. BS: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology. N'AM: Writing – review & editing, Writing – original draft, Methodology, Investigation, Data curation, Conceptualization. NM: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Methodology, Investigation. ML: Writing – review & editing, Methodology, Investigation. LA: Writing – review & editing, Methodology, Investigation. MK: Writing – review & editing, Visualization, Methodology, Investigation, Formal analysis. NA: Writing – review & editing, Methodology, Investigation, Data curation. KT: Writing – review & editing, Visualization, Validation, Supervision, Methodology, Investigation. AZ: Writing – review & editing, Writing – original draft, Software, Project administration, Methodology, Investigation.

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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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Patterns and profiles of drug resistance-conferring mutations in *Mycobacterium tuberculosis* genotypes isolated from tuberculosis-suspected attendees of spiritual holy water sites in Northwest Ethiopia

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Purpose: This study examined the patterns and frequency of genetic changes responsible for resistance to first-line (rifampicin and isoniazid), fluoroquinolones, and second-line injectable drugs in drug-resistant *Mycobacterium tuberculosis* (MTB) isolated from culture-positive pulmonary tuberculosis (PTB) symptomatic attendees of spiritual holy water sites (HWSs) in the Amhara region.

Patients and methods: From June 2019 to March 2020, a cross-sectional study was carried out. A total of 122 culture-positive MTB isolates from PTB-suspected attendees of HWSs in the Amhara region were evaluated for their drug resistance profiles, and characterized gene mutations conferring resistance to rifampicin (RIF), isoniazid (INH), fluoroquinolones (FLQs), and second-line injectable drugs (SLIDs) using GenoType®MTBDR*plus* VER2.0 and GenoType®MTBDR*sl* VER2.0. Drug-resistant MTB isolates were Spoligotyped following the manufacturer's protocol.

Results: Genetic changes (mutations) responsible for resistance to RIF, INH, and FLQs were identified in 15/122 (12.3%), 20/122 (16.4%), and 5/20 (25%) of MTB isolates, respectively. In RIF-resistant, *rpo*B/Ser**531**Lue ($n = 12$, 80%) was most frequent followed by His**526**Tyr (6.7%). Amongst INH-resistant isolates, *kat*G/Ser**315**Thr1 ($n = 19$, 95%) was the most frequent. Of 15 MDR-TB, the majority ($n = 12$, 80%) isolates had mutations at both *rpo*B/Ser**531**Leu and *kat*G/Ser**315**Thr1. All 20 INH and/or RIF-resistant isolates were tested with the MTBDR*sl* VER 2.0, yielding 5 FLQs-resistant isolates with gene mutations at *rpo*B/Ser**531**Lue, *kat*G/Ser**315**Thr1, and *gyr*A/Asp**94**Ala genes. Of 20 Spoligotyped drug-resistant MTB isolates, the majority ($n = 11$, 55%) and 6 (30%) were SIT149/T3-ETH and SIT21/CAS1-Kili sublineages, respectively; and they were any INH-resistant (mono-hetero/multi-). Of 15 RIF-resistant (RR/MDR-TB) isolates, 7 were SIT149/T3-ETH, while 6 were SIT21/CAS1-Kili sublineages. FLQ resistance was detected in four SIT21/CAS1-Kili lineages.

Conclusion: In the current study, the most common gene mutations responsible for resistance to INH, RIF, and FLQs were identified. SIT149/T3-ETH and SIT21/

CAS1-Kili constitute the majority of drug-resistant TB (DR-TB) isolates. To further understand the complete spectrum of genetic changes/mutations and related genotypes, a sequencing technology is warranted.

KEYWORDS

drug-resistant TB, rpoB, katG, gyrA, mutations, spoligotypes, pulmonary tuberculosis, spiritual holy water site attendees

Background

Drug-resistant *Mycobacterium tuberculosis* (MTB) is an escalating health problem that presents substantial challenges for public health systems across the world (1–3). The advent and transmission of drug-resistant MTB strains, especially multidrug-resistant/RIF-resistant TB (MDR/RR-TB)-which is resistant to the two key anti-mycobacterial drugs, INH and RIF- and "extensively drug-resistant TB (XDR-TB)"-which is an MDR/RR isolate that is also resistant to any FLQs and bedaquiline and/or linezolid-has made it more challenging to contain and eradicate the disease globally (1–4). It makes TB control programs less effective since resistant TB strains require specialized laboratory facilities, diagnostic tools with higher accuracy, and treatment regimens that aren't usually available in resource-constrained settings (1, 2). Globally, 10.6 million people contracted TB, and about "410,000 developed MDR or RIF-resistant TB" (5). The prevalence of DR-TB varies by geography, with India, China, and the Russian Federation having the largest burden (1). Ethiopia bears a substantial burden of TB, and the rising incidence of DR-TB has detrimental effects on the country's health systems and efforts to contain the disease (1, 6–8). Moreover, although Ethiopia is on track to transition out of the thirty nations with high rates of MDR/RR-TB (1), the burden of resistant TB is still a problem, where 0.71% of new TB cases and 12.0% of retreated TB patients were reported to have MDR-TB (9).

Drug-resistant TB strain has a severe impact on both individual and societal levels, with resistant TB being associated with higher patient mortality rates than susceptible TB strains (1, 3, 10). The treatment outcomes for DR-TB are often unfavorable due to the limited accessibility and effectiveness of second-line anti-TB medicines, lengthy treatment durations, and higher toxicity of the drugs (2, 10). Thus, patients experience prolonged suffering and there is an increasing risk of transmitting the disease to others (3, 11). Accordingly, DR-TB necessitates an accurate and timely diagnosis for the effective management of TB patients (3).

Molecular diagnostic methods have transformed the detection and identification of drug-resistance-associated genetic changes in MTB in a rapid and precise manner (2, 12), providing accurate evidence for patient-centered treatment decisions (13). The application of "PCR-based tools, like the GeneXpert®MTB/RIF (Cepheid Inc., Sunnyvale, CA, USA)," has significantly transformed the process of detecting TB and drug-resistant TB and enhanced the treatment of TB patients (14). "Line Probe Assays (LPAs) (Hain Lifescience, Germany)" are another widely used tool for the simultaneous diagnosis of TB and the anti-mycobacterial resistance profile of TB strains (14, 15). These assays target specific genetic regions linked with resistance to INH and RIF, and FLQs and SLIDs, respectively. Although LPAs have certain limitations that only detect the gene mutations within the target of interest (15), "MTBDR*plus* VER 2.0" (16), and "MTBDR*sl* VER 2.0" (17), have higher sensitivity and specificity than the first version to diagnose anti-TB drug resistance, and associated gene mutations (16, 17). Ethiopia is one of the nations that has benefited from the use of LPA diagnostic technologies to improve the management of TB patients. LPAs ("MTBDR*plus* and MTBDR*sl*") tests were integrated into the Ethiopian national TB diagnostic algorithm (18, 19). Since the rollout of LPAs in Ethiopia, TB diagnostic referral centers at the national and regional levels have been authorized to undertake those assays (19).

Genetic mutations play a crucial role in conferring anti-TB drug resistance in MTB (2). The presence of those genetic changes conferring resistance to key anti-mycobacterial drugs differs geographically and is influenced by factors like the prevalence of DR-TB strains, treatment regimens, and patient adherence to therapy (20). Thus, understanding the types and frequency of those genetic changes is critical for effective drug-resistant strain detection, guiding treatment strategies, and monitoring the emergence of new resistance patterns (20, 21).

Furthermore, it is paramount to identify populations at high risk for TB transmission and conduct TB case finding in hotspot settings (22, 23). The Ethiopian National TB Control Program identified prisoners, HIV-positive people, diabetic patients, the older adult, University residents, TB patients' contacts, the homeless, healthcare workers, and refugees as key high-risk groups for TB (22); thereby earlier studies reported the burden of TB in prison settings (24, 25), refugees (26), homeless (27, 28), and University residents (29), in Ethiopia. Although Ethiopia has identified key populations for TB and implemented WHO's policy on TB infection control in high-risk settings, targeting spiritual holy water sites as high-priority settings for TB control activities is not given much attention (30). The first national TB prevalence survey (6), and an earlier community-based TB study among key populations in hotspot settings in Ethiopia have been conducted (31). However, these studies excluded high-risk places such as holy water sites and other congregate settings, which can be potential hotspots for TB transmission. The current study was guided or aimed to answer the question, "What is the prevalence of TB, drug-resistant rate, the genotype distribution of resistant MTB isolates, and the frequency of gene mutations responsible for resistance for first and second-line anti-TB drugs among PTB suspects attending holy water site in the Amhara region?" As a baseline information, an earlier study on the prevalence of smear-positive TB among holy water site attendees was conducted in the Amhara region and found that the prevalence of TB was 7.4-fold higher in those study cohorts than in the general population in Ethiopia (32). However, this study was geographically limited and used acid-fast bacilli (AFB) smear microscopy to detect TB suspects, which has low sensitivity, thus the findings may not reflect the true TB burden among holy water site attendees in the region (32).

Spiritual holy water sites in Ethiopia are congregate settings where many people, particularly Orthodox Christian followers, come to receive holy water treatment blessed by priests for several kinds of illnesses, including TB and other respiratory diseases (32). Although studies showed that TB patients seek treatment from holy water sites, the prevalence of TB among individuals attending those settings in Ethiopia has not been thoroughly investigated. There is also scarce information on the genetic diversity of drug-resistant MTB genotypes and the types and frequency of genetic changes conferring resistance to commonly prescribed anti-TB drugs among TB isolates from PTB-symptomatic attendees of holy water sites in Ethiopia. Therefore, this study aimed to evaluate drug susceptibility profiles of TB isolates and characterize the types and frequency of mutations responsible for resistance to INH, RIF, FLQs, and SLIDs in MTB isolates, and to Spoligotype those isolates from culture-positive PTB-symptomatic attendees of holy water sites in the Amhara region, Ethiopia.

Methods

Setting and study period

A cross-sectional study was performed from June 2019 to March 2020 at nine purposively selected holy water sites found across nine administrative zones in the Amhara region. The Amhara region is found in Northwestern Ethiopia, which comprises thirteen zones and three administrative towns. Bahir Dar is the regional capital, which is 567 km far from the national capital, Addis Ababa. One holy water site was chosen from each administrative zone based on its consistent popularity for spiritual holy water treatment, its ability to accommodate a large number of attendees, and where many people visit and reside for an extended time (32). According to the criteria mentioned above, the selected holy water site from each study zone is deemed to be representative (Supplementary Table S1).

Study participants

For this study, the source population was all individuals who attended holy water sites during the data collection time. However, attendees who had PTB suggestive symptoms (33), particularly cough ≥2 weeks and having productive cough, and other symptoms such as night sweating, fatigue, fever, loss of appetite, shortness of breath, chest pain, unexplained weight loss, had contact history with active TB patients, and previous TB disease were recruited and included as the sample population. During the course of the study, at nine selected holy water sites, a total of 10,313 attendees (≥18 years of age) were screened, and 560 individuals with symptoms of PTB participated. Supplementary Table S1 illustrates the study settings, total number of attendees screened for PTB-suggestive symptoms, number of PTB suspected cases, and bacteriologically confirmed TB cases.

Eligibility criteria

Attendees (≥18 years of age) who had coughs ≥2 weeks plus the above-mentioned PTB suggestive symptoms were included, whereas individuals who were under 18 years and those who were seriously ill to provided sputum sample and necessary information were excluded. Furthermore, attendees who were on TB medications during the sample collection time were excluded.

Sociodemographic data collection

After obtaining participants' informed consent to be included in the study, their sociodemographic data including sex, age, residence, marital status, educational level, family size per household, occupation, and study area were recorded using an interviewer-administered questionnaire. We followed the national TB screening guideline during the screening of PTB suggestive symptoms (33).

Sputum specimen collection and *Mycobacterium tuberculosis* complex isolates

Using a leak-proof, sterile screw-capped falcon tube (50 mL capacity), the sputum samples were received from PTB-symptomatic attendees. We used an ice-pack carrier to transport the sputum specimens to the regional research laboratory center, "Amhara Public Health Institute, Bahir-Dar, Ethiopia." We followed the standard procedures during the mycobacterial culturing steps. In brief, the "N-acetyl-L-cysteine (NALC-NaOH)" solution was utilized to decontaminate the sputum, following the neutralization process using a phosphate buffer solution (pH 6.8). The mixture was centrifuged to prepare the inoculums for culture. Then after, the inoculum/sediment from the bottom of the tube was taken and inoculated into Löwenstein-Jensen (LJ) slant culture tubes, and incubated at a temperature of 37°C for at least 8 weeks. The growth of MTB colonies on LJ culture media was inspected weekly for up to 8 weeks, and the growth was confirmed by Ziehl-Neelsen (ZN) smear staining" (34). "Capilia TB-Neo (Tauns laboratories, Japan)" was utilized to differentiate MTB complex species (35). In each step of the test, a known H37Rv strain and sterile molecular-grade water were utilized as positive and negative controls, respectively.

Specimen preparation from MTB colonies grown on LJ culture media

From MTB colonies grown from LJ culture medium, suspensions were prepared and transferred into a transport media, 1.5 mL of "PrimeStore Molecular Transport Medium (PS-MTM; Longhorn Vaccine & Diagnostics, San Antonio, TX, USA)." The MTB suspension preparations from a positive LJ culture medium depend on the culture state (36). In summary, for intact slopes, using a sterile inoculation loop, MTB colonies were carefully scraped off and washed down (suspended) in 1 mL sterile molecular grading water in the original slant culture bottle. After pipetting off the prepared suspension, it was transferred into a 1.5 mL Eppendorf tube. The Eppendorf tubes holding suspensions were centrifuged at 13,000 g for 5 min, the supernatant was discarded (36), and the deposit was then transferred into the PS-MTM. The PrimeStore tubes holding the MTB suspension were shipped non-refrigerated to South Africa by air, where DNA

extraction, genotyping drug susceptibility testing (gDST), and Spoligotyping procedures were performed.

Mycobacterium tuberculosis genomic DNA extraction

From all LJ culture-positive isolates, the "MTB genomic DNA was extracted using the PrimeXtract™ kit (Longhorn Vaccines and Diagnostics, San Antonio, TX, USA)" following the manufacturer's protocol. In summary, MTB inoculum (200 µL), 100% ethanol (200 µL), and lysis buffer (200 µL) were transferred into a 1.5 mL microcentrifuge tube, then thorough vortexing and subsequent centrifugation was performed. Using a micro-extraction column, the entire supernatant was transferred, following centrifugation at 13,000 rpm for 1 min, and then the follow-through material was discarded. After adding wash buffer 1 (500 µL) to the extraction column, it was then centrifuged at 13,000 rpm for 1 min, followed by further addition of wash buffer 2 (500 µL) to the extraction columns, and subsequently centrifuged as described above, and the follow-through material was discarded. The extracted total MTB genomic DNA was eluted by centrifugation at 13,000 rpm for 1 min using preheated (60–70°C) 50 µL elution solution. Then, the extracted total DNA was stored at −20°C fridge for future use. Genomic MTB DNA concentration and quality were measured using a spectrophotometer at optimal densities of 269 and 280 nm.

Drug susceptibility testing

The genotype DST (gDST), MTBDR*plus* VER2.0 ("Hain Lifescience, Germany") was performed on 122 culture-positive MTB isolates (16). MTB isolates that were found RIF and/or INH-resistant were subjected to MTBDR*sl* VER2.0 to detect FLQs and second-line injectable anti-mycobacterial drug resistance (17). The entire techniques that included the preparation of the master mix, polymerase chain reaction (PCR) amplification, and hybridization were done following the manufacturer's protocol ("Hain Lifescience, Germany") (16, 17).

Interpretation of results

The MTBDR*plus* assay can detect the presence and absence of wild-type (WT) and mutant (MUT) DNA sequences (bands) within particular regions of the three resistance-conferring genes: the *rpo*B gene, which encodes the "β-subunit of the RNA polymerase," was used to determine RIF-resistance; whereas the *kat*G gene, which encodes the "catalase-peroxidase," was used to detect high-level INH resistance; and the *inh*A promoter region (encodes enoyl ACP NADH reductase), was used to identify low-level INH resistance. Rifampicin resistance was demonstrated by the absence of bands in the *rpo*B probes, whereas resistance to INH was demonstrated by the absence of *kat*G and *inh*A bands. On the other hand, when the mutant probes' bands are as strong as or stronger than the existing amplification control (AC) bands, they are considered resistant. In most cases, the absence of the WT band is associated with the presence of a MUT band, indicating resistance. Rarely, the absence of WT band (s) without the presence of a corresponding MUT band could be observed, which was attributed to "unknown" genetic changes in the probe region or mutations that exist outside of the drug-resistance-determining regions, which the assay cannot detect. The coexistence of WT and MUT bands within a single stripe may indicate the existence of hetero-resistance or mixed TB strain infection. In general, resistance was recorded where one or more WT bands were absent or where one or more WTs were missing with a corresponding mutation.

The MTBDR*sl* VER2.0 test determined the presence and absence of WT and MUT DNA sequences within a particular region of four genes: *gyr*A, which encodes the "A-subunit protein of DNA gyrase; *gyr*B (it encodes β -subunit protein of DNA gyrase)" were utilized to determine resistance to FLQs; and the *rrs* (encodes 16S rRNA) was utilized for the detection of cross-resistance to Kanamycin (KAM) and Amikacin (AMK), and Capreomycin (CPM) and Viomycin (VIO), whereas the *eis*, which encodes (aminoglycoside acetyltransferase), was used to identify low-level resistance to KAM. In short, resistance was recorded where one or more WT probes were absent or where one or more WTs were missing with a corresponding mutation type. However, the probability of strains developing mutations outside of the test regions cannot be ruled out, since the assay cannot detect genetic changes that exist outside the regions of interest.

Quality control

In each of the LPAs tests (MTBDR*plus* VER2.0 and MTBDR*sl* VER2.0), sterile water was utilized as the negative control, while the universal reference H37Rv strain, which is sensitive to all anti-TB medicines, was utilized as a positive control.

Spoligotyping

All 20 drug-resistant MTB isolates were subjected to Spoligotyping "following the manufacturer's protocol" (37), and the Spoligotyping kit supplier's instructions ("Ocimum Biosolutions Company, IJsselstein, The Netherlands"). In brief, the MTB isolate's "direct repeat (DR) region was amplified by a thermal cycler PCR machine" ("VWR International, UK") utilizing the oligonucleotide primers derived from the MTB direct repeat region. A total volume of 25 µL constituting the PCR amplification reaction mixture of 12.5 µL of "HotStarTaq Master Mix (Qiagen, UK)," 2 µL of forward primer (DRa), and 2 µL of reverse primer (DRb), 5 µL of extracted DNA, and 3.5 µL sterile molecular grading water. Then, the mixture was subjected to heat at 96°C for 3 min, and then subjected to 30 cycles for 1 min at 96°C, 1 min at 55°C, 30 s at 72°C, and 5 min at 72°C for 1 cycle.

The amplified PCR product underwent hybridization with a series of 43 immobilized oligonucleotides that are covalently bound to a membrane ("Animal and Plant Health Agency, Great Britain"). Each of these oligonucleotides corresponds to a different spacer sequence of DNA located within the direct repeat (DR) locus. The membrane was washed twice for 10 min in 2XSSPE (pH 7.7)-0.5% sodium dodecyl sulfate at 60°C after hybridization, then incubated in 1:4000 diluted streptavidin-peroxidase ("HotStar, UK") for 45–60 min at 42°. The membrane was then washed twice for 10 min in 2X SSPE-0.5% sodium dodecyl sulfate at 42°C and rinsed for 5 min in 2XSSPE at room temperature. The detection of hybridized DNA was done using

the enhanced chemiluminescence method ("Amersham Biosciences, UK") and by exposing it to X-ray film ("Hyperfilm ECL, Amersham Biosciences, UK"), following the manufacturer's instruction. The presence or absence of spacers was visualized on X-ray film through the depiction of black and white squares, respectively. Reference strains, *M. bovis* BCG and MTB H37Rv used as positive controls, while sterile Qiagen water ("Qiagen Company, Germany") was utilized as negative control.

Statistical analysis

All the laboratory test data were first documented in a prepared "Microsoft Excel spreadsheet," and checked if errors existed during recording (cleared), properly coded, then entered into STATA 15 ("Stata Corp, College Station, TX, USA") for analysis. Descriptive statistical analysis, frequency, and percentage were used to summarize the key findings, and the Chi-square test's *p*-value was reported. Findings were presented in tables and figures. The "shared international spoligotypes (SIT) number and the corresponding lineages/sublineages were obtained using the open-source spoligotype database (SpolDB4)[1]" (38). MTB Strains that exhibited similarity to a pre-existing spoligotype pattern in the database were assigned an SIT number, while TB strains without SIT numbers were categorized as "new." MTB trains with the same spoligotype patterns was called a "cluster," while a single pattern was defined as "unique" to this study.

Research ethics

The study received ethical clearance from the Human Research Ethics Committee of the University of Pretoria, Faculty of Health Sciences, South Africa (Ref: No. 600/2018) and the National Research Ethics Review Committee, Ethiopia (Ref: No. SHE/SM/14.4/708/2019). Furthermore, a written official permission letter was obtained from the Ethiopian Orthodox Tewaheido Church (Ref: No.2478/6275/2011). A verbal and/or written consent declaration with full details about the study was given to participants and signed. Participants were assured that their data would remain confidential by using the non-personal identifier. Attendees who tested TB positive were linked with nearby healthcare facilities to commence the appropriate treatment and further management.

Results

Characteristics of study subjects

Five hundred and sixty PTB suspected cases participated in the study. Of these, 122 participants were bacteriologically confirmed (LJ culture-positive) (Supplementary Tables S1, S2). The median age of PTB suspected participants was 35 years, and male individuals constituted the majority (n = 308, 55.0%). One hundred twelve (20.0%), and 191 (31.1%) were previously treated and had a contact history with active TB patients, respectively (Supplementary Table S2). Of 122 bacteriologically confirmed TB cases, the majority (n = 33, 34.0%) and (n = 28, 26.7%) of the attendees were from the North Shewa and South Gondar zones, respectively. Over one-third (37.5 and 40.8%) of participants were previously treated and had contact history with active TB patients, respectively (Supplementary Tables S2, S3).

Drug susceptibility testing

In this study, we have done gDST using "MTBDR*plus* VER2.0" on 122TB isolates. Of which 20 (16.4%%), and 15 (12.3%) were resistant to INH and RIF, respectively. Of those resistant to INH, 15 (75%) were also resistant to RIF and 5 (25%) were INH-monoresistant, while 1 (5%) isolate was INH-hetero-resistant. Three-fourths of drug-resistant isolates (n = 15, 75%) were MDR-TB (both RIF and INH resistant). The majority (n = 12, 16.0%) of them were identified from participants aged between 18 and 33 years, and male accounts (n = 9, 13.4%). Furthermore, 8 (36.4%) of those isolates identified as MDR-TB were detected from the South Wollo zone study site (Table 1).

