

Women in science: infectious diseases: epidemiology and prevention 2023

Edited by

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Women in science: infectious diseases: epidemiology and prevention 2023

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Prevalence of and risk factors for chlamydia in female outpatients with genital tract infections: a nationwide multi-center, cross-sectional study in China

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Introduction: *Chlamydia trachomatis* is the etiological agent of the commonest sexually transmitted bacterial infection. This study aimed to examine the prevalence of genital chlamydia and associated risk factors in Chinese female outpatients with genital tract infections.

Methods: A prospective, multicenter epidemiological study of genital chlamydia prevalence in 3008 patients with genital tract infections in 13 hospitals in 12 provinces of China was performed between May 2017 and November 2018. Vaginal secretion specimens were collected for the clinical diagnosis of vaginitis, whereas cervical secretion specimens were tested for *Chlamydia trachomatis* and *Neisseria gonorrhoeae*. All patients participated in a one-on-one cross-sectional questionnaire interview.

Results: Totally 2,908 participants were included. The prevalence rates of chlamydia and gonococcal infections in women with genital tract infections were 6.33% (184/2908) and 0.01% (20/2908), respectively. Multivariate analysis showed high risk factors for chlamydia were premarital sex behavior, first sexual intercourse before the age of 20 and bacterial vaginosis.

Discussion: Given that most chlamydia cases are asymptomatic and no vaccine is currently available, chlamydia prevention strategies should include behavioral interventions as well as early screening programs to identify and treat individuals with genital tract infections, especially those with the above identified risk factors.

KEYWORDS

Chlamydia trachomatis, genital tract infection, risk factor, bacterial vaginosis, China

Introduction

Chlamydia is a commonly diagnosed sexually transmitted infection (STI) caused by *Chlamydia trachomatis* (*C. trachomatis*). In 2020, the World Health Organization (WHO) estimated 129 million individuals were infected by *C. trachomatis* worldwide; meanwhile, around 374 million new STI cases were detected, indicating more than 1 million STI cases acquired daily (1), which is likely an underestimate as chlamydia infections are often asymptomatic. In the United States, the Centers for Disease Control and Prevention (CDC) estimated almost \$16 billion in direct medical costs alone, and the highest prevalence (61%) was detected among individuals aged 15–24 years (2). Most cases of chlamydia infection are generally asymptomatic. Multiple sequelae can result from chlamydia infection in women; especially if left untreated, the most serious sequelae may include pelvic inflammatory disease (PID), ectopic pregnancy, infertility and chronic pelvic pain, requiring high medical costs (3, 4). Over the past two decades, the rates of reported chlamydia cases have steadily increased in both men and women. During the COVID-19 pandemic, the temporary decreases observed in chlamydia infection rates from 2018 to 2021 in the United States were unlikely due to an actual reduction in new infections, as yearly reproductive health visits for young women also decreased during this period (3).

Genital tract infections can be classified anatomically and mainly involve vaginitis, cervicitis and PID (Table 1) (5). The pathogen targets endocervical and upper genital tract squamocolumnar epithelial cells in women, causing multiple urogenital infections such as cervicitis, acute salpingitis and PID; chlamydia is transmitted via direct contact during vaginal, anal or oral sex and may even be acquired by newborns during vaginal delivery. Moreover, chlamydia can coexist with other reproductive tract infections and STIs, including gonococcal infections, syphilis, human immunodeficiency virus infection (HIV), human Papillomavirus Infection and trichomoniasis (6). Therefore, the clinical management of urogenital chlamydia is focused on reducing the course of infection or inflammation (e.g., early diagnosis and treatment), preventing further sexual transmission and adverse reproductive health complications, and preventing *C. trachomatis* transmission to neonates during delivery (7).

Chlamydia screening programs aim at reducing chlamydia and PID rates in women by early detection and treatment. The U.S. CDC therefore recommends annual screening for all sexually active women aged ≤25 years or older with high risk of infection, including those with new or multiple sexual partners (2). Meanwhile, other high-income countries, including the United Kingdom, Australia, Sweden, Denmark, and Norway, recommend annual chlamydia screening for sexually active young adults, especially women.

Genital chlamydia was included as a reportable STI in 105 national STI surveillance sites in China in 2008 (6). Despite the lack of official data in China, reports suggest 4.7–30% of women of reproductive age experience at least one chlamydia episode, with up to 70% of infections being asymptomatic (6, 8, 9); this rate ranges from

TABLE 1 Classification of genital tract infections.