The MTBDR*sl* VER2.0 was done on 20 any INH-resistant (mono-hetero-multi/−) isolates and 15 RIF-resistant (mono-hetero-multi/−) MTB isolates. All of the isolates yielded interpretable results and were included in the study. Further resistance to FLQs was detected only in MDR-TB isolates. Accordingly, 25% (5/20) were identified as FLQs-resistant. Interestingly, all five (6.7%) FLQ-resistant isolates were detected from attendees aged between 18 and 33 years and from the South Wollo zone study area (Table 1).

Frequency of gene mutations conferring resistance to INH, RIF, and FLQs

Mutations in the RIF-resistance determining region (*rpoB*)

In RIF-resistant isolates, the gene mutations in the *rpo*B were most frequent at codon Ser**531**Lue (80%); the next mutation was seen in His**526**Tyr (6.7%). The remaining two RIF-resistant isolates (13.3%) had shown a mutation or missing wild-type band (probe absent), but no corresponding MUT band, and were classified as "unknown" mutations. Those were *rpo*BWT6 and *rpo*BWT7 (6.7% each). This could be due to the gene mutations occurring outside the analyzed codon regions which LPAs cannot detect (Table 2).

Gene mutations in the *katG* and *inhA*

Amongst INH-resistant strains (n = 20), mutations in the *kat*G (indicating high-level resistance) were most frequent at codon Ser315Thr1 (95%), while no *inh*A gene mutation was observed. From 20 isolates with INH resistance, 5 (25.0%) were detected as INH-hetero-resistance and had mutations at codon Ser315Thr1 (100%) (Table 2).

Of 20 any drug-resistant isolates, 15 (75%) were MDR-TB strains (both RIF and INH- resistant). Of the total 15 MDR-TB strains,

1 https://www.pasteur-guadeloupe.fr:8081/SITVITDemo/

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TABLE 1 Proportions of anti-TB drug resistance with different variables ($n = 122$).

Variables			Anti-TB drug resistance					
		# of LJ-positive cases (n)	INH, n (%)	Chi-square (p-value)	RIF/MDR*, n (%)	Chi-square (p-value)	FLQs, n (%)	Chi-square (p-value)
Sex	Male	67	14 (20.9)	0.138	9 (13.4)	0.673	2 (3.0)	0.494
	Female	55	6 (10.9)		6 (10.9)		3 (5.5)	
Age group (years)	18–33	75	17 (22.7)	0.016	12 (16.0)	0.052	5 (6.7)	0.195
	34–49	31	0 (0.0)		0 (0.0)		0 (0.0)	
	≥50	16	3 (18.8)		3 (18.8)		0 (0.0)	
Residence	Urban	46	10 (21.7)	0.215	7 (15.2)	0.444	2 (4.3)	0.914
	Rural	76	10 (13.2)		8 (10.5)		3 (3.9)	
Marital status	Married	85	11 (12.9)	0.118	7 (8.2)	0.038	2 (2.4)	0.141
	Single*	37	9 (24.3)		8 (21.6)		3 (8.1)	
Educational status	Cannot read & write	59	6 (10.2)	0.132	4 (6.8)	0.080	0 (0.0)	0.010
	Primary school	30	8 (26.7)		7 (23.3)		4 (13.3)	
	Secondary school & above	33	6 (18.2)		4 (12.1)		1 (3.0)	
Family size	1–5	57	13 (22.8)	0.073	9 (15.8)	0.271	3 (5.3)	0.543
	>5	65	7 (10.8)		6 (9.2)		2 (3.1)	
Study zone	North Wello	22	1 (4.5)	0.005	1 (4.5)	0.005	0 (0.0)	<0.001
	South Wello	22	9 (40.9)		8 (36.4)		5 (22.7)	
	North Shewa	33	3 (9.1)		3 (9.1)		0 (0.0)	
	South Gondar	28	6 (21.4)		2 (7.1)		0 (0.0)	
	Central Gondar & others**	17	1 (5.9)		1 (5.9)		0 (0.0)	
Types of PTB cases	Previously treated	42	3 (7.1)	0.046	3 (7.1)	0.209	1 (2.4)	0.511
	Newly diagnosed	80	17 (21.3)		12 (15.0)		4 (5.0)	
Contact history with active TB patients	Yes	78	12 (15.4)	0.689	9 (11.5)	0.735	2 (2.6)	0.255
	No	44	8 (18.2)		6 (13.6)		3 (6.8)	

*Single, divorced & widowed; **Others: Awi zone, West Gojjam, East Gojjam, and Wag-Hamra. FLQs, Fluoroquinolones; INH, Isoniazid; LJ, Lowenstein-Jensen; MDR*, Multidrug-resistant (resistant to both RIF and INH); PTB, pulmonary tuberculosis; RIF, Rifampicin; TB, tuberculosis.

mutation in the *rpo*B and *kat*G genes were most frequent at Ser531Leu ($n = 12$, 80%) and Ser315Thr1 ($n = 15$, 100%) (Table 2). The remaining 3 MDR-TB isolates had shown a gene mutation at *rpo*BWT6 (probe absent) and Ser315Thr1, *rpo*BWT7 (probe absent) and Ser315Thr1, and His**526**Tyr and Ser315Thr1 (6.7% each) (Table 2).

Gene mutations in the FLQ-resistance-determining region

The MTBDR*sl* VER2.0 was performed on 20 any drug-resistant TB strains to further assess the second-line injectable drugs (SLIDs) resistance. Accordingly, five MDR-TB strains had shown FLQs resistance, which is defined as pre-XDR-TB. All 5 FLQs-resistant isolates had mutations only in the *gyr*A genes (at codon Asp94Ala). No mutations were detected at *gyr*B, *rrs*, and *eis* high-level and low-level drug-resistance conferring gene mutations to SLIDs (Table 3).

Mycobacterium tuberculosis genotypes and profiles of genetic changes conferring resistance to anti-TB drugs

To further analyze the genotype and clustering of 20 drug-resistant MTB strains, we performed a genotyping technique using the established Spoligotyping method, following the manufacturer's protocol. All 20 drug-resistant MTB strains were successfully genotyped and have interpretable spoligo-patterns. The majority ($n = 11$, 55%) drug-resistant strains were found to be SIT149/T3-ETH

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TABLE 2 Gene mutations conferring resistance to RIF and INH.

Drug	Gene	Failing wild type band	Developing mutation band	Mutation	RR (*N* = 15) [n (%)]	MDR/RR (*N* = 15) [n (%)]
Rifampicin	*rpo*B	*rpo*BWT1	–	–	–	–
		*rpo*BWT2	–	–	–	–
		*rpo*BWT3	*rpo*BMUT1	D516V	–	–
		*rpo*BWT4	–	–	–	–
		*rpo*BWT5	–	–	–	–
		*rpo*BWT6	WT6 (absent)	Unknown	1 (6.7)	1 (6.7)
		*rpo*BWT7	WT7 (absent)	Unknown	1 (6.7)	1 (6.7)
		*rpo*BWT7	*rpo*BMUT2A	H526Y	1 (6.7)	1 (6.7)
			rpoBMUT2B	H526D	–	–
		*rpo*BWT8	*rpo*BMUT3	S531L	12 (80)	12 (80)
					MDR (Total, *N* = 15)	INH-MR (Total, *N* = 5)
					n (%)	n (%)
Isoniazid	*katG*	*kat*G WT	*kat*G MUT1	S315T1	15 (100)	5 (100)
		*kat*G WT	*kat*G MUT2	S315T2	–	–
	*inh*A	*inh*A WT1	*inhA* MUT1	C15T	–	–
			inhA MUT2	A15G	–	–
		*inh*A WT2	*inhA* MUT3A	T8C	–	–
			inhA MUT3B	T8A	–	–

INH, Isoniazid; MDR, Multidrug-resistant; INH-MR, Isoniazid Mono-resistant; MUT, Mutant; RIF, Rifampicin; RR, Rifampicin resistance; WT, Wild-type.

TABLE 3 Gene mutations conferring resistance to FLQs.

Drug/phenotypic resistance	Gene	Failing wild type band	Developing mutation band	Mutation	MDR (*N* = 15) [n (%)]
FLQs	*gyr*A	*gyr*AWT1	–	–	–
		*gyr*AWT2	*gyr*AMUT1	A90V	–
			*gyr*AMUT2	S91P	–
		*gyr*AWT3	*gyr*AMUT3A	D94A	5 (33.3%)
			*Gyr*AMUT3B	D94N	–
				D94Y	–
			*gyr*AMUT3C	D94G	–
			*gyr*AMUT3D	D94H	–
	*gyr*B	*gyr*BWT	*gyr*BMUT1	N538D	–
			*gyr*BMUT2	E540V	–
KAN/AMK/CAP	*rrs*	*rrs*WT1	*rrs*MUT1	A1401G	–
KAN/CAP/VIO				C1402T	–
KAN/AMK/CAP/VIO		*rrs*WT2	*rrs*MUT2	G1484T	–
Low-level KAN	*eis*	*eis*WT1	–	G−37T	–
		*eis*WT2	*eis*MUT1	C−14T	–
			–	C−12T	–
			–	G−10A	–
		*eis*WT3	–	C−2A	–

AMK, Amikacin; CAP, Capreomycin; FLQs, Fluoroquinolones; KAM, Kanamycin; MUT, Mutant; MDR, Multidrug-resistant; VIO, Viomycin; WT, Wild-type.

sublineages followed by SIT21/CAS1-Kili ($n = 6$, 30%). The remaining isolates were CAS1-Delhi, CAS1-family, and SIT54/MANU2. Of the total 11 SIT149//T3-ETH sublineages, 4 strains were detected from the South Gondar zone study site, while 2 and 3 isolates were detected from the South Wello and North Shewa zones, respectively. Also, from six SIT21/CAS1-Kili sublineages, four isolates were identified from the South Wello zone study site, while the remaining two isolates were from the North Shewa and North Wello zone study area (Table 4).

Furthermore, of 11 SIT149/T3-ETH sublineages, 7 strains were MDR-TB, while four were INH-mono-resistant. Amongst 6 CAS1-Kili/SIT21 and one CAS1-family sublineages, all were MDR-TB, while four of these were FLQs-resistant strains. The remaining two sublineages, SIT54/MANU2 and SIT25/CAS1-Delhi were MDR and INH-mono-resistant, respectively (Table 4).

Amongst 7 MDR SIT149 genotypes, five had genetic changes in *rpo*B and *kat*G genes (at codon Ser531Leu and Ser315Thr1), while one isolate had a gene mutation at codon His**526**Tyr and Ser**315**Thr, and four isolates were INH-mono-resistant with a gene mutation in *kat*G (at codon Ser315Thr1). All the six MDR SIT21/CAS1-Kili and CAS1-family spoligotypes had drug resistance-conferring gene mutations in *rpo*B and *kat*G genes (at codon Ser531Leu and Ser315Thr1), of these, four isolates were FLQs-resistant with a drug-resistance conferring gene mutations in *rpo*B, *kat*G, and *gyr*A genes (at codon Ser531Leu, Ser315Thr1, D94A, respectively) (Table 4).

Discussion

Early diagnosis and effective anti-mycobacterial drugs targeting the infecting MTB isolate are critical for improving patient management, increasing cure rates, and limiting further transmission of the disease (1, 3). Moreover, it is crucial to identify the key genetic changes responsible for anti-TB drug resistance and the associated drug-resistant MTB genotypes to handle patients with DR-TB effectively (2, 3, 39). Nowadays, the innovation and improvements of gDST methods capable of evaluating genes harboring antimicrobial drug resistance mutations have substantially improved the detection and management of drug-resistant TB (14, 15). In addition to XpertMTB/RIF assay, LPAs are commonly used in developing countries including Ethiopia, and come at the forefront in performing gDST and evaluating the occurrence of specific gene mutations resulting in drug resistance to some key anti-mycobacterial medicines used in the treatment of drug-susceptible and resistant TB strains (14–16, 40). Thus, the widespread gDST service is very important for tailoring TB patient treatment and hindering the transmission of the disease.

The most common gene mutations linked with RIF resistance occur in the 81 bp RIF-resistance determining region (RRDR) (codons 507–533) of the *rpo*B gene (95%), which encodes the "RNA polymerase beta-subunit" (2, 41). Those gene mutations alter the binding site of RIF, preventing its inhibitory action on RNA synthesis (13, 41, 42). Earlier studies have shown that specific mutations, at codons S531L, H526Y, and D516V are frequently observed in RIF-resistant MTB (13, 21, 43–45). In this study, among RIF-resistant TB isolates, the most frequent mutation (80%) associated with RIF resistance was at codon S531L, followed by His526Tyr (6.7%). The gene mutation at codon S531L was noted as the dominant mutation of the *rpo*B gene responsible for RIF resistance in several previous studies done in different parts of Ethiopia; which includes the Amhara region (73%) (46), Tigray region (70%) (44), Somali region (80%) (47), Addis Ababa (81.3%) (48), Southwest Ethiopia (82.4%) (49), a meta-analysis report, Ethiopia (74.2%) (21), and a recent multicenter study report, Ethiopia (59.1%) (45). In line with our result, a higher frequency of mutation at codon S531L was reported in other high TB burden countries like China (58%) (50), India (62%) (51), Pakistan (64%) (52), Sudan (64%) (53), and Iran (66%) (54). The other mutation in our study, His526Tyr was previously reported in Ethiopia, which ranged from 6.6–17.2% (21, 44, 45, 55), Sudan (12.8%) (53), India (11.1%) (51), and China (8.9%) (50). However, a similar or higher proportion to our results was noted in Sudan (12.8%) (53), India (11.1%) (51), China (8.9%) (50), South Africa, at amino acid position 526 (27.9%) (36). In this study, two RIF-resistant isolates (*rpo*BWT6 and *rpo*BWT7) had shown a mutation or missing WT band, but no corresponding MUT band, and were classified as unknown genetic changes. This proportion of isolates with unknown mutations is similar to other studies from a recent multicenter study, Ethiopia (13.6%) (45), Addis Ababa, Ethiopia (15.8%) (55), Southern Ethiopia (14.7%) (56), and elsewhere, a large, multisite diagnostic study (13%) (57). This could be due to the mutations occurring outside the analyzed codon regions (drug-resistance determining regions) which LPAs unable to evaluate/detect (58).

Isoniazid resistance is mostly due to the genetic change in the *kat*G gene (50–95%) and the *inh*A promoter region (20–35%) (2, 13, 41, 51), depending on geographical distributions (59). The gene mutations in the *kat*G gene inhibit INH activation, while the gene mutations in the *inh*A promoter region result in overexpression of the *inh*A gene, which encodes the INH target enzyme (13, 41). Prominent genetic changes associated with INH resistance include *kat*G Ser315Thr1/2, and *inh*A -15C/T (44, 60). Similarly, in the current study, amongst INH-resistant isolates, mutations in the *kat*G was most frequent at codon Ser315Thr1 (95%), while no *inh*A gene mutation was observed. In agreement with our findings, a recent multicenter study in Ethiopia reported a prevalence of 91.8% for *kat*G/Ser315Thr1 mutation (45), and a meta-analysis study that examined INH conferring gene mutations noted a prevalence of 95.8% for *kat*G315 mutation (21). The *kat*G/Ser315Thr1 gene mutation, causing high-level INH resistance was predominant (95%) in the current study and other earlier studies in different parts of Ethiopia (21, 45–48, 55, 56, 61). "Mutations in the *inh*A promoter region which are associated with low-level INH resistance are usually less frequent when compared with *kat*G mutations" (41). The *inh*A gene mutation is not observed in our study; as well as other studies in Ethiopia also reported no or low proportion of mutation in the *inh*A promotor region (21, 46, 48, 55, 61).

The indiscriminate use of FLQs in many countries including Ethiopia for various common infectious disease treatments, which might result in the development of antimicrobial resistance against these key antibiotics (62–64). In FLQ-resistant MTB isolates, mutations in the *gyr*A and *gyr*B genes, which encode the DNA gyrase enzyme, are commonly observed (20, 42, 65). These mutations alter the binding site of FLQs, reducing their inhibitory effect on DNA replication. Specific mutations, at codons, such as D94G, A90V, and S91P of *gyr*A (quinoline resistance-determining region, QRDR) have been frequently reported in FLQ-resistant TB (11, 45, 65). In the current study, amongst 15 MDR-TB isolates, 5 (33.3%) were FLQs-resistant, and all isolates had *gyr*A gene mutations at codon

TABLE 4 Drug-resistant *M. tuberculosis* lineages and profiles of the gene mutations conferring resistance to INH, RIF, MDR/RR, and FLQs.

Sample ID	Isolation zone	Spoligotypes descriptions (Binary format)	SIT No	Lineage/ clades	Any INH-r (*n* = 20)	Any RR (*n* = 15)				MDR/RR (*n* = 15)				FLQ-r (*n* = 5)
					S315T1	S531L	H526Y	WT6 (absent)	WT7 (absent)	S531L & S315T1	*rpo*BWT7 & S315T1	H526Y & S315T1	*rpo*BWT6 & S315T1	D94A, S531L & S315T1
NW91	NW	■■■□□□□■■□■■■■■■■■■□□□ □□□□□□□□□□□□□■■■■■■■	21	CAS1-Kili			–	–	–		–	–	–	–
SW02	SW	■■■□□□□■■□■■■■■■■■■□□□ □□□□□□□□□□□□□■■■■■■■	21	CAS1-Kili			–	–	–		–	–	–	
SW86	SW	■■■□□□□■■□■■■■■■■■■□□□ □□□□□□□□□□□□□■■■■■■■	21	CAS1-Kili			–	–	–		–	–	–	
SW91	SW	■■■□□□□■■□■■■■■■■■■□□□ □□□□□□□□□□□□□■■■■■■■	21	CAS1-Kili			–	–	–		–	–	–	
SW100	SW	■■■□□□□■■□■■■■■■■■■□□□ □□□□□□□□□□□□□■■■■■■■	21	CAS1-Kili			–	–	–		–	–	–	
SW43	SW	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■□□□□■■■■■■■	149	T3-ETH			–	–	–		–	–	–	–
SW94	SW	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■□□□□■■■■■■■	149	T3-ETH			–	–	–		–	–	–	–
SW97	SW	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■□□□□■■■■■■■	149	T3-ETH			–	–	–		–	–	–	–
SW67	SW	■■■□□□□■■■■■■■■■■■■■■■ □□□□□□□□□□□□□■■□□■■■■■	25	CAS1-Delhi*		–	–	–	–	–	–	–	–	–
SW69	SW	■■■□□□□■■□■■■□■■■■■□□□ □□□□□□□□□□□□□■■■■■■■	NA	CAS family			–	–	–					
NS48	NS	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■■□□□□■■■■■■■	149	T3-ETH**		–	–	–		–		–	–	–
NS73	NS	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■■□□□□■■■■■■■	149	T3-ETH		–		–	–	–	–		–	–
NS63	NS	■■■□□□□■■□■■■■■■■■■□□□ □□□□□□□□□□□□□■■■■■■■	21	CAS1-Kili				–	–		–	–	–	–
SG16	SG	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■■□□□□■■■■■■■	149	T3-ETH				–	–		–	–	–	–
SG35	SG	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■■□□□□■■■■■■■	149	T3-ETH				–	–		–	–	–	–
SG97	SG	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■■□□□□■■■■■■■	149	T3-ETH*		–	–	–	–	–	–	–	–	–
SG98	SG	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■■□□□□■■■■■■■	149	T3-ETH*		–	–	–	–	–	–	–	–	–
SG99	SG	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■■□□□□■■■■■■■	149	T3-ETH*		–	–	–	–	–	–	–	–	–
SG100	SG	■■■■■■■■■□□□□□□□□□□■■■ ■■■■■■■■■■□□□□■■■■■■■	149	T3-ETH*		–	–	–	–	–	–	–	–	–
WG31	WG	■■■■■■■■■■■■■■■■■■■■■■■ ■■■■■■■■■■□□■■■■■■■■■	54	MANU2		–	–		–	–	–	–		–

**Isoniazid hetero-resistant; *Isoniazid-mono-resistant; The presence of gene mutations conferring drug-resistance in each *M. tuberculosis* lineages/sublineages is highlighted in green color. INH-r, Isoniazid resistant; MDR, Multidrug resistance; MUT, Mutant; NA, Not assigned; NS, North Showa; NW, North Wello; SG, South Gondar; SIT, Spoligo-international types; SW, South Wello; RR, Rifampicin resistance; FLQ-r, Fluoroquinolone resistance; WT, Wild-type; WG, West Gojjam.

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Asp94Ala(D/A). Supporting our finding, a recent multisite study conducted in Ethiopia reported that *gyr*A/Asp94Ala gene mutation (14.3%) was detected in FLQs-resistant TB isolates in Ethiopia (45). However, in contrast to our observation, studies in Ethiopia reported that *gyr*A/D94G gene mutations were predominant in FLQs-resistant TB isolates (44, 66, 67), while few other studies indicated that *gyr*A/A90V gene mutations are prevalent and responsible for FLQs-resistance in Ethiopia (45, 62). Several previous studies have indicated that *gyr*A/D94G gene mutation is predominant across the corners of the globe (68–72). However, further molecular study data is necessary to fully understand the spectrum of gene mutations that confer resistance to FLQs (gyrA/B gene mutations) on MTB in Ethiopia. In our study, *gyr*B gene mutation was not observed, which is in concordance with previous studies in Ethiopia (44, 45, 62).

The observed association between MTB genotypes and drug resistance in Ethiopia highlights the importance of understanding the genetic diversity of the pathogen in combating drug-resistant TB (63, 73, 74). The diversity of MTB genotypes circulating in the country, coupled with specific mutations conferring drug resistance, underscores the need for a comprehensive approach to TB control (73, 75). A lack of comprehensive data on the molecular epidemiology of DR-TB strains in Ethiopia further complicates the implementation of molecular diagnostics. Yet, in Ethiopia, the link between resistance to anti-TB drugs and MTB genotypes is a complex and multifactorial phenomenon (75). Thus, characterizing DR-TB genotypes and understanding the prevalence and distribution of resistance-associated gene mutations for key anti-TB drugs is essential for guiding treatment strategies and monitoring the emergence of new resistance patterns in the country (21).

In the current study, 20 drug-resistant MTB isolates were successfully Spoligotyped, in which the majority (55%) were SIT149/T3-ETH Sublineages followed by SIT21/CAS1-Kili (30%). Furthermore, from 11 SIT149/T3-ETH sublineages, 7 were MDR-TB, while four were INH-hetero-resistant. Supporting our findings, the high clustering rate and predominance of drug-resistant SIT149/T3-ETH sublineages were reported in Ethiopia (56, 63, 76, 77). In agreement with our findings, earlier studies in Ethiopia reported that SIT149/T3-ETH sublineages were predominantly linked with drug resistance, particularly MDR/XDR-TB (63, 73–75, 78). However, in contrast to our findings, a recent study in Ethiopia noted that "TUR-genotype (54%) was predominant in MDR-TB strains" (79). This could be the fact that the T3-ETH/SIT149 Sublineages is an Ethiopian-specific genotype that predominantly circulates in the highlands of the country and plays an important role in TB disease transmission (73). Thus, a comprehensive study using improved genotyping techniques such as sequencing methods with high discriminatory power is warranted to tailor the clustering of these sublineages and their association with drug resistance. However, the predominance and its association with drug-resistance, SIT149/T3-ETH sublineages could indicate that these strains are becoming more important in TB disease transmission and developing drug resistance in Ethiopia. On the other hand, high proportions of holy water site attendees infected with clustered TB strains, suggest probable recent transmission of MDR-TB in the study area. Furthermore, SIT21/CAS1-Kili sublineages were the second most prevalent in the current study, in which among 6 CAS1-Kili and one CAS-family sublineages, all strains were MDR-TB, and four of these were FLQs-resistant. In line with our observation, a few studies conducted in Ethiopia (63, 80), and elsewhere, in Zambia (81), reported that SIT21 sublineages were frequently detected in MDR/XDR-TB isolates. The emergence of drug resistance in the Ethiopian strains, CAS1-Kili/SIT21 and T3-ETH/SIT149, could potentially be attributed to local factors including delayed diagnosis, inadequate compliance, insufficient contact investigations, or other unidentified factors TB prevention and care system.

Limitations

However, this study has limitations. The number of TB isolates included in our study is relatively very small, and study population selection bias might impede generalizing the findings. Since LPAs can only detect the presence/absence of specific gene mutations responsible for drug resistance for key anti-TB drugs, it will not be enough to describe the spectrum of gene mutations in the country or study region. We used only the Spoligotyping technique to characterize the genetic diversity of drug-resistant MTB isolates, which may have resulted in low discriminatory capacity and hampered identification of transmission chains.

Conclusion

The finding of our study revealed that canonical drug resistance-conferring gene mutations at *rpo*B/S531L, *kat*G/S315T1, and *gyr*A/D94G were the most frequent in RIF, INH, and FLQs-resistant isolates, respectively. In this study, higher clustering rates of drug-resistant MTB lineages were observed. Furthermore, two sublineages, SIT149/T3-ETH and CAS1-Kili showed a higher proportion of drug resistance, particularly MDR/pre-XDR-TB. However, to comprehend better the association of SIT149/T3-ETH and SIT21/CAS1-Kili sublineages with drug resistance in Ethiopia, improved genotyping techniques with high discriminatory power such as sequencing methods and a large number of molecular data is warranted to further elucidate such genotypes and mutations and predict drug-resistance. TB screening and surveillance for drug resistance among key populations are essential to effectively control the disease in the country.

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 studies involving humans were approved by the Human Research Ethics Committee of the University of Pretoria, Faculty of Health Sciences, South Africa (Ref: No.600/2018). 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

MAR: Writing – review & editing, Writing – original draft, Visualization, Validation, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. NEM: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. PBF: Writing – review & editing, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization.

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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.

Publisher's note

Supplementary material

The Supplementary material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2024.1356826/full#supplementary-material

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Development and implementation of a strategy for early diagnosis and management of scrub typhus: an emerging public health threat

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Scrub typhus, caused by *Orientia tsutsugamushi*, is a re-emerging zoonotic disease in the tropics with considerable morbidity and mortality rates. This disease, which is mostly prevalent in rural areas, remains underdiagnosed and underreported because of the low index of suspicion and non-specific clinical presentation. Limited access to healthcare, diagnostics, and treatment in rural settings further makes it challenging to distinguish it from other febrile illnesses. While easily treatable, improper treatment leads to severe forms of the disease and even death. As there is no existing public health program to address scrub typhus in India, there is an urgent need to design a program and test its effectiveness for control and management of the disease. With this backdrop, this implementation research protocol has been developed for a trial in few of the endemic "pockets" of Odisha, an eastern Indian state that can be scalable to other endemic areas of the country, if found effective. The main goal of the proposed project is to include scrub typhus as a differential diagnosis of fever cases in every tier of the public health system, starting from the community level to the health system, for the early diagnosis among suspected cases and to ensure that individuals receive complete treatment. The current study aimed to describe the protocol of the proposed Scrub Typhus Control Program (STCP) in detail so that it can receive valuable views from peers which can further strengthen the attempt.

KEYWORDS

scrub typhus, implementation research, protocol, diagnosis, disease management, public health, control program

1 Introduction

1.1 Scrub typhus disease, its diagnosis, and its treatment

Scrub typhus, also known as bush typhus, is a re-emerging but neglected zoonotic acute febrile illness. Approximately 1 million people worldwide are affected by this disease annually, with a mortality rate of nearly 50% if untreated, and 1 billion people are at risk of infection globally (1, 2). This disease is caused by *Orientia tsutsugamushi*, an obligate

intracellular gram-negative *Coccobacillus* belonging to the family Rickettsiae. Rodents are the natural reservoir, while trombiculid mites ("chiggers," *Leptotrombidium deliense*, and others) act as a vector as well as a reservoir of the causative bacteria. Scrub typhus is transmitted to humans by the bite of infected chiggers (larvae) of trombiculid mites (3, 4).

The clinical manifestations of scrub typhus range from a mild and self-limiting illness to severe or even fatal illness. The initial clinical signs include an eschar, indicating localized skin necrosis where the mite has fed (though it may not always be present), along with nearby lymph node, followed subsequently by fever, headache, myalgia, generalized lymphadenopathy, cough, gastrointestinal symptoms, transient hearing loss, and rash (5, 6). However, there are wide variations in the clinical manifestations of the disease, and the reasons for such variations are mostly unknown. Severe scrub typhus manifests as acute respiratory distress, meningoencephalitis, gastrointestinal bleeding, acute renal failure, hypotensive shock, and coagulopathy (7). Studies from Assam have reported that *Orientia tsutsugamushi* infection can lead to acute encephalitis syndrome (AES) and subsequent death (8). Occasionally, the case fatality can be as high as 30%−70% in untreated cases (9, 10). Furthermore, the wide genetic variability observed in the pathogen in India, such as Gillam, Karp, Hualien1, and Keto, and across the globe, along with complicated host–pathogen interactions, hinders the development of vaccine and improved diagnostic and therapeutic methods (8, 11–14).

The identification of an eschar at the site of mite bites serves as a highly specific (98.9%) indicator for diagnosing scrub typhus (15). However, the presence of an eschar in Indian and other Asian populations is rare, which makes it an inappropriate method for the diagnosis of scrub typhus. Therefore, the diagnosis predominantly depends on laboratory tests (11). Serological assays such as the Weil–Felix test, indirect immunofluorescence assays, indirect immune peroxidase assays, and enzyme-linked immunosorbent assay (ELISA) and immune chromatographic tests (ICTs) are the prominent tests employed to diagnose rickettsial diseases. Among all serological assays, the IgM ELISA-based method is the most reliable one for the diagnosis of scrub typhus (16). Compared to other tests, the Weil–Felix test lacks high sensitivity and specificity and can serve as a useful and inexpensive primary diagnostic tool that can be performed by laboratory technicians at peripheral hospitals (17, 18). Other assays are not widely recommended in primary care. The Indian Council of Medical Research (ICMR) has recently developed guidelines outlining the diagnosis and treatment of rickettsial diseases. These guidelines include presenting manifestations, case definitions, laboratory criteria (both specific and supportive investigations), and recommended treatments (17).

The drug of choice for the treatment of diseases within the order Rickettsiales is doxycycline, while tetracycline, chloramphenicol, and azithromycin have also demonstrated efficacy (19). However, there is growing concern about the development of antibiotic-resistant strains of *O. tsutsugamushi*, both at present and in the future.

1.2 Epidemiology of scrub typhus

1.2.1 Global

The areas where the disease is prevalent is known as the "Tsutsugamushi Triangle," encompassing Japan in the east, Afghanistan and the Middle East in the west, and various regions in between such as the Pacific Islands, North Australia, Indonesia, Southeast Asia, China, and Korea. The Tsutsugamushi triangle is home to more than half of the world's population, with 2 billion people at risk and 1 million cases of scrub typhus occurring per year (20).

Nevertheless, scrub typhus is currently increasingly identified in regions where the disease was previously unfamiliar or had been largely overlooked, including India, Sri Lanka, the Maldives, and Micronesia. Recently, scrub typhus has been reported beyond the boundaries of the Tsutsugamushi triangle, causing concerns across the globe about the pathogen (4, 21).

1.2.2 India

Scrub typhus is a serious public health problem in India causing severe morbidity and a significant number of deaths (22). It has been reported in different geographical zones of India: Kerala, Karnataka, Andhra Pradesh, and Tamil Nadu in South India; Bihar, Maharashtra, Jammu Kashmir, Himachal Pradesh, Uttaranchal, and Rajasthan in Northern India; and Meghalaya, Sikkim, and West Bengal in Northeast India (6, 23–28).

Evidence has been emerging, although mostly from small-scale regional studies, that scrub typhus is prevalent mainly in many rural areas of India, especially among vulnerable populations such as poor farmers, children, older adults, and pregnant women. In some parts of India, this prevalence has resulted in the characterization of scrub typhus as a re-emerging infectious disease in India, emphasizing a critical public health concern for this illness in the country (8, 29). In some settings in India, ∼20%−24% of all patients presenting with unexplained febrile illness has been reported to be due to scrub typhus, and 53% of patients have evidence of acute kidney injury (30–32). A study showed that the circulating genotypes of *O. tsutsugamushi* in three scrub typhus–endemic geographic regions of India: South India, Northern India, and Northeast India—provide potential resources for future region-specific diagnostic studies and vaccine development (29).

1.2.3 Odisha

Odisha is located within the endemic belt of scrub typhus, but the disease continues to remain unrecognized, underdiagnosed, and underreported. Only recently, few reports from a private tertiary care hospital in the capital city confirmed many cases of undifferentiated fever as scrub typhus (33–35). The authors of these reports observed that scrub typhus patients were hospitalized due to either prolonged fever (more than 10–30 days) or complications such as encephalopathy/AES, acute renal failure, acute respiratory distress, and multiorgan failure cases with unknown etiology.

Abbreviations: STCP, Scrub Typhus Control Program; STIS, STCP Information System; PCST, Presumptive Case of Scrub Typhus; CCST, Confirmed Case of Scrub Typhus.

Among children, AES/encephalopathy was the most common cause of hospitalization followed by hepatic involvement, shock, and renal failure.

However, none of the studies cited above were conclusive about the population burden of scrub typhus, even from any particular district or "pocket" in the state, because all of these studies were conducted in tertiary health facilities, and the cases would be only those with extreme severity requiring hospitalization and those who would have presented from all over the state. However, many cases from the Keonjhar district were found to be positive for scrub typhus by molecular tests conducted at the reference laboratory of the state, The Regional Medical Research Center (RMRC), Bhubaneswar, an institute of ICMR, which is because few physicians from the district clinically suspected scrub typhus in many prolonged fever cases that presented to them and referred them for molecular testing at RMRC. In the backdrop of these two observations, first from the tertiary hospital in the capital city and the second from the primary care setting in Keonjhar, an exploratory study was undertaken by the RMRC to examine the prevalence of scrub typhus in few suspected high-endemic pockets of Keonjhar. The study showed high prevalence (39.7%) of scrub typhus among children with prolonged fever during July–September, conventionally the peak transmission season for other diseases such as dengue, malaria, and other viral diseases. Among them, eschar was found in 17.9% of the cases (36). Meanwhile, the serological analysis undertaken by RMRC of 30 AES archived samples collected from hospitalized children during the 2016 epidemic from another district, Malkangiri, indicated that 23.3% (7/30) of the cases were positive for scrub typhus. Recently, high incidence of scrub typhus associated with acute kidney injury was reported among patients having a history of acute fever with various degrees of kidney involvement admitted to a tertiary care hospital (11).

1.3 Rationale and aims

Despite its high estimated burden, the WHO has described scrub typhus as one of the most underdiagnosed and underreported febrile illnesses requiring hospitalization. It strongly emphasizes surveillance owing to its relatively high fatality rate (up to 30% in untreated patients).

It has been suggested that deaths from scrub typhus may exceed those of dengue fever globally. However, dengue in India and elsewhere still attracts far higher public and professional awareness, whereas scrub typhus is outside the "limelight" (6). A recent editorial in the *New England Journal of Medicine* described scrub typhus as "probably the single most prevalent, under-recognized, neglected, and severe but easily treatable disease in the world" (37).

Although scrub typhus is currently increasingly found in metropolitan areas, it still remains predominantly a disease affecting the rural population of low and middle income countries (LMICs) that includes India (38). The rural populations in such settings are often mired with limited access to healthcare, diagnostics, and treatment, which further makes it difficult to differentiate scrub typhus from other febrile illnesses on a clinical basis alone. Consequently, scrub typhus tends to be largely ignored, especially amid fever outbreaks, and its progression often becomes complex, resulting in severe complications and even fatalities without proper intervention. Nonetheless, with early diagnosis and the prompt initiation of specific treatment, the chances of cure rates are significantly high (39).

As solid evidence of a considerable burden of scrub typhus in Odisha emerged from reports of tertiary care hospitals and the community-based study of the RMRC (11, 36), there was a necessity to initiate a public health program that can be instituted to control this problem in the state so that many severe health conditions and fatalities related to scrub typhus at the population level can be averted in the long term.

Therefore, the RMRC proposed to conduct an implementation research project (hereinafter referred to as the research project), the cornerstone of which would be designing and rolling-out a replicable and scalable template of Scrub Typhus Control Program (STCP) within the public health system of the state. The research project will also thereafter entail the assessment of the effectiveness of STCP. The current study aims to describe the protocol of the research project in detail so that it can receive valuable suggestions from peers, which can further strengthen the endeavor.

2 Methods and results

The research project has two distinct sections and will entail the following broad areas of activities, with each area of activity having its own set of objectives.

- STCP design and its roll-out
  - Empiric selection of the study site where the STCP will be rolled-out and which area will be considered as control unit for evaluation.
  - Preparation for STCP roll-out
  - STCP roll-out
- Evaluation of the STCP

2.1 An outline of the public health system of Indian states

The proposed STCP will be rolled-out entirely through the existing public health system of Indian states (Figure 1) without any additional resources, except the provision of logistics to equip public health laboratories for diagnosis of the disease under the designed program so that it becomes a sustainable model.

2.2 Description of scrub typhus control program (STCP)

2.2.1 Conceptual framework guiding the design of STCP

A commonly used conceptual framework, often referred to as the cascade of care, in exploring the control of diseases such as tuberculosis and hypertension was customized for

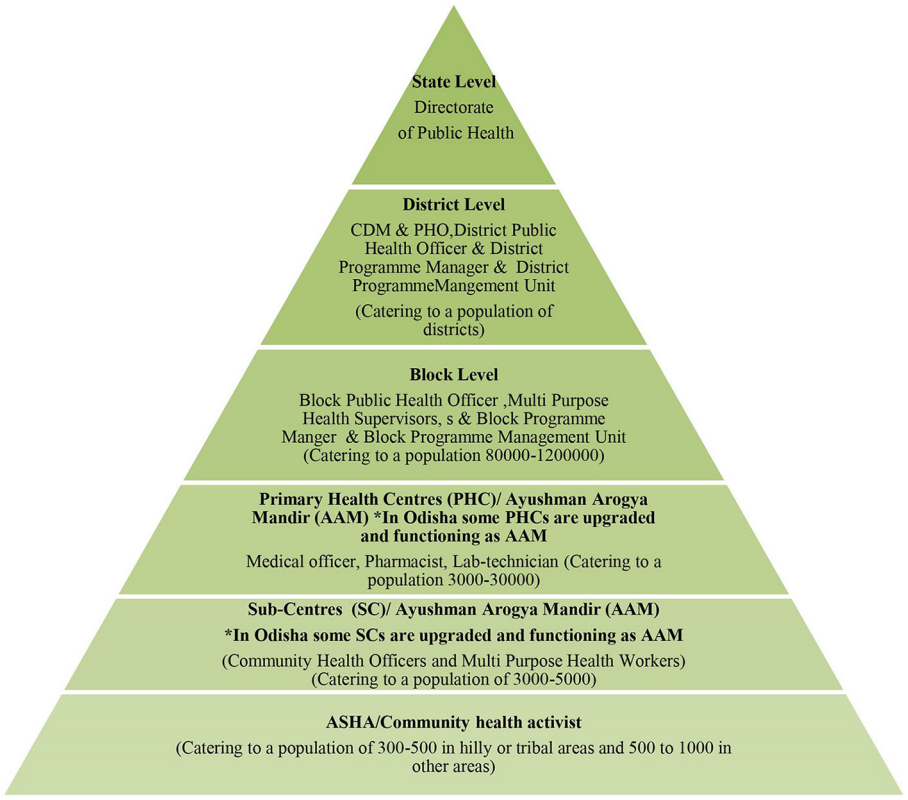

FIGURE 1
Hierarchy of the public health system in India.

designing and planning a public health program to control scrub typhus—STCP. The conceptual framework is graphically presented below (Figure 2), which comprises different steps of scrub typhus control, starting from suspecting scrub typhus in the population to completing the treatment of confirmed cases in health facilities. Other than natural attrition from the care cascade, for example, many suspected cases turn out to be not diseased after testing and hence they drop out of the program. There are certain programmatic "losses" envisaged through this framework, thus not all suspects may be identified at the community level and all identified suspects may not undergo screening/diagnosis. To summarize, it was presumed from anecdotal experiences that the suspicion index for scrub typhus among all categories of health workers, including physicians, was low and that diagnostic tests for this disease was not available in primary care (11, 36). Therefore, these two components were identified as "rate limiting steps" for scrub typhus control and guided the design of the STCP so that the potential losses from the care cascade can be preemptively plugged and program effectiveness optimized.

2.2.2 Objective of STCP

Based on the conceptual framework mentioned above, the objectives of the proposed STCP are as follows:

- Scrub typhus to be included as a differential diagnosis of fever cases in every tier of the public health system
- Screening and diagnosis among presumptive scrub typhus cases to be enhanced
- Complete treatment of diagnosed scrub typhus cases to be ensured.

2.2.3 Main activities of STCP

Based on the objectives of the STCP, the main activities to be carried out by the STCP along with their monitoring indicators are shown in Figure 3.

2.3 Diagnosis and treatment algorithm of the STCP

A diagnostic algorithm has already been developed in line with the ICMR guidelines for treating scrub typhus but is customized to the local context and STCP objectives. This algorithm will be applied to diagnose and treat cases of scrub typhus in the proposed program (Figure 4).

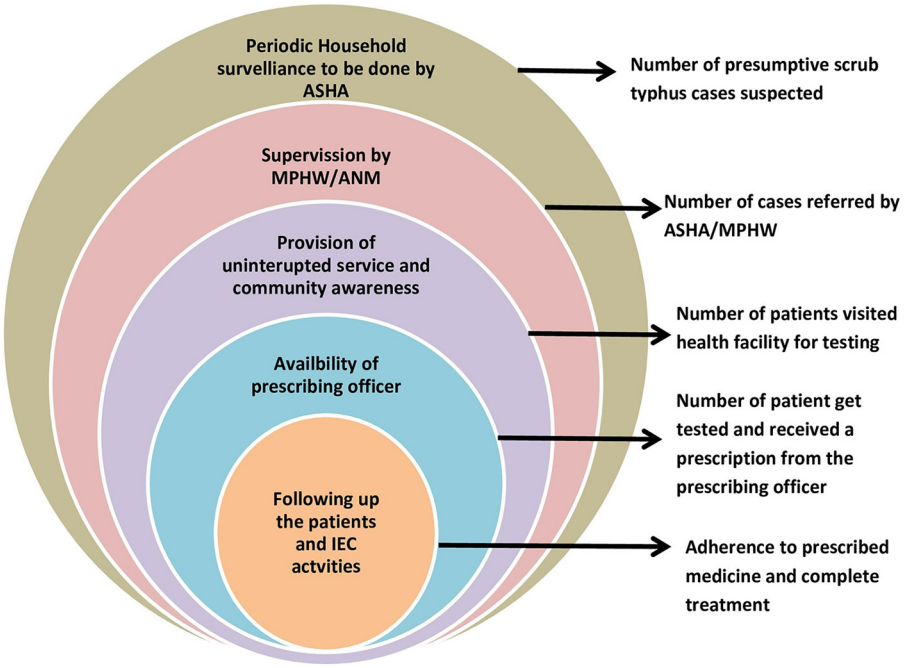

FIGURE 2
Conceptual framework for studying the continuum of care for scrub typhus across the health system.

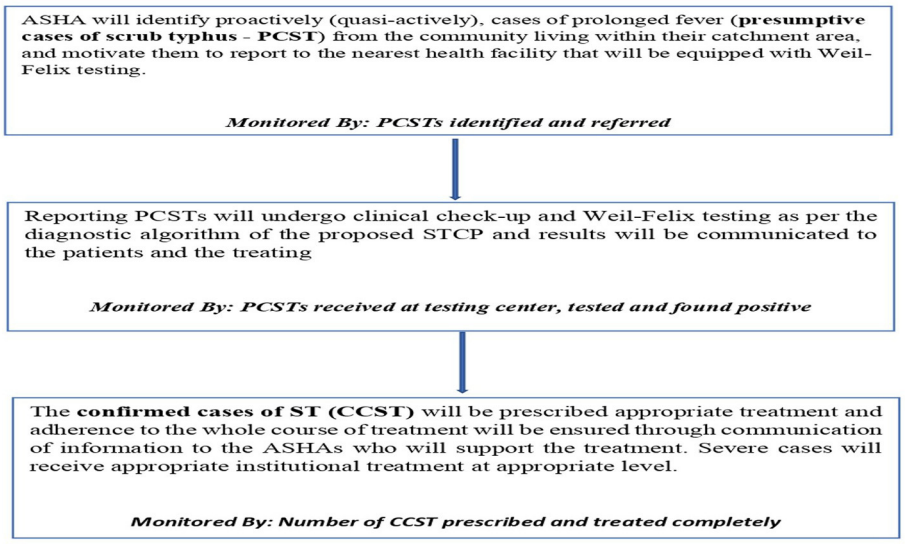

FIGURE 3
Flowchart showing the main activities of the STCP.

2.4 Outline of research project

Herein, we delineate four broad areas of activities (with their respective objectives and micro-actions mapped to them (Table 1) for the proposed implementation research project:

The broad areas of activities of the implementation research project are described. Then, we mapped the objectives to them and planned the micro-level actions to be carried out to achieve the objectives. All these three verticals are summarized in the matrix below. Some of the putative activities are elaborated later. The first activity of the STCP has already been carried out, which was essential for the selection of the STCP setting.