Infectious diseases	Anatomical site	Clinical syndrome
Vaginitis	Vagina	Trichomoniasis ^a
		Vulvovaginal candidiasis ^b
		Bacterial vaginosis ^c
		Aerobic vaginitis ^d
		Nonspecific vaginitis
Cervicitis	Cervix	Mucopurulent cervicitis
		Cervical neoplasia
Pelvic inflammatory disease	Endometrium	Endometritis
	Fallopian tubes	Salpingitis
	Ovaries	Oophoritis
Others	Vulva	Vulvar vestibulitis
	Vulva	Genital warts
	Bartholin glands	Bartholinitis

^aA type of vaginitis which caused by infection with the protozoan parasite *Trichomonas vaginalis*.

^bA type of vaginitis which caused by infection with *Candida* species.

^cBacterial vaginosis refers to the relative lack of vaginal endogenous lactobacilli and synergistic anaerobic microbial growth in the vaginal epithelium.

^dRefers to the relative lack of vaginal endogenous lactobacilli and synergistic aerobic microbial growth in the vaginal epithelium.

10 to 13% in pregnant women (10). Other studies revealed (8, 11, 12) chlamydia infection rate is even higher in women visiting sexually transmitted disease and gynecology clinics, reaching 10–20%. Considering the geographic variation of *C. trachomatis* infection, the severe situation of chlamydia and the lack of robust epidemiological studies, the current prospective, multicenter epidemiological study aimed to determine the epidemiological features and risk factors associated with chlamydia infection in gynecology clinics in China. Targeted screening is the most cost-effective strategy to reduce the prevalence of chlamydia (13). The current study provides guidance for nationwide chlamydia screening programs to reduce the burden of chlamydia in China.

Materials and methods

Patients

This multi-center, cross-sectional study was conducted at 13 hospitals in 12 provinces of China between May 2017 and November 2018. During this period, 6,125 women with gynecological complaints (e.g., vulvovaginal itching, abnormal discharge, bleeding or abdominal pain) seeking sexual and reproductive health care were assessed. Among them, totally 3,008 women with genital tract infections were eligible for this study, and all of them were willing to participate face-to-face interview. All enrolled patients in the selected hospitals were required to participate in a questionnaire interview at the time of first visit after meeting the eligibility criteria and providing written informed consent. Inclusion criteria were: (1) with one or more types of genital tract infections, including vaginitis, cervicitis, pelvic inflammatory disease and so on; (2) ever engaged in sexual intercourse; (3) no antibiotic use in the last 2 weeks. Exclusion criteria

Abbreviations: STI, sexually transmitted infection; *C. trachomatis*, *Chlamydia trachomatis*; WHO, World Health Organization; CDC, the Centers for Disease Control and Prevention; PID, pelvic inflammatory disease; HIV, human immunodeficiency virus infection; BV, bacterial vaginosis; *N. gonorrhoeae*, *Neisseria gonorrhoeae*; OR, odds ratio; CI, confidence interval; VVC, vulvovaginal candidiasis.

were: (1) antimicrobial therapy during the previous 21 days; (2) previous history of hysterectomy; (3) menopause; (4) contraindications to the Papanicolaou test or cervical sampling.

Chlamydia trachomatis questionnaire

A close-ended questionnaire was generated to assess potential risk factors associated with chlamydia. All enrolled patients in the selected hospitals were required to complete a face-to-face questionnaire interview at the time of enrollment after meeting the eligibility criteria and providing written informed consent. The questionnaire included five primary domains: (a) demographic data such as age, housing situation, monthly income, education level, marital status, and single parenthood; (b) personal hygienic behaviors, including the frequencies of vulva cleansing, vaginal douche, sanitary pads, and wearing cotton underwear; (c) sexual behaviors, including premarital sex, sexual orientation, age at first intercourse, frequency of sex, number of sex partners, experience of masturbation, experience of orgasm and use of a contraceptive device during sex; (d) history of gynecological diseases, including pregnancy, abortion and genital tract infections; (e) self-reported symptoms and signs such as vulvar pruritus, vulvar pain, increased vaginal discharge, vaginal odor, abdominal pain, lower urinary tract symptoms, postcoital bleeding, vaginal pH and vaginal cleanliness.

Gynecological examination and clinical diagnosis of genital tract infections

Vaginal secretions were obtained and rolled onto glass slides with aseptic cotton swabs for microbiological tests. Cervical swab specimens were collected with the Cobas PCR Sample Kit (Roche Molecular Systems) following speculum examination.