Some of the actions mentioned above are elaborated below:

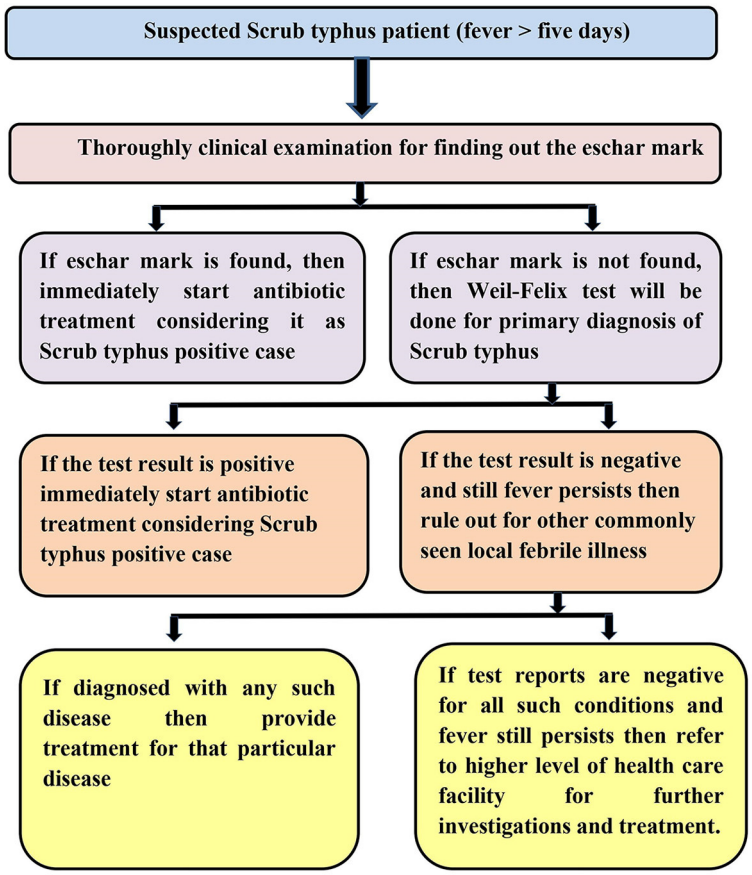

FIGURE 4
Flowchart showing the diagnostic algorithm of the STCP.

2.4.1 Secondary data analysis and selection of the study site

A secondary data analysis has already been conducted for exploring the burden of scrub typhus in various sub-district units (known as "blocks" in India which represent the lowest administrative unit in Indian states) of the Keonjhar district of Odisha with a view to select the study site. This was planned as a forerunner to the design, implementation and evaluation phase of STCP as mentioned above. Since there is no scrub typhus specific data currently available in the health information system and a previous study by RMRC has confirmed that scrub typhus cases are hidden amid the unidentified fever cases only, only cases of prolonged fever (of 5 days or more) was decided to be considered as presumptive cases of scrub typhus. Year-wise data for the last 3 years on prolonged febrile illness available with Integrated Disease Surveillance Program cell of Odisha, Directorate of Health Services, Govt. of Odisha, and hospital records of all the 15 Community Health Center (CHC) and district headquarter hospital were collected, collated, and analyzed to assess the magnitude and prepare the distribution map. By analyzing the data, four blocks of Keonjhar with highest prolonged fever cases and with >60% tribal population has been selected as the study setting (described below).

The total population of the district is 1,801,733(2011 census), of which > 44.5% belongs to tribal communities. There are 59 Primary Health Center (PHC) and 226 sub-centers under 15 CHC, 2 sub-divisional hospitals, and one district headquarter hospital in the districts. The district has been selected based on our preliminary observation that scrub typhus is one of the major cause of unexplained febrile illness among children < 15 years of age (36). Four CHC, two for implementation at Jhumpura and Patana and two for comparison (control) at Harichandanpur and Sainkul CHC, are selected. Allocation of blocks to control and intervention arms were done randomly (Figure 5).

2.4.2 Baseline survey

A baseline survey will be conducted among the patients visiting the outpatient department in selected CHC and PHC under them with complaints of prolonged fever. This survey will provide an overview of scrub typhus burden. Following this survey, a community assessment survey will be conducted using the cross-sectional study design with the objective of finding out the knowledge attitude and practices of people regarding the disease and their health seeking behavior toward febrile illness. Additionally, a health system assessment will be performed to understand the readiness of the existing public health system in implementing the proposed STCP.

TABLE 1 Broad area of activities, purposes, and the corresponding micro-plans of the implementation research project.

Broad areas of activities	Purpose	Micro-plan of actions to be taken
Empiric selection of study sites	To select the site where the proposed program will be implemented and subsequently evaluated	• Collection of secondary data for prolonged fever for last 3 years from the Integrated Disease Surveillance Program (IDSP) cell and its analysis
Preparation for STCP roll-out	To ensure preparatory activities for seamless roll-out of the STCP at the intervention site(s)	• Advocacy meetings and stakeholder mapping • Development of different training modules for health workers at different levels and IEC materials to educate the community • Development of the STCP information system • Introduction of the Weil-Felix test in the existing public health laboratory network • Setting of a molecular testing facility at the RMRC • Training of frontline health workers for identification of suspects and referrals • Training of lab technicians for the Weil-Felix test • Training of MOs for complete treatment
STCP roll-out	• To roll-out STCP • To monitor whether the implementation is progressing as per plan/design • To roll-out STCP MIS • To ensure quality assured lab services—carry-out molecular research	• IEC in the community level using different mediums in the local language • Monitoring of the referral system and • Supportive supervision • Data collection, extraction, and analysis of STCP information system data • Reorientation or refresher training for health workers • Monthly meeting at the sector level • Quality assurance of lab services • Transportation of samples to the RMRC for molecular tests
Program evaluation	• To estimate the effectiveness of STCP	• Baseline community survey • Health system assessment • End-line survey • Analysis of time-series information system data

2.4.3 IEC and training material/module development

The training modules for healthcare workers will be prepared with the help of experts using the existing literature. A total of four training modules will be prepared for medical officers, laboratory technicians and pharmacists, community health officers (CHOs), multi-purpose health workers (MPHWs), and accredited social health activists (ASHAs). These training modules will contain a brief overview about scrub typhus biology, the burden of the disease, its transmission cycle, signs and symptoms, clinical manifestations, diagnosis, case management, treatment, controlling strategy, primary prevention, protocols for sample collection, and the Weil-Felix test procedure and their role in project implementation.

The Information, Education, Communication (IEC) materials (posters, leaflets, and audio-visuals) will be prepared in local languages to deliver precise, simple, comprehensive and practicable messages. The IEC materials and the communication strategy will be developed through the 'participatory learning and action' approach by assessing the knowledge, practices, taboos, beliefs, and misconceptions prevalent in the community related to treatment and prevention of the disease.

2.4.4 Training

The healthcare providers at all levels will be trained by organizing district-level sensitization workshops with the help of experienced clinicians, rickettsia experts, and public health experts. Following those training, individual training workshops will be organized at the CHC and sector levels. Regular meetings/workshops will be organized at the field level on epidemiology and public health importance of scrub typhus and clinical features, diagnosis, clinical management, and treatment of those patients. Refresher training sessions will be organized periodically.

2.4.5 IEC

IEC activities, such as street plays called "*Pala*" in the local language, wall paintings, sensitization programs at schools, meetings with villagers at the village health nutrition day, *Village-welfare* meetings, and meetings with mothers on immunization day, will be conducted for sensitizing and creating awareness regarding scrub typhus.

2.4.6 Setting up the laboratory network and quality assurance

The CHCs and primary health centers (now referred to as "health and wellness centers" in the Odisha context) will be established as referral and diagnostic units. These are already equipped with basic logistics for treatment and diagnosis along with manpower, such as community health officers, to facilitate the same. The existing public health laboratory network will be equipped with the Weil-Felix test for the primary diagnosis of scrub typhus at the peripheral level. Laboratory technicians will be trained for conducting the test and result interpretation. For quality assurance of the testing, samples will be shipped to the ICMR-RMRC molecular laboratory via proper cold chain for conducting IgM ELISA followed by PCR.

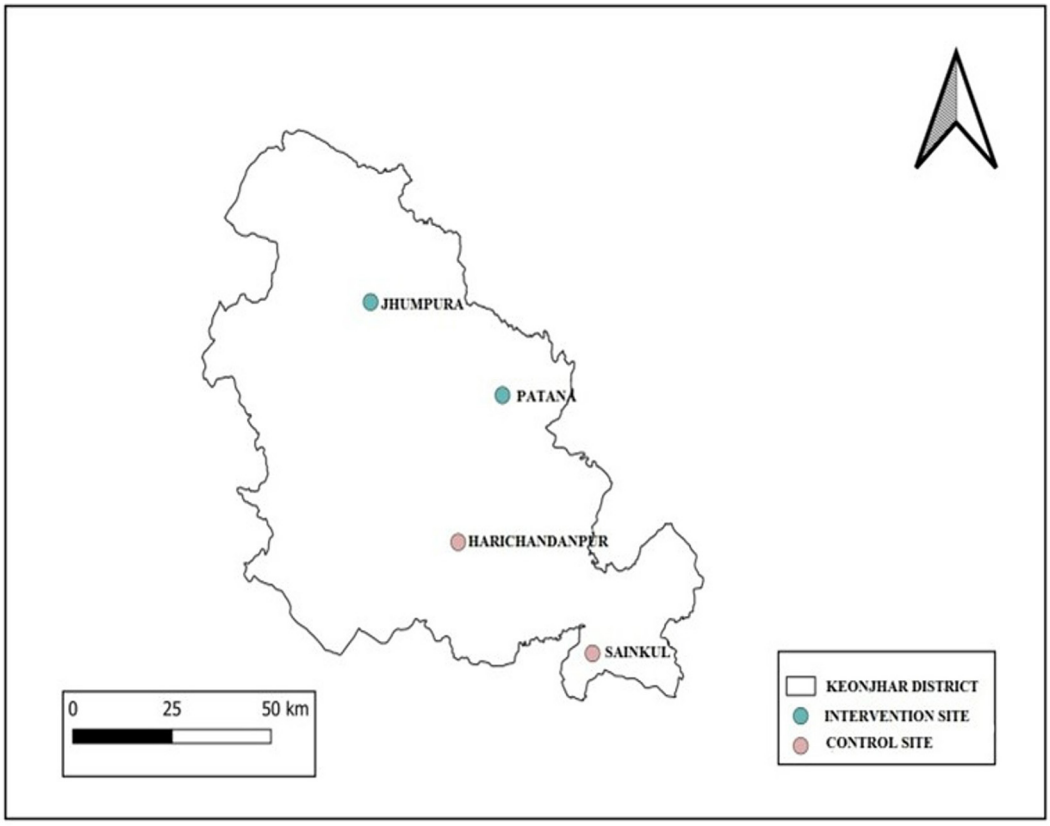

FIGURE 5
Map showing the study sites.

2.4.7 STCP information system

An information system (STIS) will be developed for the STCP using the forms and registers at different levels of the healthcare system, as described in Table 2, to track the functioning of the referral system. The indicators will be computed using the data extracted from the STIS for the monitoring and evaluation of the STCP.

2.4.8 Supervision and monitoring

Monitoring and supervision of the planned activities under the STCP will be done periodically for ensuring the optimum implementation of the STCP as per the plan. The project team will be attending every meeting of the field staff for discussing the functioning of various components of the programs and trying to find solutions to challenges and barriers. Meetings will be also held periodically with higher-level, sub-district, and district-level officials to discuss the ongoing STCP, the challenges faced, and their possible solutions.

2.4.9 End-line survey

An end-line survey will be conducted in the same population where the baseline survey was conducted using same methods and tools. The findings of this survey will be used during the evaluation of the proposed STCP.

TABLE 2 Forms, registers, and indicators used at different levels of STIS.

Level	Document	Indicators
ASHA	• Register: The existing minor element register will be used for registering PSTC • Referral form • Monthly reporting format	• Number of PSTC identified • Number of PSTC referred • Number of PSTC receiving tested for scrub typhus • Number of PSTC tested positive for scrub typhus • Number of CCST receiving complete treatment • Number of testing done at the laboratory
Lab	• Lab register • Lab form • Monthly Lab Reporting Format	
Medical Officer	• Treatment form	
Block	• Monthly reporting format	

2.5 Evaluation

The evaluation will be carried out using a difference-in-difference framework. The framework will use knowledge and

practice outcomes of community members and health system personnel from baseline and end line cross-sectional surveys. The evaluation process will analyze the time series of seminal indicators recorded by the STCP information system as follows:

- Number (percentage) of Presumptive Scrub typhus Case (PSTC) suspected.
- Number (percentage) of PSTC referred.
- Number (percentage) of PSTC tested.
- Number (percentage) of CCST.
- Number (percentage) of CCST cases that received complete treatment.

The evaluation will also examine the input and the process involved in the roll-out of the STCP such as training, community engagement, and IEC.

2.6 Ethical clearance

The study has been approved by the Institute of Human Ethics Committee (ICMR-RMRCB/IHEC-2020/030) and Research & Ethics Committee, Department of Health and Family Welfare, Government of Odisha [22516/MS-2-IV-04-2020 (PT-1) Dated 23/11/2021].

3 Discussion

Recent recognition of the substantial burden of scrub typhus in the South Asian region, including India, can be ascribed not only to increased reporting of the infectious disease but also to perhaps increasing incidence of the infection due to the ongoing social, environmental, and economic changes, which has left the region more vulnerable to such zoonotic diseases. Human activities, such as clearing of forest, aggressive agricultural practices, urbanization, continued poor sanitization, and close proximity of people to livestock, along with climate change, are accelerating human interaction with natural hosts of such diseases. The 'One Health' approach, which advocates the transdisciplinary collaborative relationship between humans, animals, and environment health partners to address the emergence and re-emergence of the zoonotic disease, is unquestionably the best possible strategy for overall control of scrub typhus (40, 41). The current proposed program described in this protocol, however, is yet to adopt the 'One Health' approach. The proposed program primarily focuses on establishing the structures for Early Diagnosis and Complete Treatment (EDCT) for reducing severe morbidity and mortality rates caused by scrub typhus. The premises for this strategy is that, once this basic EDCT infrastructure is well established and functioning optimally, in the subsequent phases of the project, the endeavor should be to include the control of non-human components of the disease for its wholistic control, namely, animal host of the disease and environment, by employing "One Health" and climate-adaptive approaches. The proposed study is the first of its kind in the country which entails designing, roll-out, and assessment of a scrub typhus control program from a tribal district of Odisha. The choice of a district, which is remote and challenging, infrastructure-wise and designing the project to run entirely through the existing local public health system ensure its sustainability, replicability, and scalability in any part of the country where it is needed.

Ethics statement

The studies involving humans were approved by Institute Human Ethical Committee (ICMR-RMRCB/IHEC-2020/030) and Research Ethics Committee, Department of Health and Family Welfare, Government of Odisha [22516/MS-2-IV-04-2020 (PT-1) Dated 23/11/2021]. 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

HJ: Data curation, Investigation, Methodology, Supervision, Writing—original draft, Visualization. ADa: Investigation, Methodology, Writing—review & editing. SD: Writing—review & editing, Data curation, Investigation. HK: Writing—review & editing, Project administration, Funding acquisition, Resources. SP: Funding acquisition, Project administration, Supervision, Writing—review & editing. MR: Conceptualization, Funding acquisition, Investigation, Project administration, Writing—original draft, Writing—review & editing, Methodology, Resources, Supervision, Visualization. ADu: Conceptualization, Investigation, Methodology, Project administration, Writing—original draft, Writing—review & editing, Visualization. MB: Investigation, Methodology, Conceptualization, Funding acquisition, Project administration, Resources, Supervision, Visualization, 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.

Publisher's note

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Measles outbreak investigation in Berhet District, North Shewa, Ethiopia

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Introduction: Measles, though usually self-limiting, can have severe consequences influenced by factors such as vaccination and nutrition, notably vitamin A deficiency and malnutrition. Despite progress, contextual changes and implementation issues have hampered efforts, resulting in increased outbreaks and cases of measles. This study seeks to pinpoint outbreak features, risk factors, and strategies for preventing and controlling measles.

Methods: A descriptive cross-sectional study and a 1:2 unmatched case-control study design were employed. All 101 suspected measles cases listed on the line-list were included in the descriptive research, with 60 measles patients and 120 controls included in the case-control investigation. Line-list data were cleaned and analyzed using a pivot table in Microsoft Excel 2016. Subsequently, the data were cleaned, entered into Epi Info 7.2, and exported to SPSS 26 for analysis.

Results: Twenty cases occurred per 10,000 individuals. Men accounted for 67.3% of cases, with ages ranging from 5 months to 45 years and mean and standard deviations of 9.6 and 7.6, respectively. Age group of 5–14 years comprised 57.4% of cases, followed by 1–4 years with 24.8%. Being unvaccinated against measles showed an adjusted odds ratio (AOR) of 12.06 (95% CI: 3.12–46.52). Travel history to regions with active cases had an AOR of 5.73 (95% CI: 1.78–18.38). Contact with a measles patient showed an AOR of 10.3 (95% CI: 3.48–30.5). Understanding the measles transmission mechanism had an AOR of 0.164 (95% CI: 0.049–0.55), and awareness of the disease's preventability had an AOR of 0.233 (95% CI: 0.67–0.811). All factors were independently associated with the illness.

Conclusion: This outbreak affected a broader age range with a high attack rate, mainly in the age group of 5–14-years. Over 35% of cases lacked measles vaccination, indicating low administrative vaccine coverage. Factors contributing to the outbreak include lack of measles vaccination, travel to areas with active disease, contact with cases, and insufficient knowledge of measles transmission and prevention strategies among mothers and caregivers.

KEYWORDS

measles, outbreak, Berhet district, Northshewa, Ethiopia

Introduction

The measles virus causes the acute, highly contagious disease known as measles. The measles virus belongs to the Paramyxoviridae family's genus Morbillivirus (1). There is little indication that the viral antigens have altered considerably over time, suggesting that the virus is antigenically stable (2). Nevertheless, viral genome sequencing has revealed that different lineages (genotypes) of wild-type measles viruses exist (3). The discovery of a particular virus genotype, when taken into account alongside epidemiological data, can indicate the source of an outbreak. The measles virus is susceptible to drying, heat, and UV light. The virus can only survive for around 2 h in the air or on surfaces and objects (4). The upper respiratory tract, or the conjunctiva, is the primary site of infection for this extremely infectious virus, which is mostly spread by respiratory droplets or airborne spray. The measles virus only naturally occurs in humans. While monkeys are susceptible to infection, there does not seem to be a mechanism for the virus to spread among them in the wild. Children who visit endemic places or come into contact with travelers who have been there are among the risk factors for measles virus infection, regardless of their vaccination status (5). These include children with vitamin A deficiency and immunodeficiency brought on by HIV or AIDS, leukemia, alkylating drugs, or corticosteroid medication, as well as newborns who lose passive antibodies before the age of normal vaccination. Malnourished people and young children are especially vulnerable to complications and mortality from measles infection (3).

Leukopenia is linked to the incubation phase, which is thought to span 10–14 days. Fever that is accompanied by cough, coryza, and/or conjunctivitis indicates the prodromal phase. The rash typically appears 3–5 days after the fever appears. Before the rash appears, during the prodromal phase, the virus starts to shed. Nectin-4 causes the respiratory epithelium to become basolaterally infected after viremia caused by infected lymphocytes, and viral transmission persists through respiratory secretions. From around 4 days before the development of the rash to 4 days following it, people are thought to be contagious. The entire simple illness course takes 17–21 days, starting at the feverish onset (3, 6).

Although measles recovery results in lifetime immunity, the patient paradoxically undergoes temporary immunosuppression during and after acute infection, which is supported by the suppression of delayed-type hypersensitivity responses (4, 7). The primary cause of measles-related morbidity and mortality is pneumonia or gastrointestinal infections, which are typically brought on by secondary bacterial infections brought on by immunosuppression (8).

Although the measles is self-limiting, several serious consequences have been reported. Morbidity and mortality of measles are complex, influenced by vaccination as well as nutritional status; severe results are associated with deficiencies in vitamin A and malnutrition. There is a rare chance that measles will cause problems with the central nervous system (CNS). Acute disseminated encephalomyelitis and primary measles encephalitis are two of the severe diseases that have been reported (7).

The estimated global measles case burden exceeded 9.7 million cases in 2015, with 254,928 reported cases across all six regions of the WHO, for an estimated total of 134,200 measles deaths (7). One known limitation of measles case surveillance is that it is subject to underreporting. Studies have demonstrated underreporting in outbreak settings (7).

The WHO regions with the highest incidence were those in Africa, the Eastern Mediterranean, and Europe in 2014–2015, as a result of significant measles outbreaks. The regions with the highest number of cases reported were Africa (98,621; incidence of 100/million), the Americas (423; 0.6/million), the Eastern Mediterranean region (21,335; 33/million), the European region (25,974; 31/million), South-East Asia (29,109; 17/million), and the Western Pacific region (65,176; 35/million) (4, 7). The region of the Americas verified the elimination of measles in 2016, demonstrating the feasibility of elimination in low- and middle-income countries (1).

Every Member State in all six WHO Regions has set goals to eradicate measles by 2020 or earlier, and the WHO Global Vaccine Action Plan for 2012–2020 has set the target of eliminating measles and rubella in at least five WHO Regions by 2020 (9). Despite ongoing viral incursions from other parts of the world, the Region of the Americas was able to achieve and maintain the eradication of endemic measles transmission in 2002, lasting for over 10 years (10, 11). This outstanding accomplishment has inspired efforts to attain elimination in other regions and resulted in many lessons being learned. The Americas are currently setting the standard for measles prevention, having been the first region to eradicate polio. The event has also brought attention to the continuous difficulties in maintaining elimination; measles is a global issue that affects everyone (11).

Over 11 years (2004–2014), 7,296 samples were collected in Amhara Regional State, with 2,412 (36.7%) testing positive for measles IgM. Those aged 10 years and above were most affected, and in 2014, all 11 zones reported cases, with a peak during the hot, dry season (9). This study aims to investigate the measles outbreak in the Berhet district and North Shewa zone of Ethiopia's Amhara region in 2022.

Methods and materials

Study area and period

The research was conducted in Berehet, one of the 24 districts in North Shewa, Amhara, Ethiopia. It is located 230 km from Addis Ababa, Ethiopia's capital, 830 km from Bahir Dar, the Amhara Region's seat, and 145 km from Debre Berhan Town, which serves as the capital of the North Shewa Zone. The Woreda has a population of 45,349, comprising 20,472 men, 18,723 women, 4,626 individuals aged <5 years, and 1,528 children aged <1 year. Berehet Woreda shares borders with Asagirt Woreda to the north, the Afar region to the east, Haile Mariam Kesem Woreda to the west, and Minjar Woreda and Oromia region Fentalie Woreda to the south. The Woreda consists of nine rural and nine urban kebeles, four health centers, nine health posts, and three medium-sized private clinics. Health posts provide outreach services, while health centers in the district offer static immunization services. The data collection period for the descriptive study was from 13 February

2022 to 20 May 2022. For the case-control study, data for the cases were collected from 4 April 2022 to 10 April 2022 and for the controls from 6 May to 16 May 2022, after the two incubation periods for the cases under investigation (Figure 1).

Study design

We utilized an unmatched case-control study design at a ratio of 1:2 alongside a descriptive cross-sectional study.

Sampling procedures and techniques

The descriptive research included all 101 suspected measles cases that were listed on the line-list. For the case-control study, 60 cases were listed throughout the investigation. We used all 60 cases and 120 controls.

Operational definitions

During the outbreak investigation, we used suspected, confirmed case, epidemiologically linked cases, and measles-related death definitions (12).

Suspected case: A person with fever and maculopapular (non-vesicular) generalized rash and cough or coryza (runny nose) or conjunctivitis (red eyes).

Confirmed case: A possible case that has been confirmed by testing (positive IgM antibody).

Epidemiologically linked case: Any unconfirmed case of suspected measles that is linked (by person, place, and time) to a laboratory-confirmed case; that is, living in the same or neighborhoods where a laboratory-confirmed case is located and where there is a chance of transmission; the rash onsets in the two cases happening within 30 days of each other.

Measles-related death: It is a death that happens in a person who has been diagnosed with confirmed measles and that is not related to any other illness, such as trauma or accident, and happens within 30 days of the rash starting.

Kebele: The smallest administrative unit in Ethiopia.

Variables of the study

Dependent variable

Measles case (Yes/No).

Independent variable

Socio-demographic factors (age, sex, marital status, educational status of the client and mother, and occupational status), vaccination status, contact history, travel history, knowledge of the mode of transmission of measles, and knowledge that measles is vaccine-preventable.

Inclusion and exclusion of cases and controls

Cases: Individuals living in the Berhet district who exhibit clinical indications and symptoms consistent with a proven case, as defined by the national measles guidelines; these cases can be laboratory-verified, suspected, or epidemiologically linked to confirmed cases. Those on paper who did not meet the criteria for a suspected or confirmed case were excluded.

Controls: People who live in the same neighborhood as the case but do not meet the criteria for a measles case in Bereket.

Excluded individuals were those who had a known history of measles disease.

Data collection methods

In the line-listing used for the descriptive study, we identified cases of measles. The national standard case definition was used to identify cases for the case-control study. After locating the cases on the line-list, we interviewed the head of the household, Berhet district health office personnel, health center PHEM focal, and health extension workers. After two incubation periods, data were gathered for controls—those who do not exhibit measles signs and symptoms in the same neighborhood as the case. A structured questionnaire was adopted from the CDC outbreak investigation tool, which comprises three sections (the first section covers socio-demographic information, the second section focuses on clinical features, and the third section explores the associated factors of measles infection). Parents or caregivers were interviewed on behalf of their children if their age was <18 years. Five health personnel participated in data collection, with four health officers as data collectors and one MPH in epidemiology holder as supervisor.

Data processing and management

For the descriptive study, line-list data were utilized. Microsoft Excel 2016 pivot tables were used to clean, arrange, and analyze the data. The case-control data were cleaned, then imported into Epi Info version 7.2 and exported to SPSS version 26 for further analysis.

Data analysis

For the descriptive study, line-list data were cleaned, and Microsoft Excel 2016 pivot tables were used for analysis. Descriptive analysis was employed to calculate and compile measles cases based on person, time, and place.

For the case-control study, all categorical variables were cross-tabulated with the outcome variable, and the frequencies and proportions of each variable were reported for the case and control groups. All explanatory variables that were significantly associated with the outcome variable in the bivariate logistic

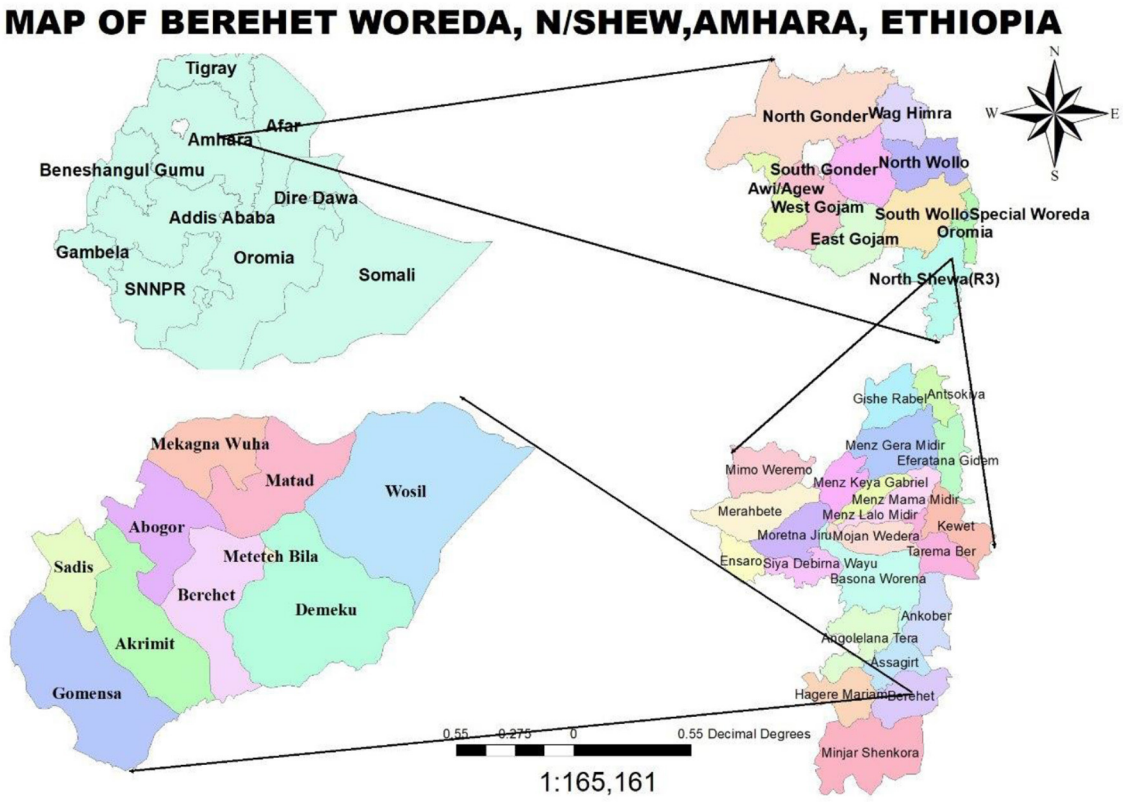

FIGURE 1
Map of the study area.

regression at $p < 0.20$ were added to the multivariable logistic regression model to identify independent factors associated with measles infection.

The model's appropriateness was evaluated using the Omnibus test, the Hosmer and Lemeshow goodness-of-fit test, and other methods. The adjusted odds ratios (AORs) and corresponding confidence intervals (CIs) were used to assess the strength of the correlations between the predictor and outcome variables for a $P < 0.05$. To present the results, tables, images, and text were utilized.

Ethical consideration

An ethical approval support letter was obtained from the Amhara Public Health Institute, which is an autonomous sub-national public health institute. One of the responsibilities of the institute is to provide ethical approval and consent letters for research projects conducted in the region. Additionally, permission was obtained from the Berhet district health office, and we investigated the measles outbreak with documentation from the North Shewa zone health department. After addressing the study objectives and confirming participants' willingness, all involved—participants, parents, or caregivers—provided verbal informed consent, ensuring confidentiality.

Results

Descriptive analysis

Between 13 February 2022 and 20 May 2022, a total of 101 measles cases were reported in the Berhet district, North Shewa, comprising three rural kebeles and one urban kebele. No fatalities were recorded. Among the cases, 68 (67.3%) were male, with respective attack rates of 0.3% for men and 0.1% for women. The age of affected individuals ranged from 5 months to 45 years, with a mean of 9.6 years and a standard deviation of 7.6 (refer to Figure 2).

Description of cases by person

Forty (57.4%) of the cases occurred in the age group of 5–14 years, followed by the age group of 1–4 years (24.8%). No deaths were reported in any age group. The overall attack rate was 20 per 10,000. Most of the cases (67%) were male.

Description of cases by place

The cases were reported from four kebeles: Methbila, Demeqo, Mafudand, and Wosil. The majority of cases (85 or 84.2%) were reported from Methbila kebele,

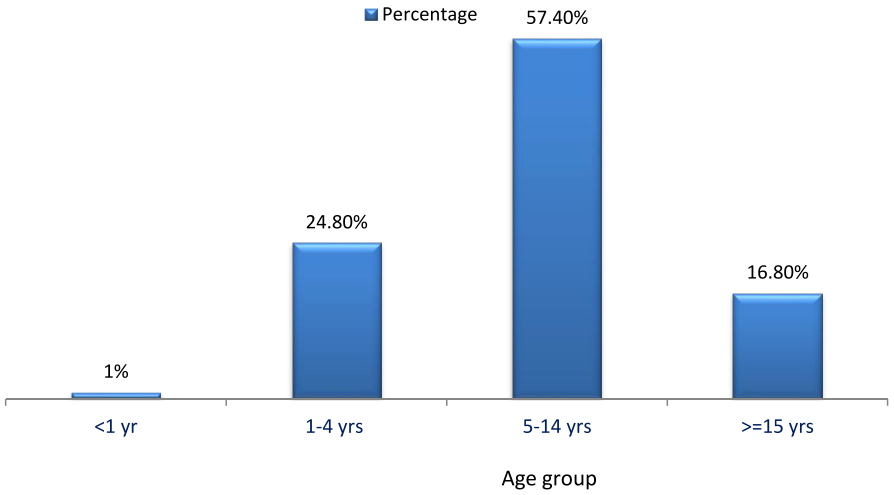

FIGURE 2
Proportions of measles cases by age group in Berehet district, Amhara region, Ethiopia, 2022.

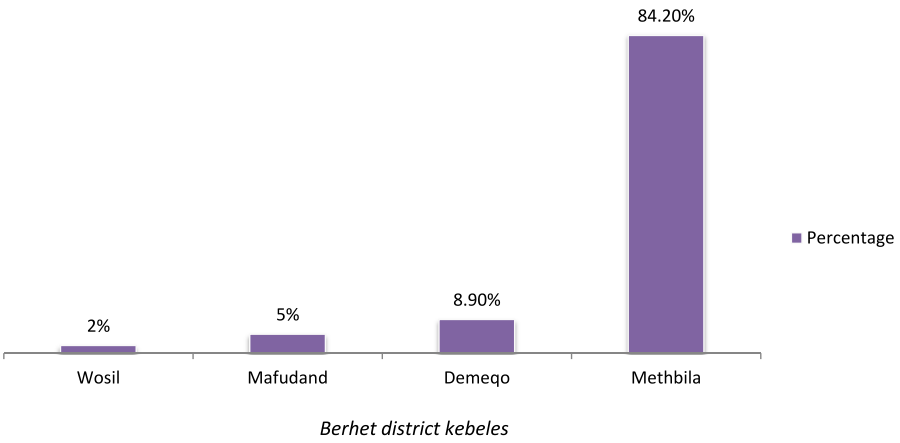

FIGURE 3
Distribution of measles cases by kebele in Berhet district, Amhara, Ethiopia, 2022.

which is the urban kebele in the district (refer to Figure 3).

Description of cases by time

On 27 February 2022, the initial case was observed at the Methbila health center, originating from Methbila kebele, 10 days after the onset of the rash. The index case, a 13-year-old female, had no record of vaccination. The patient had a travel history to a confirmed measles outbreak in an adjacent district before the onset of the rash. The patient did not experience any complications. After the index case, different cases were reported, and five samples were sent to the national laboratory for confirmation on 3 March 2022. The number of cases increased after active surveillance started. The case count experienced a swift rise from the initial week of April to the concluding week of April, as illustrated in Figure 4.

Vaccination status of the cases: Out of the overall cases, 36 (35.6%) had not received the measles vaccine, while 14 (13.9%) either were unaware of their vaccination status or had missing information about it, as depicted in Figure 5.

Vaccination coverage and cold chain management in the Berhet district

Throughout the outbreak investigation, we assessed the cold chain management of both the health office and health facilities in the Berhet district. The district health offices lack refrigeration, with all vaccines stored in the health center.

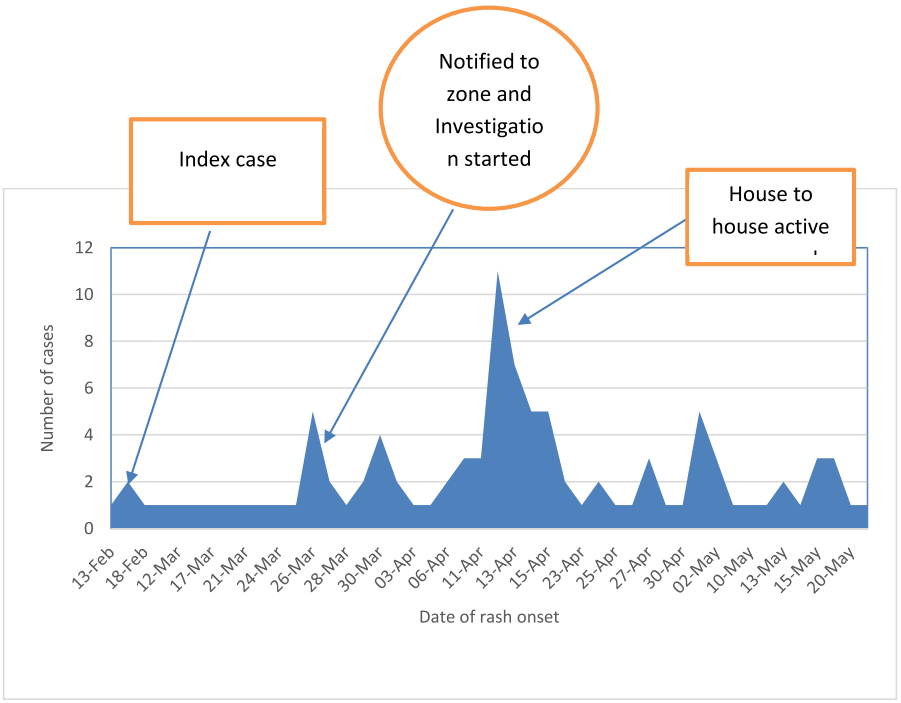

FIGURE 4
Epi Curve of the outbreak based on the date of onset of rash in Berhet district, Amhara region, Ethiopia, 2022.

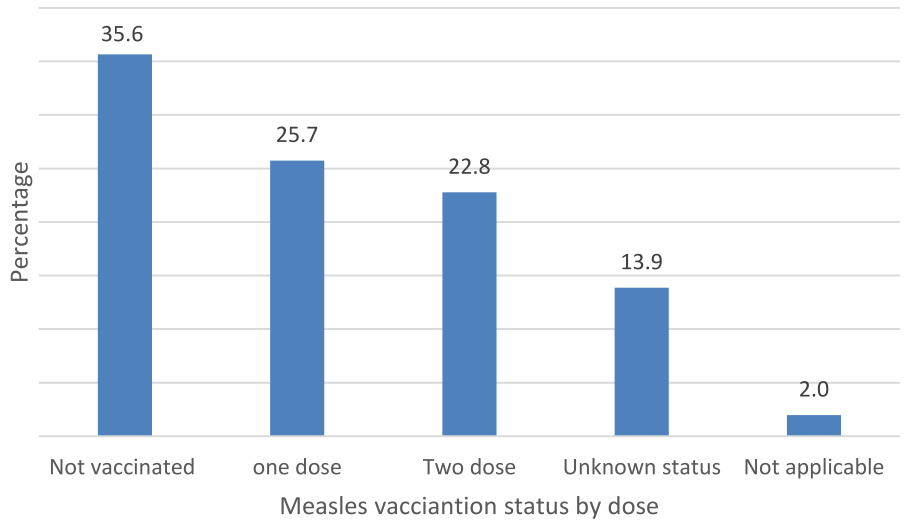

FIGURE 5
Vaccination status of measles cases in Berhet district, Amhara, Ethiopia, 2022.

However, each health center is equipped with a functioning fridge. Among the nine health posts, one does not have a functional fridge. Notably, the district lacks health workers trained in fridge maintenance, with only one EPI focal individual trained in cold chain management. Additionally, the administrative coverage of the district was below 60%, as indicated in Figure 6.

Laboratory results

In March 2022, five blood samples were collected by laboratory technicians from individuals suspected of having measles in the Berhet district to validate the outbreak. Subsequently, these samples were sent to the laboratory at the Ethiopian Public Health Institute for confirmation of cases. Measles IgM antibodies were detected

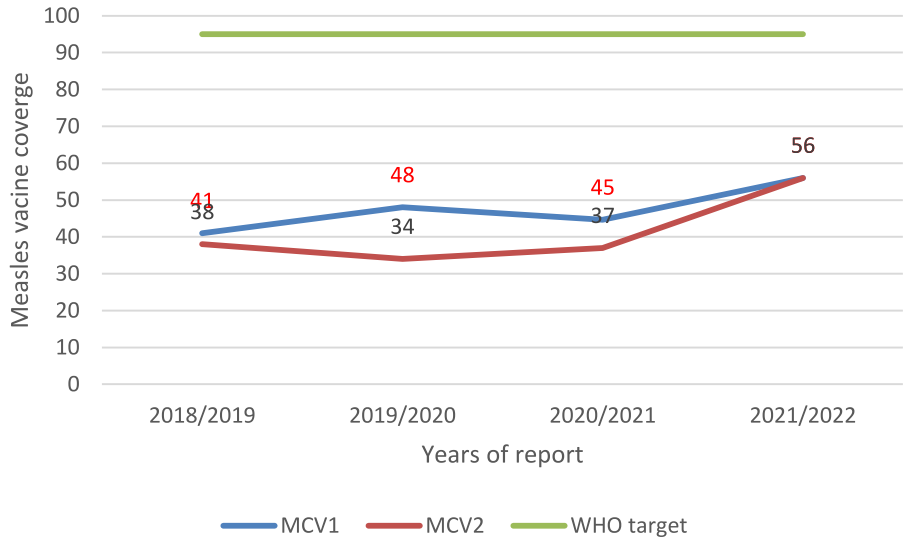

FIGURE 6
Coverage of MCV1 and MCV2 in Berhet district, North Shewa, Amhara region, 2022.

in one out of five samples; the other cases were epidemiologically connected to a known epidemic district nearby. Following the epidemiological and clinical declaration of a measles outbreak, five additional samples were sent to EPHI from various Methbila kebele goes for confirmation; four of the samples were negative, and one was positive for rubella IgM. Five more samples were submitted to confirm whether there had been an outbreak of rubella, but all came back negative. In total, 10 nasopharyngeal swabs were taken from Menjar district, the outbreak source district, to identify the virus strain, and the results revealed the presence of the measles virus strain in four samples.

Analytic epidemiology

During the outbreak investigation, 60 measles cases and 120 recruited controls were asked a range of questions about measles transmission, prevention strategies, affected age groups, immunization status, contact history, travel history, home conditions, health-seeking behavior, and education level. Male participants made up 70% of the cases and 55% of the control group in the study. In terms of age distribution, 55% of controls and 66.1% of cases belonged to the age range of 5–15 years. Notably, 31.4% of cases and 26.9% of controls with vaccination history had a vaccination card. In total, 98% of the patients exhibited measles signs and symptoms. Six variables were found to be significant risk factors for contracting measles infection during the bivariate analysis. These variables included being male, not having received a measles vaccination, having traveled within 7–18 days before the onset of symptoms, having previously come into contact with someone who had the disease, not knowing that measles is a preventable illness, and not knowing the mode of transmission. Variables with $p \leq 0.20$ were included in a multivariable logistic regression analysis to identify factors significantly associated with measles, using a significance threshold of <0.05. Following adjustment for confounding effects in the multivariable model, five variables were identified as significantly associated with measles infection. Notably, a lack of vaccination against measles emerged as an independent risk factor for contracting the disease. A child's chance of contracting measles was 12 times higher among those who had not received the vaccination than in those who had [AOR = 12.06, 95% CI (3.12–46.52)]. People who had previously been to a location where measles was active were almost six times more likely to contract the illness than people who had never visited [AOR = 5.73, 95% CI (1.78–18.38)]. An independent risk factor for contracting measles was having contact with a patient who had the illness. When compared to those who had no contact history, the odds of contracting measles were ten times greater for those who had contact with someone who had the signs and symptoms of the illness [AOR = 10.3, 95% CI (3.48–30.5)]. Additionally, it was discovered that being aware of the measles' route of transmission protected against contracting the infection. People who knew the measles' mode of transmission, as learned from their mothers or caregivers, were 83.6% less likely to contract the illness than people who did not [AOR = 0.164, 95% CI (0.049–0.55)]. Similarly, 76.7% fewer people had measles than those who believed it was not a preventable disease. These people knew that measles was a preventable disease [AOR = 0.233, 95% CI (0.67–0.811)] (Table 1).

Outbreak response

Following the announcement of a measles outbreak in the Berhet district, the district health office of Berhet convened an emergency meeting, provided onsite orientation, assigned a team to assist the primary health facility rapid response team,

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TABLE 1 Measles outbreak analysis, Berhet district, Amhara region, Ethiopia, May 2022.

Variables	Measles status		Bivariable analysis		Multivariable analysis	
	Case N. (%)	Control N. (%)	COR (95% CI)	*P*-value	AOR (95% CI)	*P*-value
Sex						
Female	18 (30%)	54 (45%)	0.524 (0.271–1.012)	0.054*	0.349 (0.115–1.064)	0.064
Male	42 (70%)	66 (55%)	1		1	
Age						
<1 year	1 (1.7%)	1 (0.8%)	1			
1–4 years	7 (11.7%)	12 (10%)	0.583 (0.031–10.8)	0.718		
5–14 years	40 (66.7%)	66 (55%)	0.606 (0.037–9.96)	0.726		
≥15 years	12 (20%)	41 (34.2%)	0.293 (0.17–5.03)	0.397		
Distance from a health facility						
<5 km	38 (63.3%)	74 (61.7%)	1			
>5 km	22 (36.7%)	46 (38.3%)	0.931 (0.49–1.76)	0.828		
Vaccination status						
Yes	21 (35%)	63 (52.5%)	1		1	
No	23 (38.3%)	11 (52.5%)	6.273 (2.6–15)	0.000*	12.06 (3.12–46.52)	0.000*
Unknown status	16 (26.7%)	46 (38.3%)	0.912 (1.04–0.94)	0.912	0.851 (0.27–2.6)	0.782
Travel time (7–18 days)						
Yes	52 (86.7%)	52 (43.3%)	8.5 (3.7–19.4)	0.000*	5.73 (1.78–18.38)	0.003*
No	8 (13.3%)	68 (56.7%)	1			
Contact history						
Yes	53 (88.3%)	31 (25.8%)	21.7 (8.9–52.8)	0.000*	10.3 (3.48–30.5)	0.000*
No	7 (11.7%)	89 (74.2%)	1			
Do you think measles is preventable?						
Yes	37 (61.7%)	108 (90%)	0.179 (0.81–0.394)	0.000*	0.233 (0.67–0.811)	0.022*
No	23 (38.3%)	12 (10%)	1			
Know the transmission mood of measles						
Yes	31 (51.7%)	110 (91.7%)	0.097 (0.43–0.221)	0.000*	0.164 (0.049–0.55)	0.003*
No	29 (48.3%)	10 (8.3%)	1			

Unknown status: Either the individuals affected by the outbreak have not been vaccinated against measles, or their vaccination status is unknown due to incomplete records or other factors. In bivariable analysis *(p-value < 0.20), while in multivariable analysis *(p-value < 0.05).

assessed the situation, improved the active case search, provided the standard format for a measles line-list, and reviewed the available resources to determine whether more were needed. Alerts were sent out, and the reporting kebele was asked to provide more information to the fast-reaction team. The district health office, in collaboration with the health center's RRT and HEW, actively searched for cases of measles in local communities, schools, religious institutions, and private clinics. The assessment team from the North Shewa Zone Health Department was then dispatched to the Berhet District Health Office to investigate the incident. The district health officer and the outbreak investigation team met and discussed how to execute measles outbreak prevention and control. Approximately 20 cases of measles were found in the neighborhoods during an inquiry and active surveillance.