Vaginitis was defined as inflammation or infection of the vagina, and had a broad differential types, including trichomoniasis, vulvovaginal candidiasis (VVC), bacterial vaginosis (BV), aerobic vaginitis (AV), and nonspecific vaginitis (Table 1) (14). The clinical diagnoses of trichomoniasis was based on wet mount for direct examination of *Trichomonas* in vaginal secretions, while the diagnosis of candidiasis was based on Gram staining for the detection of *Candida* hyphae, pseudohyphae or spores in vaginal secretions; the diagnosis of BV was performed as described by Nugent et al. (15) and scores valued 7–10 are interpreted as BV flora; the diagnosis of AV was performed as described by Donders et al. (16) and scores valued 3–10 accompanied by genital complaints are interpreted as AV (4, 14). The type of vaginitis that does not meet the criteria presented above but involved some of the features of vaginitis, was diagnosed as nonspecific vaginitis (17). Mixed vaginitis was defined as the simultaneous presence of at least two above types of vaginitis (18).

Cervicitis was clinically diagnosed according either or both major signs: (1) a visibly purulent or mucopurulent endocervical exudate, or (2) sustained endocervical bleeding easily induced by gentle contact of a cotton swab (4). PID was clinically diagnosed according one or more of the following three minimum clinical criteria present on pelvic examination: (1) cervical motion tenderness, (2) uterine tenderness, or (3) adnexal tenderness (4).

Chlamydia trachomatis testing

Cervical specimens were transferred to tubes containing cervical cell preservation solution provided in the kit and stored at -80°C until analysis as described by the manufacturer. All samples were transported to Peking University First Hospital for the detection of *C. trachomatis*/*Neisseria gonorrhoeae* (*N. gonorrhoeae*) with a qualitative nucleic acid amplification test on the Cobas 4,800 system (Roche, Switzerland) as described by the manufacturer.

Ethical approval of the study protocol

The study followed the Declaration of Helsinki and was approved by the Ethics Committee of Peking University First Hospital, Beijing, China (reference number: 2016[1156]). Written informed consent was provided by all eligible participants before enrolment. The participating institutions included Peking University First Hospital, Peking University Shenzhen Hospital, Beijing Tsinghua Changgung Hospital, The Second Affiliated Hospital of Chongqing Medical University, The First Affiliated Hospital of Guangxi Medical University, The Second Hospital of Hebei Medical University, Nanjing Drum Tower Hospital, Qinghai Red Cross Hospital, Qilu Hospital of Shandong University, The Second Hospital of Shanxi Medical University, Tianjin Medical University General Hospital, Women's Hospital of Zhejiang University and The Third Xiangya Hospital of Central South University.

Statistical analyses

IBM SPSS Statistics 23 (IBM Corp. Released 2016, IBM SPSS Statistics for Windows, Version 23, Armonk, NY; IBM Corp.) was used for statistical analysis. Data were reported as number (percentage) or odds ratio (OR) with the corresponding 95% confidence interval (CI). The chi-square test was performed to compare categorical variables between groups. Logistic regression analysis and raw ORs in univariate analysis were used to assess the associations of chlamydia with demographic and behavioral variables. $p < 0.05$ indicated statistical significance.

Results

Patient population

Totally 3,008 women were enrolled in this study, and 100 (3.3%) were excluded for poor sample quality or a lack of personal data. Finally, 2,908 (96.7%) evaluable patients were included in the analysis, including 2,222 women diagnosed with vaginitis, 393 with cervicitis, 131 with PID, and 271 with other genital tract infections (e.g., vulvitis and bartholinitis). The demographic and baseline characteristics of the patients are shown in Table 2. The prevalence rates of *C. trachomatis* and gonococcal infections in women with genital tract infections were 6.33% (184/2908) and 0.01% (20/2908), respectively; 6 cases were co-infected with *C. trachomatis* and *N. gonorrhoeae* (diagnosed with BV), nonspecific vaginitis,

TABLE 2 Characteristics of the study patients with *C. trachomatis* and *N. gonorrhoeae* infections.