Case management

All suspected cases were treated using vitamin A capsules. One dose was given at the time of diagnosis, and for adult patients, the second dose was given to take the next day, with the last dose administered by day 14. Antibiotics were administered to treat carriers through house-to-house visits based on their clinical manifestations. Community mobilization activities were carried out in collaboration with local community representatives and kebele leaders to encourage routine vaccine uptake. An active case search was conducted in the community by staff members of the health center, health post, zonal, and district health departments to prevent the spread of the disease to other kebeles. Health centers received vitamin A to treat patients without charging for their care, and all medical staff received technical support for managing

cases, documenting information, and reporting findings. To halt the spread of the measles outbreak and reduce morbidity, cases were treated.

A district-wide mass vaccination campaign was launched for high-risk age groups, including 9 months to 2 years and up to 15 years, in Methbila kebele, the area with the highest measles incidence.

Discussion

This study aimed to delineate the extent of the measles outbreak and identify the factors contributing to disease contraction in the Berhet district. After detecting three serum samples with positive measles-specific IgM antibodies in the neighboring district, along with one confirmed measles case and other cases linked epidemiologically to the laboratory-confirmed ones, the outbreak was officially declared. The results of the descriptive analysis demonstrate that the measles outbreak had an overall attack rate of 20 cases per 10,000 people, and the age group most affected was from 5 to 15 years. We found a lower attack rate than the attack rate of the measles outbreak investigation conducted in the Ginner district, 63/10,000 (13), and higher than a study in the Kabridehar district of Somali Regional State, Ethiopia, which reported an AR of 4/10,000 (14), and a study in Guji zone, Oromia region, 8.1/10,000 (15). The concentration of vulnerable individuals likely accelerated the spread of the disease, leading to a higher attack rate. Additionally, the district's vaccination rates for MCV1 and MCV2 have been below 60% for the past 3 years due to low vaccination rates. The widespread, high attack rates across a larger age range may indicate that routine immunization rates have been consistently low for a number of years, which may have contributed to the present outbreak. This conclusion implies the need for routine immunization and supplementary immunization activities (SIAs), as well as for monitoring the buildup of susceptible persons, to safeguard both target and non-target age groups (16). No fatalities have been documented in this outbreak. The current case fatality rate (CFR) estimates utilized by the World Health Organization (WHO) in low-income countries span from 0.05% to 6% (17). In complex emergencies or isolated areas where there is either low natural immunity or low vaccination coverage, the CFR is often between 10% and 30% (18, 19). This zero CFR observed in our study was totally different from different findings of measles outbreak investigations conducted in Ethiopia (3, 13, 15, 20). It is possible that early reactions and case management were implemented in the impacted kebeles, which is why this study had no fatalities. It could also be because deaths that occur within the community go unreported. A child's chance of contracting measles was 12 times higher for those who had not received the vaccination compared to those who had. This is consistent with the results of a case-control study carried out in the Somali Regional State, Ethiopia, in the districts of Kabridehar and Kabridehar town, where it was found that having received the measles vaccine protects against contracting the disease (3, 14, 21).

Likewise, our results align with a case-control study conducted in Uganda, suggesting that the lack of measles vaccination was a primary factor contributing to most measles infections in children (22). This is because measles vaccination is crucial for preventing measles infection (23, 24). Achieving 95% population immunity is essential to prevent measles outbreaks, disrupt transmission, and promote the elimination of measles (7). This discovery implies that the measles outbreak may be attributed to the accumulation of individuals susceptible to measles infection. Individuals with a history of traveling to regions with active measles were six times more likely to contract measles compared to those who did not travel. This observation is corroborated by comparable studies conducted in the Bale zone, Oromia, Ethiopia, and in the Ginner district (13, 20).

This arises from either direct contact with an infected individual or an elevated risk of contracting the disease in an area currently experiencing measles activity. Another distinct risk factor for measles acquisition was exposure to an individual already diagnosed with the illness. Those who had direct contact with a measles case patient faced an almost tenfold higher risk of contracting the disease compared to those with no prior contact. This observation is substantiated by the findings of a case-control study conducted during an outbreak investigation in Southwest Ethiopia and by other studies in various rural districts of Ethiopia (13, 15, 20, 21, 25) and other similar study done in Uganda (9), showing the likelihood of contracting the disease was more than three times higher when in touch with a case. This is due to the transmission of measles through respiratory droplets or direct/indirect contact with the nasal and throat secretions of infected individuals. Moreover, the secondary attack rate for measles surpasses 90% in the presence of susceptible individuals (6).

It has been discovered that mothers' and caregivers' knowledge about measles transmission protects against contracting the illness. The findings align with a study conducted in the Artuma Fursi Oromia zone of the Amhara region of Ethiopia. In that study, it was observed that women who recognized the importance of measles vaccination were three times more likely to immunize their children compared to mothers who were not aware of the vaccine (25). A study in ten high-burden countries shows similar findings (23). Furthermore, the research carried out in Southwest Ethiopia indicated that mothers with sufficient knowledge of preventive measures against measles were at a reduced risk of contracting the infection (26).

Limitations of the study

Most individuals did not have vaccination records. We asked the participants or caregivers whether their children had received the measles vaccination at 9 months of age or older on the upper left arm. The lack of a vaccination card made it difficult to ascertain the precise date and status of immunization, which is likely why recall bias exists.

Conclusion and recommendations

The outbreak exhibited a higher incidence rate, affecting a wider age range, predominantly children aged 5–14 years. More than 35% of cases lacked measles vaccination, revealing a low administrative vaccination rate. Several factors contributed to

the outbreak, including non-vaccination, travel to areas with active measles, contact with cases, and insufficient knowledge among mothers and caregivers about measles transmission and prevention. To address this, efforts should focus on reaching remote areas, enhancing surveillance, supporting community immunization drives, and establishing isolation facilities. The vaccination program should extend measles immunization up to the age of 15 years, coordinated by the APHI and regional health authorities.

Data availability statement

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

Author contributions

YS: Visualization, Data curation, Writing – review & editing, Methodology, Formal analysis, Conceptualization. AA: Writing – original draft, Project administration, Visualization, Methodology, Formal analysis, Data curation, Conceptualization. TT: Writing – original draft, Resources, Methodology, Formal analysis, Data curation, Conceptualization. SF: Writing – review & editing, Writing – original draft, Methodology, Formal Analysis, Conceptualization.

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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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Unveiling the occurrence of COVID-19 in a diverse Bangladeshi population during the pandemic

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Introduction: COVID-19 pandemic hit Bangladesh with relatively low intensity, unlike its neighbors India and European countries and USA.

Methods: The present report included data of 8,480 individuals tested for COVID-19 RT-PCR of the workers and officials from readymade garments (RMG) industry in Chandra area in Gazipur. The present data looked into the clinic-demographic factors associated with the susceptibility of the condition.

Result: The data elucidated the susceptibility of the individuals to SARS-CoV-2 based on age, gender, pre-existing health conditions, and the presence of symptoms. It was observed that individuals aged over 60 had the highest rate of COVID-19 positivity, and men exhibited a higher infection rate compared to women. Regardless of age, fever and cough were the most frequently reported symptoms. Two-thirds of the individuals included in this report appeared to be asymptomatic carriers. The prevalence of comorbidities among individuals who tested positive for COVID-19 was notably higher, and this exhibited a gender-specific pattern.

Discussion: Although our study provides important epidemiological insights into the initial year of the pandemic among Bangladeshi populations, it can also add value for future drug and vaccine development. However, it is essential to acknowledge the limitations like - restriction of public movement, unavailability of vehicle yielding a selection bias, due to the lockdown conditions imposed owing to the pandemic and the diverse characteristics of the participants. The report emphasizes the significance of figuring out how age, gender, and underlying health conditions impact susceptibility to and transmission of COVID-19, thereby providing valuable insights for public health strategies and future research initiatives.

KEYWORDS

SARS-CoV-2, COVID-19, Public Health, RMG, Bangladesh

1 Introduction

The 21st century was introduced with the two most contagious global epidemics caused by the corona virus family, Severe Acute Respiratory Syndrome (SARS) in 2001–2003 and Middle East Respiratory Syndrome (MERS) in 2012–2015. The SARS was reported first in November 2002 in Foshan, Guangdong, China, and spread to 29 countries worldwide, leaving 774 death cases (1, 2). Since the MERS was first isolated in 2012, 27 countries reported cases till 2019 with 858 deaths (3, 4). Afterward, several different species of corona virus have been sequenced and categorized into four major genera known as *Alphacoronavirus, Betacoronavirus, Gammacoronavirus and Deltacoronavirus* (5).

The corona virus genome is known as the largest (comprised of ~32 kbp) among the plus-strand (except retrovirus) RNA viruses (HCov-229E: 27,317 nt to MHV-A59: 31,357 nt). Their unique replication strategy is usually associated with various diseases in versatile range of hosts (6). In December 2019, in Wuhan city, China, the most pathogenic and transmissible, a novel member, SARS-CoV-2 (*Betacoronavirus*), caused an outbreak termed COVID-19 (Corona Virus Disease 2019) that turned into a global pandemic by 2020 (7). The SARS-CoV-2 is the seventh member among the corona virus family infecting humans (8) that is confirmed to be 96% identical to a bat corona virus (nonpathogenic to humans) by Whole Genome Sequencing (9, 10). The SARS-CoV-2 uses the human Angiotensin Converting Enzyme-2 (ACE II) as a cellular entry receptor, thus being infectious to the human (11, 12). This novel virus has caused the unprecedented outbreak of COVID-19 that infected cumulatively about 216 million people and nearly 4.5 million death cases by August 2021 worldwide (13). Several COVID-19 diagnostic procedures have been introduced targeting either ORF (Open Reading Frame) encoding 27 different non-structural proteins or other conserved sequences of major structural proteins such as the nucleocapsid protein (N), spike surface glycoprotein (S), membrane protein (M) and small envelope protein (E) (14, 15).

The principal mode of transmission of corona virus is respiratory droplets (coughing or sneezing) with close contact an infected person and possibly a long time exposure in a closed environment by aerosol transmission (16–18). A wide spectrum of clinical complications were identified in COVID-19 patients, and few of them could not have recognized any symptoms (asymptomatic) (10). In mild to moderate cases, the most observed symptoms were fever, cough, rhinorrhea (runny nose), pharyngalgia (sore throat), myalgia (muscle pain), headache, fatigue, dyspnea (shortness of breathing), ageusia (loss of taste), anosmia (loss of smell) and diarrhea. In particular, severe cases of COVID-19 were identified as critical conditions with the Acute Respiratory Distress Syndrome (ARDS) that require extended care in an Intensive Care Unit (ICU) with ventilation support, septic shock, coagulopathy, arrhythmias, thrombocytopenia, and multiple organ dysfunction syndrome (19, 20).

Chronological age is one of the most important predictors of COVID-19 disease severity, especially a reliable biomarker for vaccine design with the highest patient satisfaction (21). However, comorbidities, for instance, diabetes mellitus, hypertension, asthma, Cardio Vascular Disease (CVD), or renal diseases, are observed to be associated with higher mortality rates, indicating that the biological age is a more pertinent risk factor for disease severity than the chronological age (22, 23). Most of the studies have reported male-biased COVID-19 cases, in addition to hospitalization, ventilation support, and fatality rate measurement of critical conditions (24, 25).

In early March of 2020, the Institute of Epidemiology, Disease Control and Research (IEDCR) first identified three cases of SARS-CoV-2 in Bangladesh. The Government imposed a nationwide lockdown in late March of 2020 (26, 27). To keep the GDP (Gross Domestic Product) trending, private sectors, especially readymade garments industries (RMG), play a key role in earning foreign currency. In the lockdown situation, running the garment factories and maintaining the health and safety of the workers was a great challenge. Hence, following the WHO guidelines, a fully dedicated COVID-19 RT-PCR laboratory was established in May 2020, closest to the EPZ (Export Processing Zone) near the capital Dhaka in Bangladesh. A several contagious variants of SARS-CoV-2 evolved globally over the time, and classified by Whole Genome Sequencing process (28). Even after mass vaccination program, a swift surge of the delta variant, later replaced by the omicron variant of SARS-CoV-2 caused a fatal increase in hospitalization (29). Furthermore, different types sub-variant of the omicron (Eris, Pirola) are identified in symptomatic subjects (30, 31). Hence, it is obvious that the COVID-19 has not been fully eradicated.

There is a high chance to get the positive result even in randomly selected asymptomatic subject due to the frequent mutation(s) of the virus. And, these mutations can be compared with the demographic and clinical data of the COVID-19 for further effective vaccine or drug developments. Recently, it has been reported using the data of 2020–2021 that even after the treatment with COVID-19 convalescent plasma, the post covid complications has been reduced compared with the demographic and clinical characteristics but could not be absolutely eradicated (32). Here, we are presenting the random population-based screening data of COVID-19 from June 2020 to August 2021 which can be used as a baseline data to understand the characteristics and transmission of the disease in Bangladesh.

2 Materials and methods

2.1 Study design

With the approval of the ethical review committee of the Department of Biochemistry and Molecular Biology, University of Dhaka, we performed a cross-sectional and descriptive population-based study to understand the baseline characteristics of COVID-19 and its epidemiology among the population predominantly from Bangladeshi garments and industrial sectors. This study evaluates a well-designed comparison between the prevalence rate and the COVID-19-associated symptoms and comorbidities according to the gender of various age groups.

2.2 Sample and data collection

From June 2020 to August 2021 (15 months), 8504 samples were diagnosed at our laboratory (Dr. Farida Huq Memorial Ibrahim General Hospital COVID-19 Diagnostic Laboratory, Chandra, Gazipur). Of these 8,504 samples, 24 were run as the Internal Laboratory Quality Control Assay. Hence, data from 8,480 (eight thousand four hundred and eighty) samples were compiled for this study (Figure 1). The clinical information was uniformly recorded

before the sample collection along with written consent of every individual for future research purposes. Accumulated information was scrutinized under several variables: demographic data (age, gender, and residence area), and clinical data (exposure history, symptoms, comorbidities, or any undergoing medication, including the COVID-19 vaccine). The whole population is divided into four age groups (Table 1) with additional subdivision by gender as male and female.

2.3 Specimen collection

Following the instruction of the CDC (Centers for Disease Control and Prevention), both the Oropharyngeal Swab (OPS) and Nasopharyngeal Swab (NPS) samples were collected in the sterile VTM (Viral Transport Medium) and transported to the molecular laboratory maintaining the cold chain (33). Each sample tube was labeled with the unique Laboratory ID and sanitized before being delivered inside the double-door protected BSL-II lab via the dynamic pass-box.

2.4 Laboratory investigation

The Reverse Transcriptase Polymerase Chain Reaction (RT-PCR) is a gold standard molecular technique for identifying the SARS-CoV-2 from OPS and NPS samples. The test kit used was from Primer

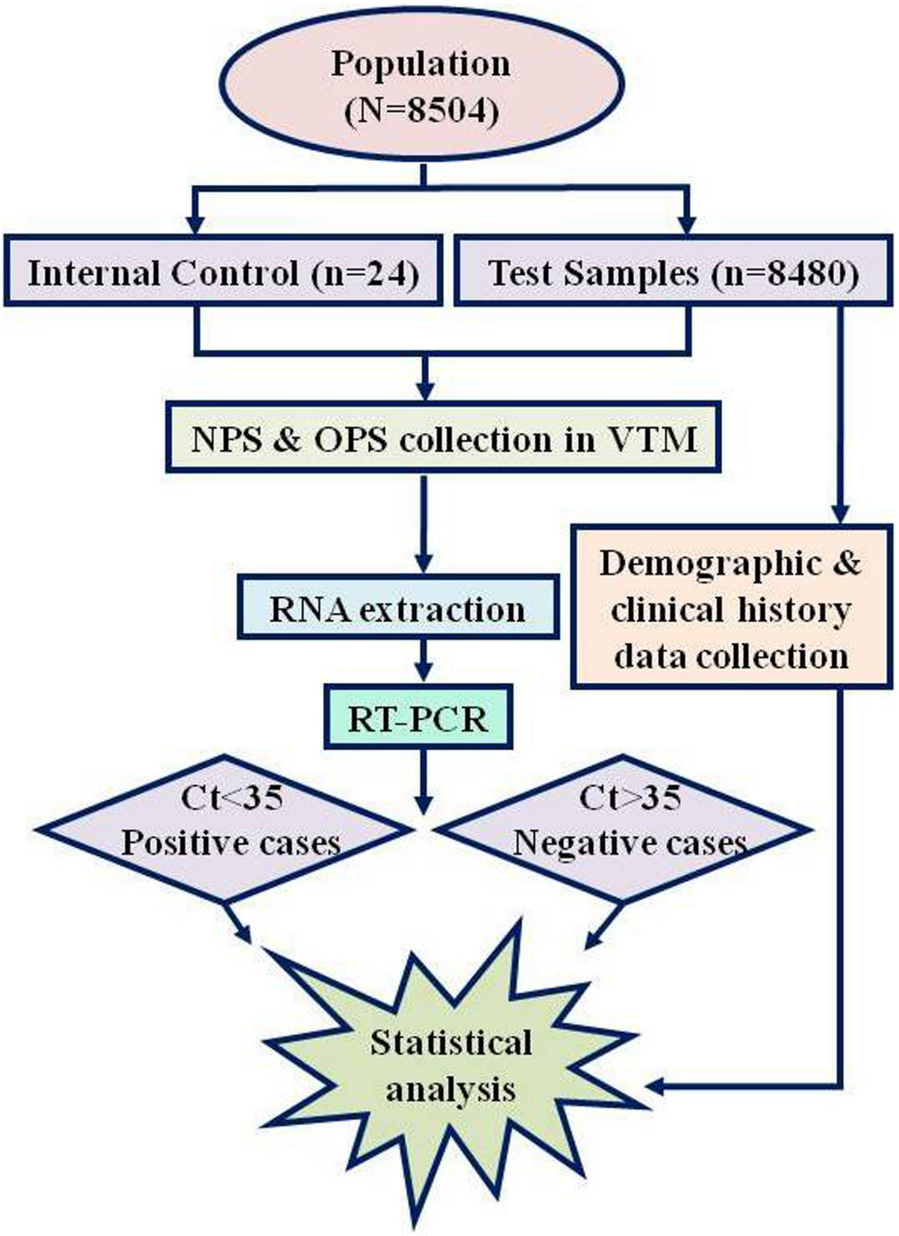


FIGURE 1
Flow diagram of COVID-19 study.

TABLE 1 Demographic characteristics of study participants with the prevalence of COVID-19 cases.

Characteristics	Study participants (*N* = 8,480)	COVID-19 Positive cases
	N (%)	(%)
Age (years)		
Below 20 years	710 (8.37)	19.30 (137/710)
21–40 Years	5,762 (67.95)	19.77 (1,139/5762)
41–60 Years	1789 (21.10)	24.09 (431/1789)
Above 61 Years	219 (2.58)	36.53 (80/219)
Sex		
Male	5,669 (66.85)	23.09 (1,309/5669)
Female	2,811 (33.15)	17 (478/2811)
Residence		
Gazipur	3,589 (42.32)	25.94 (931/3589)
Dhaka	4,334 (51.11)	16.5 (715/4334)
Tangail	274 (3.23)	40.51 (111/274)
Others	283 (3.34)	10.6 (30/283)
Symptoms		
Asymptomatic	5,738 (67.67)	13.96 (801/5738)
Symptomatic	2,742 (32.33)	35.96 (986/2742)
Comorbidity		
No	7,838 (92.43)	20.29 (1,590/7838)
Yes	642 (7.57)	30.69 (197/642)
COVID-19 vaccination		
Non-vaccinated	8,308 (97.97)	21.07 (1751/8308)
Vaccinated	172 (2.03)	20.93 (36/172)

Methods: Pearson chi-squared test was performed to test the measures of association between outcome and explanatory variables ($p < 0.001$).

Design Ltd., UK. The RNA was extracted from the cell-free fluid samples either manually or automatically. The amplification kit was designed based on the TaqMan RT-PCR principle targeting two genes-Nucleocapsid (N) and ORF1ab regions, labeled probes with FAM (Fluorescein amidites, 465-510 nm) and ROX (6-carboxyl-X-Rhodamine, 576–601 nm) fluorescent dyes for the two amplicons. Following the literature on the detection kit, the amplification was performed using Bio-Rad CFX (USA).

For the validation of the RT-PCR, Negative Extraction Control (NEC) is required to produce cycle threshold, Ct < 30 in the VIC (2′-chloro-7′-phenyl-1, 4-dichloro-6-carboxyfluorescein) or HEX (Hexachloro fluorescein, 533–580 nm) channel, whereas Positive Control Template (PCT) dilution at 1.7 copies/µl produces Ct of 14–22 in the FAM channel. With this comparison, the Ct value of Internal Extraction Control (IEC) produced by the individual's sample should be within the range of the Ct value peaked by the NEC (±6). The Ct < 35 was considered positive for SARS-CoV-2 (Figure 1). Internal Laboratory Quality Control Assay was regularly conducted and compared with another COVID-19-dedicated laboratory. Furthermore, for RT-PCR test result analysis validation, representative samples were delivered to the Institute of Epidemiology, Disease Control & Research (IEDCR), Mohakhali, Dhaka, every month who runs the national COVID-19 validation and optimization surveillance under the Ministry of Health and Family Welfare of Bangladesh. A concordance of ≥95% was obtained while samples were tested in a reference laboratory to ensure the quality of the test and result analysis parameters.

2.5 Data analysis

Pearson chi-squared test was performed to test the measures of association between outcome and explanatory variables, and a binary logistic regression model was performed to examine the unadjusted and adjusted effects of covariates on COVID-19 (34). All statistical analyses were performed using STATA 14.

3 Results

3.1 Demographic analysis

In the present study, both symptomatic and asymptomatic people randomly visited the laboratory requesting for COVID-19 test from different zones, mainly from Gazipur (42.32%), Dhaka (51.11%), Tangail (3.23%), and other areas (3.34%) of Bangladesh. Despite the majority of samples being gathered from the Dhaka region, the Gazipur area exhibited a higher COVID-19 prevalence, with Tangail recording the highest prevalence at 16.50, 25.94, and 40.51%, respectively ($p < 0.001$) (Table 1). Out of the samples received, approximately two-thirds were male (66.85%), and they exhibited a COVID-19 positivity rate of 23.09% ($p < 0.001$). In contrast, female participants, accounting for 33.15% of the samples, displayed a COVID-19 positivity rate of 17.00% ($p < 0.001$) (Table 1).