	<i>C. trachomatis</i>			<i>N. gonorrhoeae</i>		
	Negative	Positive	<i>P</i> -value	Negative	Positive	<i>P</i> -value
Mean age, years	34.98 ± 8.824	30.50 ± 7.087	<0.001	34.69 ± 8.771	36.00 ± 11.549	0.618
Age at menarche, years	13.84 ± 1.629	13.97 ± 1.653	0.686	13.85 ± 1.633	13.50 ± 1.249	0.359
Parturition frequency	1.62 ± 1.568	1.25 ± 1.689	0.548	2.37 ± 1.11	2.37 ± 1.11	
Region, n (%)						
Beijing	410 (90.71%)	42 (9.29%)	0.024	446 (98.67%)	6 (1.33%)	0.610
Changsha	231 (92.77%)	18 (7.23%)		247 (99.20%)	2 (0.80%)	
Taiyuan	188 (94.47%)	11 (5.53%)		198 (99.5%)	1 (0.50%)	
Nanning	185 (92.50%)	15 (7.50%)		199 (99.5%)	1 (0.50%)	
Xining	158 (92.94%)	12 (7.06%)		168 (98.82%)	2 (1.18%)	
Nanjing	234 (98.32%)	4 (1.68%)		238 (100.0%)	0 (0.00%)	
Tianjin	185 (92.50%)	15 (7.50%)		200 (100.0%)	0 (0.00%)	
Shenzhen	364 (93.09%)	27 (6.90%)		387 (98.98%)	4 (1.02%)	
Hangzhou	165 (97.06%)	5 (2.94%)		170 (100.0%)	0 (0.00%)	
Shijiazhuang	233 (94.72%)	13 (5.28%)		245 (99.59%)	1 (0.41%)	
Jinan	190 (95.48%)	9 (4.52%)		198 (99.50%)	1 (0.50%)	
Chongqing	180 (93.26%)	13 (6.73%)		191 (98.96%)	2 (1.04%)	

vulvovaginal candidiasis (VVC), cervicitis, BV + PID and cervicitis, respectively. As shown in Table 2, individuals with chlamydia were significantly younger than uninfected women (30.50 ± 7.087 yrs. vs. 34.98 ± 8.824 yrs., $p < 0.001$). Significant differences in *C. trachomatis* prevalence were found among regions in China, and women with genital tract infections in Beijing may have the highest burden in *C. trachomatis* prevalence, followed by Tianjin, Nanning, and Changsha (Table 2 and Figure 1).

To examine the role of the microecological environment of the female genital tract in chlamydia, we discussed the associations of genital tract infections.

with chlamydia. As shown in Table 3, BV prevalence was 34.27% (61/178) in *C. trachomatis* positive women, which was significantly higher than that of *C. trachomatis* negative women [27.43% (727/2,650)]. Chlamydia was associated with BV (OR = 1.379, 95%CI 1.000–1.901; $p = 0.049$). The prevalence of cervicitis was 30.00% (6/20) in *N. gonorrhoeae* positive women, which was significantly elevated than that of *N. gonorrhoeae* negative women [13.78% (387/2,808); Table 3]. *N. gonorrhoeae* infection was associated with cervicitis with an odds ratio of 2.681 (95% 1.024–7.019, $p = 0.037$). These results indicated tight associations of *C. trachomatis* and *N. gonorrhoeae* infections with genital tract infections.

Factors associated with chlamydia

Based on univariate analysis, risk variables associated with chlamydia in women with genital tract infections included unstable housing situation, unmarried/widowed/divorced marital status, premarital sex behavior, first sexual intercourse before the age of 20, having ≥3 sex partners, previous history of pregnancy, and previous history of abortion.

Basic information

Our data demonstrated salient differences in individual *C. trachomatis* risks between women living in stable residences, including own houses or apartments, and those living in unstable situations (rooming houses, hotels or shelters). Individuals with a stable housing situation showed a 5.54% positivity rate, versus 8.40% in those with an unstable housing situation. Besides, the positivity rate of chlamydia in the unmarried/widowed/divorced group was 11.9%, which was significantly higher than 5.0% obtained in married women.

Sexual behavior

In this study, another risk factor significantly associated with chlamydia was the sexual behavior. The prevalence of chlamydia in patients with the first sexual intercourse at an age below 20 years was significantly higher than that of patients having the first sexual intercourse at an age above 20 years (9.48% vs. 5.14%; $p < 0.001$). The prevalence of chlamydia in patients with 1 or 2 sexual partners over the lifetime was 10.05%, which was significantly lower than that of patients with 3 or more sexual partners (6.24%). The prevalence of chlamydia in patients with premarital sexual behavior was significantly higher than that of participants without premarital sexual behavior (7.76% vs. 3.81%; $p < 0.001$).