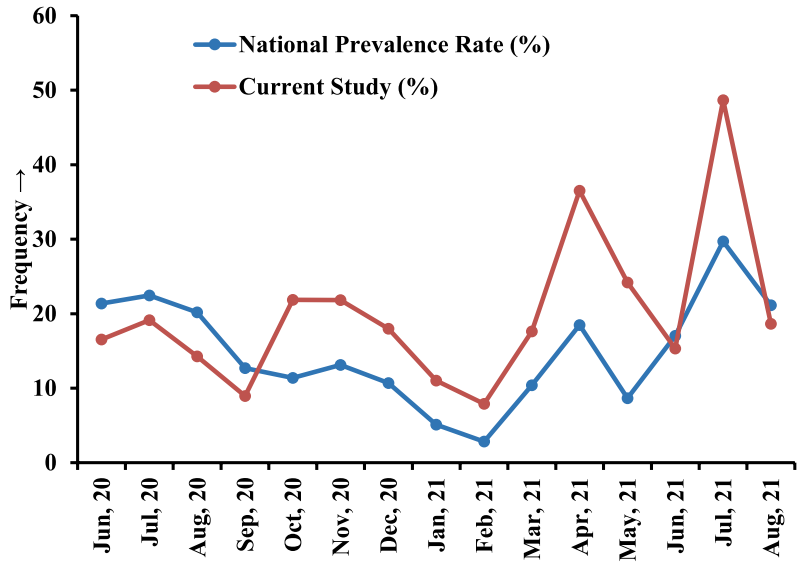
The test-participants' age spanned from 1.5 months to 95 years, with a mean age of 33.9 ± 11.8 years. They were categorized into four groups based on age, below 20 years (8.37%), 20–40 years (67.95%), 40–60 years (21.10%), and above 60 years (2.58%). The higher prevalence rate of COVID-19 was observed in individuals belong to higher age group; 19.30, 19.77, 24.09, 36.53%, respectively ($p < 0.001$) (Table 1). Nevertheless, the subjects randomly enrolled in our study within 15 months, showed similar prevalence patterns (month-wise) with the national prevalence rate (Figure 2).

After the initiation of mass COVID-19 vaccination on early February 2021 in Bangladesh, we recorded only 2.03% vaccinated cases, and the positivity rate was almost similar for both vaccinated (20.93%) and non-vaccinated (21.08%) subjects (Table 1).

3.2 Clinical characteristics of SARS-CoV-2 positive subjects

Almost one-third of the total population (32.33%) showed mild to severe COVID-19-associated symptoms such as fever, cough, runny nose, headache, muscle pain, breathing problems, nausea, vomiting, abdominal pain, sore throat, diarrhea, loss of taste, loss of smell, and weakness (Table 1; Figure 3). Among these symptomatic population 35.96% were diagnosed as COVID-19 positive whereas 13.96% showed in asymptomatic subjects ($p < 0.001$) (Table 1).

Analyzing the symptoms associated with COVID-19, fever (57.66%) and cough (55.07%) were counted as the highest along with headache



Difference	Test Statistic	p-value	Decision
Mean	t −1.5186	0.1401	Do not Reject H (p-value >0.05)
Variance	F 0.4791	0.1810	Do not Reject H (p-value >0.05)

FIGURE 2
Prevalence of COVID-19 in the laboratory compared with the national scenario. Within the 15 months (June 2020–August 2021) study period, this graph showed a similar month-wise pattern to the national frequency. T-test and variance (F) tests were performed to check whether any difference exists between our laboratory data and the national case frequency rate where no significant difference was found.

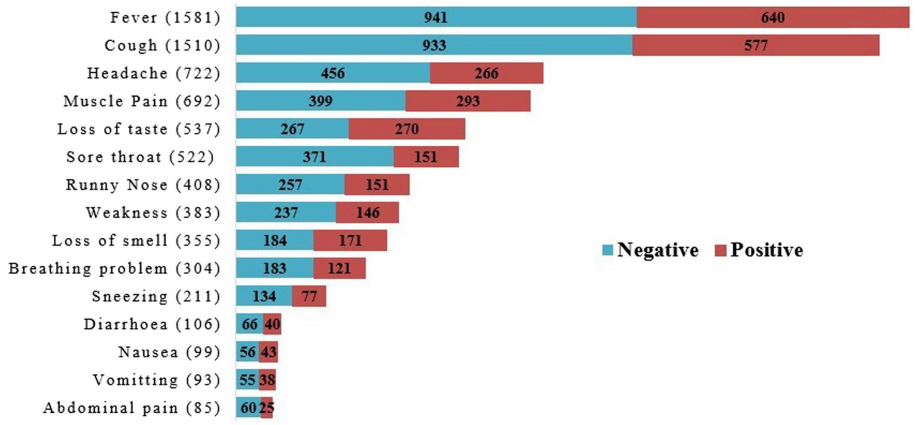


FIGURE 3
Symptoms associated with COVID-19 (both positive and negative cases) in study population.

(26.33%) and muscle pain (25.23%) (Figure 3). Relatively lower number of subjects noticed two of the most relevant COVID-19 symptoms-loss of taste (19.58%), and loss of smell (12.95%) despite, around 50% cases were found positive for both symptoms (Figure 3). Rest of the nine symptoms occurred in about 30–40% positive cases, gradually as nausea (43.43%), vomiting (40.86%), weakness (38.12%), diarrhea (37.74%), runny nose (37%), sneezing (36.49%), breathing problem (39.80%), abdominal pain (29.41%) and sore throat (28.93%) (Figure 3).

3.3 Gender and comorbidities relating to COVID-19 cases

Gender variation showed a noticeable difference in RT-PCR results, presence of symptoms, and pre-existing medical history (Tables 1, 2). With the observation of the RT-PCR results of the whole population (N=8480), two-thirds of positive samples were from male individuals (n=1309, 15.44%) compared with the females (n=478,

5.64%) (Table 1). Comorbidities were reported in 7.57% of the total population, wherein 30.69% were tested as COVID-19 positive (Table 1). The reported comorbidities were asthma, diabetes, hypertension, cardiovascular diseases, chronic renal diseases, immunocompromised, neural, and other diseases (constipation, typhoid, spinal pain, jaundice, sinusitis, major surgery, etc.).

Among the comorbidities individuals, diabetes mellitus (47.19%) and hypertension (46.57%) upheld the same highest frequency, followed by asthma (18.22%). Male biased cross tabulation was analyzed in each of the parameters: 78.21% diabetes, 79.26% hypertension, 67.52% asthma, 86.79% cardiac diseases, and 66.67% neural diseases showed being doubled than the female respondent. Immunocompromised patients (1.09%) were found to have the highest positivity rate in females (71.43%), along with the confirmed cases of tonsilitis (100%) and hypothyroidism (50%) (Table 2).

3.4 Association of covariates with the COVID-19

A binary logistic regression model was performed to examine the adjusted and unadjusted influences (the adjusted odds ratios-AOR and unadjusted odds ratios-UOR) along with the 95% confidence intervals of demographic and health-related factors in the occurrence of corona virus disease (Table 3). Respondent's residence, gender, age, and symptoms of COVID-19 were found to have a significant association with COVID-19 before or after adjusting the effects of covariates.

Regarding the geographical distribution of COVID-19 cases, respondents from Tangail had 2.48 times (AOR: 2.48, 95% CI: 1.90, 3.24), and from Gazipur had 1.48 times (AOR: 1.48 and 95% CI: 1.32, 1.66) more likelihood to be positive for COVID-19 compared to respondents from Dhaka regions. Older people had a higher risk of COVID-19 as the analysis depicted that individuals with 60 or older age had 95% (AOR: 1.95, 95% CI: 1.36, 2.79) higher odds of being COVID-19 positive compared to the individual with the age of below 20 years old (Table 3).

Male individuals were 42% (AOR: 1.42, 95% CI: 1.25, 1.61) more likely to be affected by COVID-19 compared to females. Furthermore, the respondents with symptoms had 3.22 times more likelihood to suffer from COVID-19 (AOR: 3.22, 95% CI: 2.88, 3.61) than the asymptomatic individuals (Table 3). The effect of comorbidity on COVID-19 was found to be significant when the effects of other factors were not controlled. However, there is no significant association between comorbidity and COVID-19 exposure when the effects of other covariates were considered (Table 3).

TABLE 2 Different comorbidities in gender variation.

Comorbidities (n = 642, 7.57%)**		Male (n = 483, 75.23%)	Female (n = 159, 24.76%)
Diabetes mellitus	(n = 303, 47.20%)	237 (78.21%)	66 (21.78%)
Hypertension	(n = 299, 46.57%)	237 (79.26%)	62 (20.74%)
Asthma	(n = 117, 18.22%)	79 (67.52%)	38 (32.48%)
CVD	(n = 53, 8.26%)	46 (86.79%)	7 (13.21%)
Renal disease	(n = 21, 3.27%)	16 (76.19%)	5 (23.81%)
Cancer	(n = 13, 2.02%)	6 (46.15%)	7 (53.85%)
Immunocompromised	(n = 7, 1.09%)	2 (28.57%)	5 (71.43%)
Ulcer	(n = 4, 0.62%)	3 (75%)	1 (25%)
Tonsillitis	(n = 3, 0.47%)	0	3 (100%)
Neural disease	(n = 3, 0.47%)	2 (66.67%)	1 (33.33%)
Hypothyroidism	(n = 2, 0.31%)	1 (50%)	1 (50%)
Others	(n = 27, 4.21%)	13 (48.15%)	14 (51.85%)

**Percentages and totals are based on respondents.
(Comorbidities are dichotomy groups tabulated at value 1).

4 Discussion

Over the last 50 years, several corona virus species have introduced various human and livestock diseases. Due to their recombination capability, random mutation, and multiple species infection ability, new variants continue to strike affecting human health. The novel SARS-CoV-2 corona virus has threatened human life and global health, causing the COVID-19 pandemic since December 2019. The report has focused on investigating the various aspects of this viral infections and the clinical outcome on key epidemiological parameters by interpreting the clinical history of every individual who has given samples for the COVID-19 test at the laboratory during the nationwide lockdown situation in Bangladesh.

The pre-requisite condition to reopen the readymade garments companies for their regular production and exportation process, was to ensure a COVID-19 negative office environment (35, 36). We established the laboratory exclusively for the detection of COVID-19, and diagnosed random samples from a large population (N = 8480) from several garments and industries around the EPZ (Dhaka Export Processing Zones) at Gazipur for occupational health and safety purposes. As per the CDC guideline, we enlisted the current residence address of every individual (33). We found many of them became infected from their working environment and spread the virus to their families. Hence, all the samples were marked as human-transmitted and mostly inhabited in the cluster zone of Dhaka region, the capital of Bangladesh. The IEDCR also published surveillance showing that 45% of dwellers of Dhaka had been exposed to COVID-19 by October 2020 (37).

Globally, the chronological age is considered a highly significant predictor in measuring disease severity, future disease occurrence assumption, and reason for death rates (22, 23); although the biological age plays a potential role, especially two biomarkers-the epigenetic and glycan clock (21). Glycan regulates many immunological pathways modified with different age ranges, which are most susceptible to SARS-CoV-2 attachment. The primary receptor of SARS-CoV-2, ACE II is highly glycosylated; even the ABO blood group system is based on the diversity of the glycan molecules (22). This could be a predominant reason for our study to observe the highest COVID-19 positivity rate at the age range of more than 60 years old, compared to the other three age range categories (Table 1), although the minimal number of total cases (2.58%) in the present study.

Similarly, gender influenced viral infection effectively, more than two-fold higher in men than women, where biological factors could impact the total number of cases, hospitalization, duration of recovery,

TABLE 3 Association of participant's demographic and clinical characteristics and COVID-19 positivity.

Variables	COVID-19			
	UOR	95% CI	AOR	95% CI
Place of residence				
Gazipur	1.78***	(1.59, 1.98)	1.48***	(1.32, 1.66)
Dhaka	1.00	–	1.00	–
Tangail	3.45***	(2.67, 4.44)	2.48***	(1.90, 3.24)
Others	0.60**	(0.41, 0.88)	0.73	(0.49, 1.08)
Gender				
Female	1.00	–	1.00	–
Male	1.47***	(1.30, 1.65)	1.42***	(1.25, 1.61)
Respondent's age				
0–20 years	1.00	–	1.00	–
21–40 Years	1.03	(0.85, 1.26)	0.89	(0.72, 1.10)
41–60 Years	1.32**	(1.07, 1.65)	1.22	(0.96, 1.54)
Above 60 Years	2.41***	(1.72, 3.53)	1.95***	(1.36, 2.79)
Symptoms of COVID-19				
No	1.00	–	1.00	–
Yes	3.46***	(3.11, 3.36)	3.22***	(2.88, 3.61)
Comorbidity				
No	1.00	–	1.00	–
Yes	1.69***	(1.42, 2.01)	1.08	(0.88, 1.31)

*p-value < 0.05; **p-value < 0.01; ***p-value < 0.001.
Method: binary logistic regression model was used to observe the adjusted effects of covariates on COVID-19. Adjusted odds ratios-AOR; Unadjusted odds ratios-UOR; 95% Confidence Interval: CI.

and fatal rate (38). One of the most fundamental biological factors is the activation of the sex hormones, especially estrogen, which influences both the innate and adaptive immune response in females faster than in males despite being highly susceptible to autoimmune diseases (24). We have also observed the same difference in our analysis: two-thirds of samples were collected from males, and the prevalence of COVID-19 was 2.74 times higher than in females. The immunization schedule started precisely 8 months after the establishment of our laboratory, albeit people were frightened to receive vaccines for the post-vaccination complications. Thus, we inscribed only 2.03% of vaccination information from the enrolled population.

The vital principle of our laboratory was early monitoring of random people with or without symptoms as per the requirement of rejoining to the workplaces and being socialized by maintaining accurate health and safety protocol (36). Therefore, we have collected two-thirds of asymptomatic samples and successfully diagnosed a significant number of cases as SARS-CoV-2 positive (13.96%) at the latent period. Conversely, one-third of symptomatic individuals were reported as positive (35.96%), although this was the most remarkable indicator of SARS-CoV-2 susceptibility. The rest of the two third symptomatic people probably expressed post-COVID-19 complications. Fever and cough were the most commonly reported symptoms among all ages.

Our laboratory and the designated hospital were not authorized for critically ill patients, or as the COVID-19 support center. Therefore, we received a limited number of samples with a history of chronic underlying diseases and estimated the prevalence to evaluate it as an essential co-factor. One-third of individuals with comorbiddities had a likelihood of being COVID-19 positive, while other factors were not adjusted statistically. Interestingly, three-fourths of individuals with pre-medical history were male, highlighting diabetes mellitus, hypertension, CVD, renal disease, and asthma. Alternatively, autoimmune disease and cancer were prevalent in the case of female individuals, similarly as explained in other study (39).

Our study enrolled a large, healthy Bangladeshi population with diversified responses in susceptibility to the novel virus SARS-CoV-2 in the first year of the pandemic (2020–21). The objective of this research was to explore COVID-19 through the general public with great concern in the restricted environment of nationwide lockdown. Therefore, the limitation of this study was not being able to follow up with every individual for continuous data collection in case of hospitalization or discharge dates, recovery, and mortality rate, as our hospital was not an isolation center. Several individuals rarely could have remembered any symptoms at the onset before the test. Thus, we could not estimate the duration of the incubation period to the length of long COVID-19 cases. Our laboratory work was circumscribed for pediatric and sensitive patient sample collection rather than the pedestrians that affect the age range being heteroscedastic in regression analysis. Moreover, many people were illiterate and unable to mention their regular drug intake related to specific comorbidities that might interfere with the test result. Overall, the result provides a broad range of epidemiological analysis that would be simultaneously supportive for future experiments of drug and vaccine designing of new variants of SARS-CoV-2 for the people of the South Asian region.

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 ERC of Department of Biochemistry and Molecular Biology, University of Dhaka, Bangladesh. 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

MH: Conceptualization, Project administration, Supervision, Writing – original draft, Writing – review & editing. RM: Data curation, Formal analysis, Investigation, Project administration, Writing – original draft. SI: Writing – original draft, Writing – review & editing. LB: Writing – original draft, Writing – review & editing. AJ: Formal analysis, Software, Writing – original draft, Writing – review & editing.

ZH: Project administration, Supervision, Writing – original draft. SE: Supervision, Writing – original draft. SD: Project administration, Supervision, Writing – original draft. AA: Project administration, Supervision, 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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Rift Valley Fever outbreaks in the East African Community: insights from ProMed data (2010–2024)

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Background: Rift Valley Fever (RVF) is a mosquito-borne zoonotic disease that poses a serious threat to both humans and livestock across various regions, particularly in Africa, the Arabian Peninsula, and parts of the Indian Ocean Islands. This study seeks to analyze the spatial and temporal distribution and trends of RVF outbreaks within the East African Community (EAC) countries, offering insights into the patterns and progression of these outbreaks in the region.

Methods: We conducted a retrospective analysis of the Program for Monitoring Emerging Diseases (ProMed), a digital, event-based disease surveillance system, to identify reports of outbreak events in Uganda, Kenya, Rwanda, Burundi, Tanzania, and South Sudan from 2010 to 2024. Outbreak events were systematically tabulated by year, and each record was reviewed to assess RVF outbreak characteristics, locations, trends, and spatial-temporal distribution over the past 14 years.

Results: Between 2010 and 2024, 67 RVF outbreaks were documented across Uganda, Rwanda, Kenya, Tanzania, Burundi, and South Sudan, impacting both animal and human populations with confirmed cases and fatalities. Key interventions to contain these outbreaks included restricting animal movement, vaccination campaigns, disease awareness initiatives, enhanced surveillance, contact tracing, isolation, and treatment. Reporting of these outbreaks varied across regions, with a notable monthly increase in cases during May and June and the highest annual incidence observed in 2018.

Conclusion: The recurrent and widespread outbreaks of Rift Valley Fever across East Africa highlight an urgent need for increased investment in research, surveillance, prevention, and control efforts to combat this disease.

KEYWORDS

Rift Valley Fever, outbreak, ProMed, mosquito-borne disease, East African Community

Introduction

Rift Valley Fever (RVF) virus is a mosquito-borne zoonotic arbovirus that causes disease in livestock and humans (1). RVFV is harbored and transmitted by multiple genera of mosquitos, such as the *Aedes, Anopheles, Culex* and *Mansonia* and may also be spread to humans via ingestion or contact with contaminated blood, bodily fluids, or tissues of

infected animals (2). The RVF manifestation in livestock and ruminants is primarily acute and generally more severe for young and juvenile animals, where sheep and goats are the most susceptible animals to developing severe disease caused by the virus. Clinical symptoms for infected animals include hemorrhagic fever, lymphadenitis, nasal, and ocular secretions, vomiting, high abortion frequency, and a high rate of mortality in young animals (3, 4). The RVF in humans usually result in a self-limiting febrile illness, where roughly 50% of infected individuals report no clinical symptoms; importantly, however, a small percentage of human cases progress to severe disease, causing hemorrhagic fever, hepatic disease/failure, encephalitis, and death (3, 5).

A global risk map for RVF has highlighted potential transmission zones in Africa and the Middle East. This assessment considers geographic factors and a suitability indicator based on the Aedes mosquito, one of the RVF virus vectors, and the presence of susceptible hosts (6–8).

Furthermore, limited numbers of production animals in the arabian peninsula region leads also some countries like Yemen to import animals from the African horn. Recent reports have shown that some of the imported animals have undergone veterinary control screens whereas more than million animals are annually illegally imported via sea without any health control measures (9, 10). Although RVF primarily affects livestock, it also significantly threatens the stability of large-scale agricultural economies (11). Factors such as the expansion of the *Aedes* mosquito's range into Europe and the Americas, climate change and extreme weather events, and global travel could facilitate the spread of RVF to new regions, increasing the risk of infections in both animals and humans. The risk of an RVF outbreak is governed by a number of factors, including environmental conditions. Heavy rainfall periods have been classified as important risk factors for outbreaks as they create a suitable environment for mosquito proliferation (12). Moreover, linked global-trade chains have contributed by introducing infected livestock into non-endemic regions (13). Other economic factors such as informal and unregulated herding and the lack of sanitary mandates in abattoirs also facilitate infection (14). Since its causative agent, Rift Valley fever phlebovirus, was first isolated in 1930 in the Rift Valley region in Kenya, several major outbreak events have been reported in Africa and the Arabian Peninsula (15–17). Adequately controlling RVF spread relies on a variety of methods, such as monitoring, surveillance, and vaccination. Many of these methods are cost prohibitive and should therefore be approached using transdisciplinary solutions for disease management.

In Sub-Saharan Africa, specifically in East Africa, RVF outbreaks remain a significant public health concern (18, 19), especially in regions with high livestock populations where the virus is endemic. Recent reports indicate an upsurge in cases, largely attributed to seasonal rain patterns that create favorable breeding conditions for the Aedes and Culex mosquitoes responsible for transmitting the virus. Kenya, Uganda, and Tanzania have each seen periodic outbreaks, with concerns growing around cross-border transmission due to the high mobility of livestock and people in the region. Health officials are emphasizing the importance of ongoing surveillance, rapid response capabilities, and community awareness to mitigate the spread, alongside research into RVF immunogenicity and vaccine development to protect at-risk populations.

In East African countries (i.e., Burundi, Kenya, Rwanda, South Sudan, Tanzania, and Uganda), an unexpected RVF outbreak occurred in 2018 (20). This recent outbreak was unexpected as RVF outbreak events typically occur during El Niño periods with excessive rainfall. However, in 2018, there was an unusual deluge following a prolonged drought. Despite initial skepticism due to the late timing of the flooding, RVF outbreak events were reported in Kenya, Rwanda, and Uganda, with significant rainfall creating conditions conducive for the propagation of RVFV mosquito vectors. Risk maps indicated a broader area of potential activity, highlighting the unpredictability of climate-related disease outbreaks (8).

Outbreak events, case numbers, and human deaths caused by infection with RVFV have been exacerbated by the COVID-19 pandemic in East Africa (21, 22). Therefore, this review seeks to describe RVF outbreak events distribution and trends in the six East African countries over the past 14 years. Generated evidence will serve as a foundation for modeling future epidemiological studies, as well as providing baseline information to enhance mitigation strategies.

Methods

Data source

We conducted a retrospective analysis of the Program for Monitoring Emerging Diseases (ProMed), a digital disease monitoring system hosted by the International Society for Infectious Diseases (ISID) (23, 24), to identify reports of RVF outbreak events from the East Africa Community Partner States i.e., Uganda, Kenya, Rwanda, Burundi, Tanzania, and South Sudan. The ProMed database holds a record of major global outbreaks of emerging and re-emerging infectious diseases named the "ProMed mail database." The ProMed database is also subdivided into regional official language specific databases, which included ProMed Anglophone Africa for English speaking countries (ProMed-EAFR), and Francophone Africa for French speaking countries (ProMed-FRA). Despite instances of overlap between posts recorded in ProMed-EAFR and ProMed-FRA for some EAC countries, both databases served as data sources for this review. We considered RVF outbreak events documented both in English and French in the period of 2010–2024. All RVF outbreak reports with suspected or requesting additional information were excluded. Specifically, reports were excluded if they lacked essential epidemiological details or if key data elements (such as case counts, location, or timeline) were missing or inconsistent, thus requiring additional verification. Our aim was to ensure that only fully verified and reliable reports were included in our analysis to maintain data integrity.