Gynecological history

Among women with genital tract infections, the positivity rate of chlamydia in those without a previous pregnancy was 10.48%,

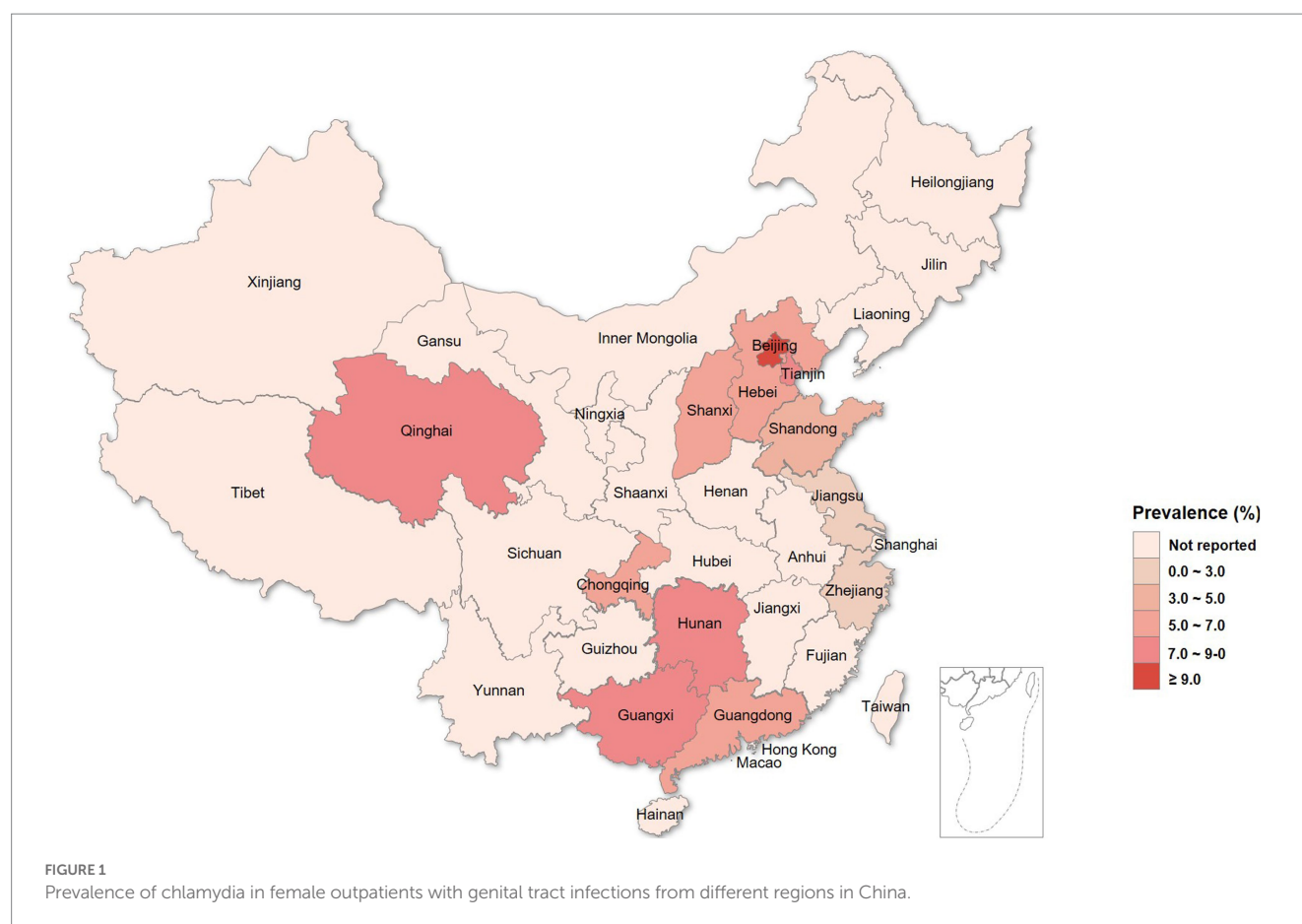


TABLE 3 Prevalence rates of *C. trachomatis* and *N. gonorrhoeae* infections in 2828 patients with gynecological infectious diseases.

	n ^a	<i>C. trachomatis</i> , n (%)			<i>N. gonorrhoeae</i> , n (%)		
		Negative	Positive ^b	<i>P</i> -value	Negative	Positive	<i>P</i> -value
Vaginitis	2,222	2079 (78.45%)	143 (80.34%)	0.553	2,205 (78.53%)	17 (85.00%)	0.482
TV	121	113 (4.26%)	8 (4.49%)	0.883	121 (4.31%)	0 (0.00%)	0.343
BV	788	727 (27.43%)	61 (34.27%)	0.049	779 (27.74%)	9 (45.00%)	0.086
VVC	715	675 (25.47%)	40 (22.47%)	0.373	712 (25.36%)	3 (15.00%)	0.288
AV	212	201 (7.58%)	11 (6.18%)	0.491	212 (7.55%)	0 (0.00%)	0.201
Nonspecific	765	715 (26.97%)	50 (28.09%)	0.745	760 (27.06%)	5 (25.00%)	0.837
Mixed vaginitis	312	290 (10.94%)	22 (12.36%)	0.559	312 (11.11%)	0 (0.00%)	0.114
Cervicitis	393	366 (13.81%)	27 (15.17%)	0.612	387 (13.78%)	6 (30.00%)	0.037
PID	131	124 (4.68%)	7 (3.93%)	0.646	129 (4.59%)	2 (10.00%)	0.252
Others	271	251 (9.43%)	20 (10.87%)	0.520	269 (9.52%)	2 (10.00%)	0.942

^aNumbers did not always add up to the expected total because of 80 missing data.

^b178 cases with *C. trachomatis* infection had data on reproductive tract infection status.

TV, Trichomonas vaginitis; BV, Bacterial vaginosis; VVC, Vulvovaginal candidiasis; AV, Aerobic vaginitis; PID, Pelvic inflammatory disease.

which was significantly higher than that of participants who reported a past pregnancy (4.91%). A pregnancy history was inversely correlated with chlamydia risk (OR=0.441, 95%CI 0.324–0.600; $p<0.001$; Table 4). Similarly, the positivity rates of chlamydia were 7.64 and 5.21% in patients without and with a history of abortion, respectively.

Others

However, no significant associations were found of chlamydia with income, education level, single parenthood, personal hygienic behaviors, sexual orientation, frequency of sex, history of genital tract infections and self-reported symptoms and signs.

TABLE 4 High-risk factors of *C. trachomatis* infection.

Variable		n ^a	<i>C. trachomatis</i> Positive n (%)	<i>P</i> -value
Basic information				
Age, years	≤25	404	31 (7.67%)	0.232
	>25	2,503	153 (6.11%)	
Housing situation	Stable	2074	115 (5.54%)	0.005
	Unstable	786	66 (8.40%)	
Monthly income, RMB	<5,000	1,629	102 (6.26%)	0.768
	≥5,000	1,023	67 (6.55%)	
Education	High school or below	962	54 (5.61%)	0.245
	Bachelor or higher	1900	128 (6.74%)	
Marital status	Unmarried/Widowed/divorced	539	64 (11.87%)	<0.001
	Married	2,320	116 (5.00%)	
Single parenthood	No	2,687	167 (6.22%)	0.205
	Yes	187	16 (8.56%)	
Personal hygienic behaviors				
Frequency of vulvar cleansing	Never	118	11 (9.32%)	0.524
	Less than once a week	239	14 (5.86%)	
	2–6 times a week	474	32 (6.75%)	
	Daily	2057	125 (6.08%)	
Frequency of vaginal douche	Never	1,599	95 (5.94%)	0.771
	Less than once a week	570	36 (6.32%)	
	2–6 times a week	306	22 (7.19%)	
	Daily	368	26 (7.07%)	
Use of sanitary pads	<5 days a month	2,374	140 (5.90%)	0.110
	≥5 days a month	486	38 (7.82%)	
Cotton panties	No	386	17 (4.40%)	0.097
	Yes	2,511	166 (6.61%)	
Sexual behavior				
Premarital sex behavior	Yes	971	145 (7.76%)	<0.001
	No	1869	37 (3.81%)	
Sexual orientation	Heterosexuality	2,731	173 (6.33%)	0.844
	Homosexuality	49	4 (8.16%)	
	Bisexuality	1	1 (0.00%)	
Age at first intercourse, yrs	≤20	781	74 (9.48%)	<0.001
	>20	2,103	108 (5.14%)	
Frequency of sex	≤ once a week	1,383	86 (6.22%)	0.404
	2–3 times a week	1,268	85 (6.70%)	
	>4 times a week	172	7 (4.07%)	
Number of sex partners	1–2	2,386	149 (6.24%)	0.030
	≥3	219	22 (10.05%)	
Experience of masturbation	No	2,480	151 (6.09%)	0.169
	Yes	336	27 (8.04%)	
Experience of orgasm	No	597	46 (7.71%)	0.109
	Yes	2,200	130 (5.91%)	
Using a contraceptive device during sex	No	1970	132 (6.70%)	0.197

(Continued)

TABLE 4 (Continued)