Search strategy

To conduct searches in the ProMed database, we first defined relevant keywords and synonyms. This included the disease name (Rift Valley Fever or RVF or Fièvre de la vallée du Rift or FVR), associated syndromes (e.g., hemorrhagic fever), and countries within the East African Community (Rwanda, Uganda, Kenya, Tanzania, Burundi, and South Sudan). The search parameters covered a specified timeframe from January 2010 to July 2024 and allowed for both English and French language searches. Searches utilized logical operators "AND/OR" to refine results.

The initial search was conducted in the ProMed-EAFR database, enabling a targeted search strategy reviewed by two parallel reviewers. Any discrepancies in inter-rater assessments were resolved by a third-party reviewer. Additional searches were completed in ProMed-FRA and ProMedmail, with reference numbers from each post recorded in Microsoft Excel. As ProMed is an open-access database, ethical approval was not required for this review.

Data extraction and management

The reviewers developed an Excel template including the list of variables of interest for data collection. The list of variables compiled from the ProMed databases contained the outbreak ID, day, month, and year of outbreak occurrence, geographical location (district/province/county, and country), population (Human, animal, or both), mortality status (Yes/No), and major interventions (awareness, contact tracing, isolation, movement restriction, surveillance, treatment, and vaccination). All extracted data were cross validated by two independent reviewers. An additional dataset of spatial coordinates (latitude/longitude) were collected from geocoded locations via Earth Pro (7.3 Google LLC) to ascertain the specific geographical locations of each outbreak. The African regional administrative boundaries, water bodies, and rivers were obtained in a shapefiles format from the Africa Open Access library. The collected shapefiles data were managed in qGIS (V 3.10.3-A Coruña) software (25).

Data analysis and visualization

The dataset gathered from the ProMed database was reviewed, structured and assessed for errors. A descriptive analysis allowed us to observe the characteristics of each outbreak event, including a trend- and spatial distribution analysis. The number of RVF outbreak events that occurred were recorded per month, year, population, and geographic location. RVF outbreak events that reported mortality were counted per country. Outbreak events that had major interventions, such as movement control or vaccination, were recorded per country. These analyses were performed using a pivot table in Microsoft Excel. A description of the geographical distribution of RVF outbreak events was performed by visualizing outbreak occurrence per geographical location point. This visualization was completed by comparing the spatial coordinate data (latitude/longitude) with the number of outbreak events per country in qGIS. Following this, a trend analysis of RVF outbreak events were observed by monthly and yearly line plotting.

Results

In total, 65 RVF outbreak events were reported in EAC countries (Table 1). Uganda and Rwanda each reported 17 RVF outbreak events. Burundi had 14 RVF outbreak events, followed by Kenya, which had 12 RVF outbreak events. Tanzania and South Sudan, on the other hand, reported 3 and 2 RVF outbreak events, respectively. Among the RVF outbreak events reported, 43 were in animals, 17 in Humans, and three in both populations. The mortality rate in animals were reported in 26 RVF outbreak events, whereas a human fatality rate was reported in 15 RVF outbreak events. Various major interventions were reported; these included movement control during 17 RVF outbreak events, animal vaccination campaigns during 13 RVF outbreak events, disease awareness during five RVF outbreak events, active surveillance during three RVF outbreak events, and contact tracing, supportive therapy/care, and isolation were reported during one RVF outbreak event.

Geographical distribution of RVF outbreak events in EAC countries varied both within and between the countries (Figure 1). We first report that RVF outbreak events in Burundi were predominantly reported in the northern areas of the country. These areas included the Ngozi district, which had a high number of four RVF outbreak events. Next, in Rwanda, RVF outbreak events were largely distributed in the south and south-eastern areas, where six RVF outbreak events were reported in the Ngoma district (southeast). RVF outbreaks in northern Burundi (Ngozi district) and southeastern Rwanda (Ngoma district) are likely influenced by specific ecological and environmental factors, such as livestock density, proximity to wetlands, and agricultural practices. However, limitations in available data prevented a detailed, granular analysis of these factors.

Moreover, the RVF outbreak events in Uganda have primarily been reported throughout the southern and central areas of the country, where the district of Isingiro (south) had reported three RVF outbreak events, which exceeds outbreak numbers in other southern areas. In Kenya, RVF outbreak events have been reported across the country's western, central, northern, and eastern regions. Finally, RVF outbreak events in Tanzania were reported in north-eastern regions of the country, including the Arusha, Manyara and Tanga areas.

In order to analyze the temporal distribution of RVF outbreak events, we quantified the year and month of occurrence of each outbreak in EAC (Figures 2, 3). The monthly trend demonstrated fluctuations in the occurrence of RVF outbreak events within the EAC partner states (excluding DRC). The number of outbreak events increased from three in the month of January to the peak with 16 RVF outbreak events in May–June. The month of August had two RVF outbreak events and the number increased to four in the month of September. The trend characterized by low

TABLE 1 Characteristics of RVF outbreak events reported in EAC partner states through the Program for Monitoring Emerging Diseases (ProMed), 2010–2024.

Variable/country	Description	Burundi	Kenya	Rwanda	South Sudan	Tanzania	Uganda	Grand total
No. outbreak event		14	13	18	2	3	17	67
Population	Animal outbreak	14	7	17		3	3	44
	Human outbreak	–	3	–	1	–	13	17
	A* and H*	–	1	1	1	–	1	4
Death	Animal	14	1	9	–	–	3	27
	Human	–	4	1	–	–	11	16
Intervention	Awareness	–	1	–	1	–	3	5
	Contact tracing	–	–	1	–	–	1	1
	Isolation	–	–	1	–	–	1	1
	Animal movement control	10	4	–	–	–	3	17
	Surveillance	–	–	4	–	–	–	4
	Supportive therapy/care	–	–	1	–	–	–	1
	Animal vaccination	–	–	13	–	1	–	14

A* and H*: Animal and Human outbreaks.

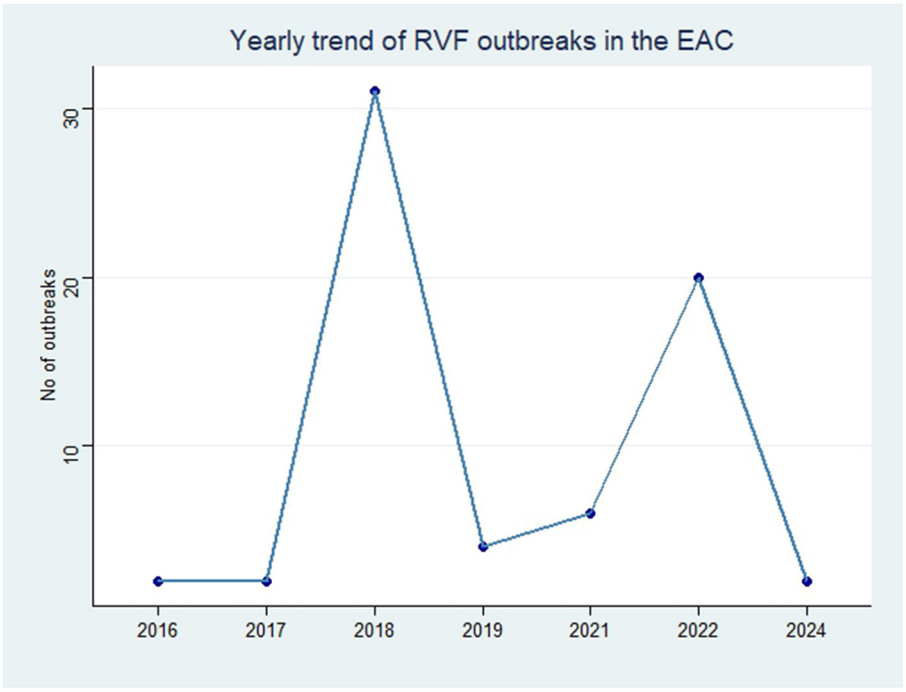

FIGURE 1
Geographical distribution of RVF outbreak events reported in EAC partner states through the Program for Monitoring Emerging Diseases (ProMed), 2010–2024. The number in parentheses represent the number of RVF outbreak events per region.

occurrence of RVF outbreak events was in the month of October, November and December with the number ranging from 0 to 2 outbreak events (Figure 2).

The annual trend indicated that from 2010 to 2024 the peak of RVF outbreak events was in 2018 which recorded occurrence of 30 outbreak events, and the lowest record of zero RVF outbreak events in 2020 (Figure 3).

Discussion

In our study, we used data from ProMed to obtain the number of RVF outbreak events in the member countries of the Eastern Africa Community from the years 2010 to 2024. From a spatial point of view, our results indicated that more than 65 RVF outbreak events were reported in EAC countries.

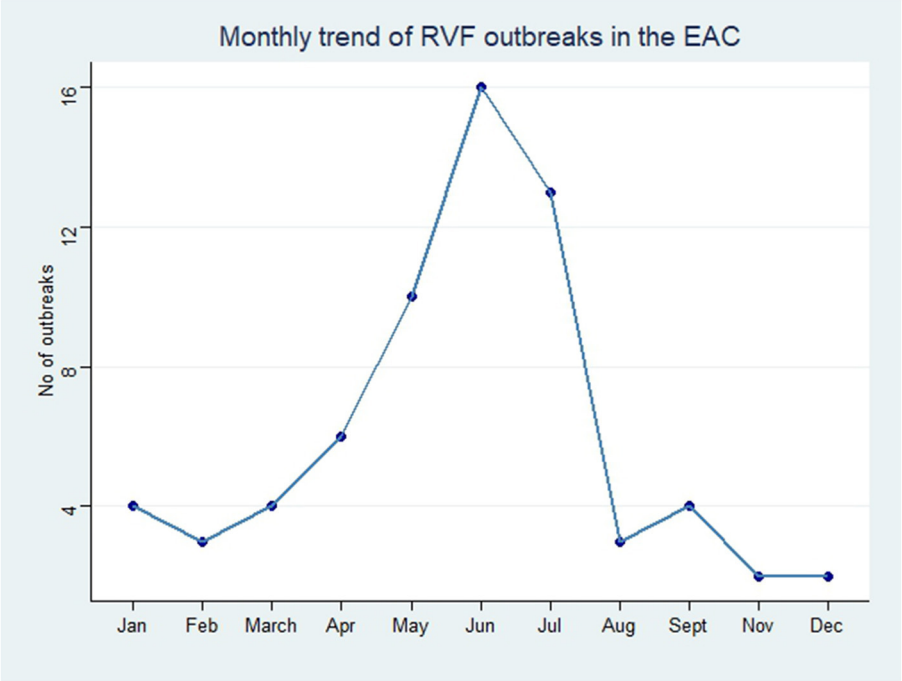

FIGURE 2
Monthly trend of RVF outbreak events reported in EAC partner states through Program for Monitoring Emerging Diseases (ProMed), 2010–2024.

In a temporal perspective, our review suggests that the month of June corresponds to the greatest likelihood of an outbreak event occurring. Our retrospective analysis has also highlighted the substantial increase in outbreak events in the year of 2018. We highlight the fact that the distribution of RVF outbreak events in EAC tends to be substantially higher in the months of May and July, indicating an established zoonotic cycle. The regular enzootic cycle, with limited transmission among animals, and the more severe epizootic/epidemic cycle during years/months of heavy rainfall.

The distribution of RVF outbreak events in Eastern Africa countries has been associated with specific climatic and environmental conditions. These outbreak events are reoccurring at irregular intervals following heavy rainfall with periods of flooding, which create ideal breeding conditions for the mosquito species that transmit the virus (26–28). The spread of RVF in recent years has been a major public health threat in the EAC region. Burundi had not previously documented larger RVF outbreak events. However, in 2022, an explosive RVF outbreak occurred, affecting ∼13 provinces within the country. This outbreak posed a significant challenge to livestock farming and underscored the fragility of food security.[1] It is possible that RVF outbreak events have been undetected and undocumented prior in Burundi, and this could be due to the inadequacy of surveillance systems, the country's capacity for diagnosis, and other contingency measures.[2] However, neighboring countries including Rwanda had epidemiological evidence of experiencing larger RVF outbreak events that were reported in 2018 (20).[3] Similarly, RVF outbreak events in Rwanda could have been under reported, as prior evidence indicated sero-prevalence in humans and animals (29). In Tanzania, RVF outbreak events have been re-occurring with an epidemics interval of 10–20 years since the 1930s (30, 31). In such endemic settings, sporadic RVF outbreak events are expected to occur, hence the 2018 RVF outbreak in Tanzania was detected promptly by the national surveillance (32). The wide spread 2018 RVF outbreak has been reported in Uganda through the National Viral Hemorrhagic Fever Surveillance System (33). The impact of the 2018 RVF outbreak has been confirmed by the Ministry of Health for Kenya, and affected humans and animals in several areas of the country (34). Similarly, this larger 2018 RVF outbreak was declared by the Ministry of Health and the Ministry of Livestock and Fisheries in South Sudan.[4]

The yearly trend of RVF outbreak events reported in EAC peaked in 2018 following the excessive rainfall caused by El Niño-Southern Oscillation (ENSO), and flooding mosquito vector habitats, similar results were reported in another study.[5] These

1 https://reports.unocha.org/en/country/burundi/

2 https://www.ilri.org/news/burundi-steps-surveillance-and-response-efforts-control-rift-valley-fever

3 https://apps.who.int/iris/bitstream/handle/10665/273028/OEW27-300606072018.pdf

4 https://www.afro.who.int/news/south-sudan-declares-rift-valley-fever-outbreak-parts-eastern-lakes-state#:~:text=South%20Sudan%20declares%20Rift%20Valley%20fever%20outbreak%20in%20parts%20of%20Eastern%20Lakes%20State-12%20March%202018&text$=$Juba%2C%2012%20March%202018%20%E2%80%93%20The,Counties%20of%20Eastern%20Lakes%20State

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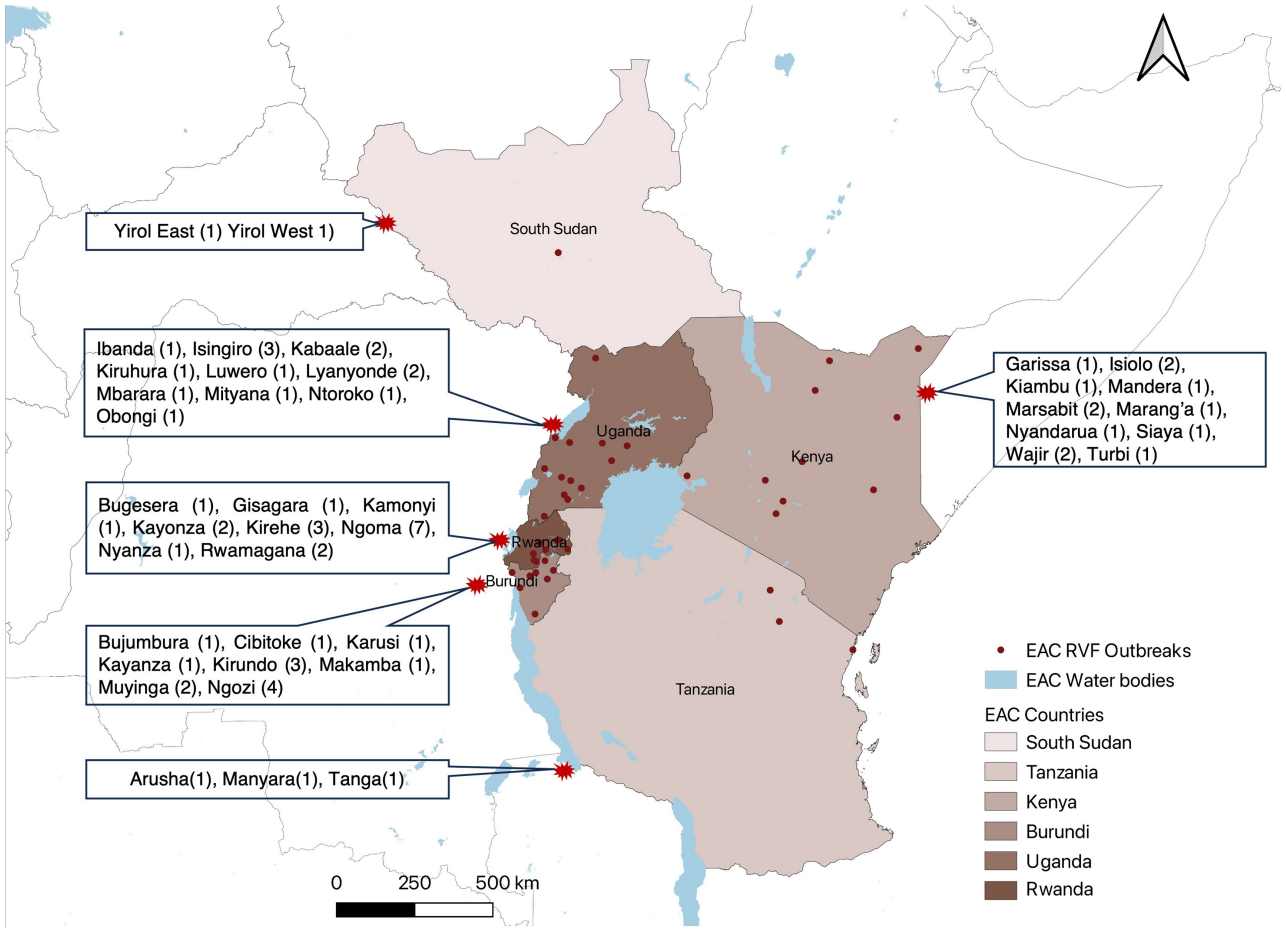

FIGURE 3
Yearly trend of RVF outbreak events reported in EAC partner states through Program for Monitoring Emerging Diseases (ProMed), 2010–2024.

outbreak events usually occur during peak ENSO conditions (December–January) and subside by February to March. However, in 2018, there was an unanticipated deluge following a prolonged and widespread drought from September 2017 to February 2018. Despite early warnings of potential RVF outbreaks, the peak in May-June was unexpected, aligning with findings from other studies (see text footnote 5). The eastern part of Africa has been reported to experience a rainy season from March to May (35). Here, we speculate that the wet months from March to May augment the proliferation of RVF vectors (i.e., mosquitoes), thus contributing to a greater number of outbreak events in the months following the rainy season as indicated by Nosrat et al. (36). Additionally, extreme climatic events have been described as a catalyst for mosquito-borne diseases in an era of climate change (37, 38). The increased mosquito population, and consequently increased RVF-infected mosquitoes can cause significant outbreak events in both livestock and humans, especially for individuals in close contact with infected animals.

In addition to mosquito transmission, RVF can spread to humans through direct contact with infected livestock or through inhalation of virus particles during animal slaughter (39, 40). During RVF outbreak events, the reported mortality in human and animals was not surprising. RVF case fatality rates in humans was estimated to be high in a systematic review and meta-analysis study that pooled contemporary epidemiological data in Africa (41).

Interventions and control measures implemented during RVF outbreak events in East African countries such as the control of animal movement, animal vaccination campaigns, disease awareness, active surveillance, contact tracing, supportive therapy/care, and isolation are well known.[6] However, the breadth and complexity of factors contributing to the risk of an RVF outbreak include climate change, the shifting distribution of vector and host species, absence of an approved human vaccine, insecticide resistance, and international travel and trade, which remain major challenges to controlling the spread of the virus (11). As climate change and extreme weather events increase in frequency, there is growing concern that RVF outbreaks will become more severe and numerous in the near future.

5 https://ui.adsabs.harvard.edu/abs/2018AGUFMGH14A..07A/abstract

6 https://www.woah.org/en/disease/rift-valley-fever/

The limitations of this paper stem from its reliance on data gathered solely from a single event surveillance database. However, the authors justify this choice by highlighting that this event surveillance system, known as ProMed, is the largest global system for monitoring emerging diseases. Additionally, it is worth noting that ProMed employs a combination of artificial intelligence and human data moderation, enhancing its data curation capabilities. Additionally, while we recognize the importance of various environmental and socio-economic risk factors in contributing to RVF outbreaks, this study focused on the epidemiological patterns and spatial distribution of outbreaks. We plan to include a more detailed analysis of these risk factors in future studies to better understand RVF dynamics.

Conclusion and recommendations

The recurrent and widespread outbreaks of RVF in East Africa underscore the urgent need for a coordinated, interdisciplinary approach to effectively control and prevent this disease. This approach must emphasize robust surveillance and early detection, comprehensive research and monitoring, seamless data integration, and targeted capacity building. Additionally, integrating policies, fostering community engagement, advancing vaccine and treatment development, strengthening climate resilience, enhancing education, and encouraging international collaboration are essential pillars in building a resilient and responsive global health system. Together, these efforts will empower both local and global communities to respond swiftly and effectively to RVF and other emerging health threats.

Data availability statement

Publicly available datasets were analyzed in this study. This data can be found at: https://promedmail.org/.

Author contributions

PN: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. TU: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. FZ: Writing – review & editing, Writing – original draft, Data curation, Methodology, Formal analysis, Supervision. MNd: Writing – review & editing, Data curation, Methodology, Formal analysis, Project administration. BU: Writing – review & editing, Data curation, Methodology, Formal analysis. MNg: Investigation, Methodology, Writing – review & editing. AT: Conceptualization, Methodology, Writing – review & editing. FN: Conceptualization, Formal analysis, Methodology, Writing – review & editing. GS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. DK: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – review & editing. JU: Conceptualization, Writing – review & editing. BH: Investigation, 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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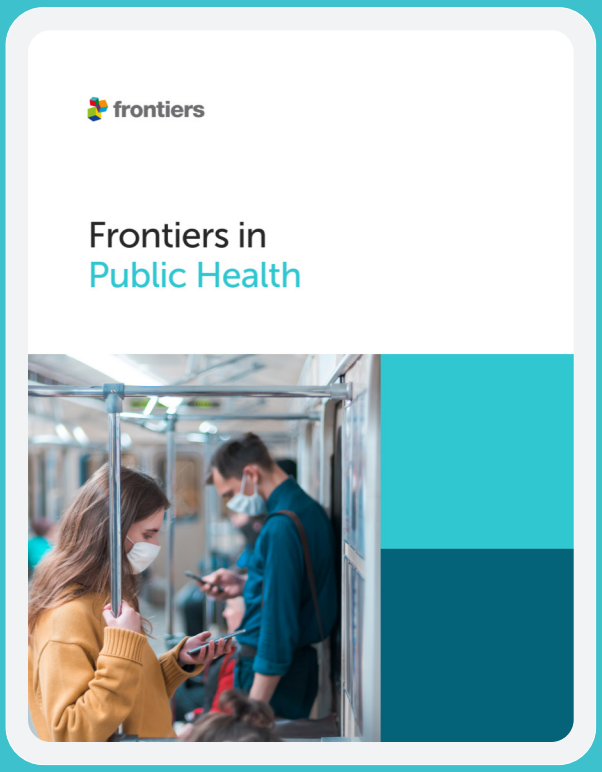

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