Variable		n ^a	<i>C. trachomatis</i> Positive n (%)	<i>P</i> -value
	Yes	833	45 (5.40%)	
Gynecological history				
Pregnancy	No	735	77 (10.48%)	<0.001
	Yes	2098	103 (4.91%)	
Abortion	No	1,296	99 (7.64%)	0.008
	Yes	1,535	80 (5.21%)	
Genital tract infections	No	697	48 (6.89%)	0.505
	Yes	2,153	133 (6.18%)	
Self-reported symptoms and signs				
Vulvar pruritus	No	1,594	102 (6.40%)	0.466
	Yes	1,187	68 (5.73%)	
Vulvar pain	No	2,572	161 (6.26%)	0.257
	Yes	209	9 (4.31%)	
Increased vaginal discharge	No	1,515	90 (5.94%)	0.674
	Yes	1,265	80 (6.32%)	
Vaginal odor	No	1928	115 (5.96%)	0.613
	Yes	851	55 (6.46%)	
Abdominal pain	No	2,422	153 (6.32%)	0.252
	Yes	357	17 (4.76%)	
Lower urinary tract symptoms	No	2,617	159 (6.08%)	0.743
	Yes	164	11 (6.71%)	
Postcoital bleeding	No	2,629	159 (6.05%)	0.523
	Yes	150	11 (7.33%)	
Vaginal pH	Normal (3.8~4.4)	1,021	51 (5.00%)	0.044
	Abnormal	1,312	92 (7.01%)	
Vaginal cleanliness ^b	Normal (I–II)	905	46 (5.08%)	0.034
	Abnormal (III–IV)	1,584	115 (7.26%)	

^aNumbers did not always add up to the total expected because of missing data.

^bVaginal cleanliness was used to judge inflammation status in wet preparations of vaginal swab samples, degree I–II representing normal vaginal microecological status while degree III–IV representing abnormal status (19).

RMB, Chinese Yuan Renminbi.

Multivariate analysis

Significant variates in univariate analysis were further assessed by multivariate analysis, including age, housing situation, premarital sex behavior, age at first intercourse, number of sex partners, vaginal cleanliness, and BV. In the multivariate logistic model, factors remaining significantly associated with chlamydia in women were positive premarital sex behavior, first sexual intercourse before the age of 20 years and BV (Table 4).

Discussion

This cross-sectional epidemiological study was performed to assess the epidemiology of chlamydia and to identify potential risk factors that could elevate the incidence of chlamydia in mainland China. We previously reported a *C. trachomatis* prevalence of 2.2% among married women in Beijing (20), versus 17.30% in female sex

workers (21), and 10.1% in patients visiting gynecology and reproductive health clinics (12). This study showed that the prevalence of chlamydia has now increased by 3-fold in Chinese female outpatients with genital tract infections compared that married women. This change appears to be mostly affected by co-existing genital tract infections, especially vaginitis, suggesting *C. trachomatis* screening is particularly important in patients with vaginitis, and the vaginal microecology may play a key role in chlamydia. Screening for *C. trachomatis* is largely dependent on the available tests and accessible to the high-risk population (11); thus, in this research we assessed female outpatients with genital tract infections as the target population (Table 5).

This study also indicated chlamydia is more prevalent in females with lower age at first intercourse. The tendency for chlamydia to concentrate geographically and its associations with social and neighborhood factors have long been established. Though the current case-based data do not include migrant status, we noted relatively high prevalence rates in the Beijing–Tianjin–Hebei urban agglomeration

TABLE 5 Odds ratios (ORs) in multivariate analysis.

Variable		P-value	OR	Upper limit	Lower limit
Age, years	≤25		1.000	1.000	1.000
	>25	0.544	0.869	0.553	1.366
Housing situation	Stable		1.000	1.000	1.000
	Unstable	0.134	1.313	0.919	1.876
Premarital sex behavior	Yes		1.000	1.000	1.000
	No	0.005	1.903	1.218	2.972
Age at first intercourse, years	≤20		1.000	1.000	1.000
	>20	0.024	0.665	0.467	0.948
Number of sex partners	1–2		1.000	1.000	1.000
	≥3	0.471	0.827	0.492	1.388
Vaginal cleanliness	Normal (I–II)		1.000	1.000	1.000
	Abnormal (III–IV)	0.185	1.288	0.886	1.874
BV	No		1.000	1.000	1.000
	Yes	0.050	1.435	1.000	2.058

BV, bacterial vaginosis.

and southwestern coastal regions that are considered national migration destinations with intensive social-economic exchanges and frequent population movements, which may accelerate the spread of this sexually transmitted pathogen.

In this study, univariate analysis indicated that patients with unstable housing situation were disproportionately affected by the *C. trachomatis* epidemic. Unstable housing situation was associated with homelessness, poor physical health, mental illness and high risk of HIV (22). These findings indicate the importance of circumstances beyond individual behaviors for chlamydia risk, and environmental conditions and stable housing must be considered. Programs focusing on improving housing situation, might in turn exert indirect effects that may prove beneficial in preventing or reducing risky sexually transmitted behavior.

Multivariate analysis showed that premarital sex, younger age at the time of first sexual intercourse, and number of sex partners were associated with a higher risk of chlamydia, which may yet contribute to increasing the prevalence of STIs. Therefore, chlamydia control programs applying sex education strategies should focus on younger individuals, as well as other people with identified risk factors, including reducing early sex experience and the number of sex partners, to reduce the prevalence and economic burden of chlamydia. Furthermore, the prevalence of *C. trachomatis* was much higher in infertile patients compared with women with previous history of pregnancy or abortion. This result further supports the data demonstrating that chlamydia imposes a substantial reproductive burden in China.

The clinical condition of BV, in which the characteristic vaginal microbial communities *Lactobacillus* spp. are instead dominated by multiple anaerobes, is associated with an increased risk of *C. trachomatis* (23). Some of these anaerobes produce metabolites, e.g., biogenic amines and short chain fatty acids, which bolster chlamydia persistence (24). BV can therefore help identify individuals at high risk of chlamydia. Opportunistic screening for *C. trachomatis* among young sexually active adults has been recommended in outpatients with genital tract infections.

In terms of self-reported symptoms and signs, we found no symptoms or signs were significantly associated with the prevalence of chlamydia, indicating it is hard to detect *C. trachomatis* via

healthcare seeking behaviors, and screening for the high-risk population is important.

This study had several limitations, and the results should be interpreted with caution. First, the study participants were not randomly recruited, and may not be an accurate representation of the target study population. Secondly, the history of genital tract infections was self-reported; over- or underreporting due to self-reporting bias may impact the association estimates.

In summary, this study provides novel insights into specific risk factors associated with chlamydia in 12 provinces of China. Given that most chlamydia infections are asymptomatic and no vaccine is currently available (25), the backbone of chlamydia prevention should include not only behavioral interventions but also early screening programs to identify and treat individuals with genital tract infections (26) detecting missed infections that could lead to reproductive complications, reducing the prevalence and economic burden of trachomatis infections.

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 the Ethics Committee of Peking University First Hospital, Beijing, China. The patients/participants provided their written informed consent to participate in this study.

Author contributions

ZL and QL: conception and design of the study. DZ, ZL, QL, SE, MH, YH, XH, HW, ZX, HX, FX, MX, XY, and JZ: investigation and data collection. DZ, ZL, and TL: data analysis. TL and DZ: drafting the

manuscript. All authors commented on the draft and decision to submit.

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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Cervical HPV infection in Yueyang, China: a cross-sectional study of 125,604 women from 2019 to 2022

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Objective: Human papillomavirus (HPV) infection is currently the main cause of cervical cancer and precancerous lesions in women. The aim of this study was to investigate the epidemiological characteristics of HPV genotypes among women in Yueyang city and to provide a basis for the prevention and treatment of cervical cancer in this city.

Methods: A cross-sectional study was conducted on 125,604 women who had received treatment from eight hospitals in Yueyang city from September 2019 to September 2022. Analysis of the prevalence of HPV in patients.

Results: The prevalence of HPV was 20.5% (95%CI: 20.2–20.7%), of which the high-risk type (HR-HPV) accounted for 17.5% (95%CI: 17.3–17.7%) and the low-risk type (LR-HPV) accounted for 5.0% (95%CI: 4.9–5.1%). Among the HR-HPV subtypes, the top five in prevalence, from the highest to the lowest, were HPV52 (5.1%), HPV16 (2.7%), HPV58 (2.6%), HPV53 (2.4%), and HPV51 (1.7%). The main LR-HPV infection types were HPV81 (2,676 cases, OR = 2.1%; 95%CI, 2.0–2.1%). Among the infected patients, 19,203 cases (OR = 74.3%; 95%CI, 73.8–74.9%) had a single subtype, 4,673 cases (OR = 18.1%; 95%CI, 17.6–18.6%) had two subtypes, and 1,957 cases (OR = 7.6%; 95%CI, 7.3–7.9%) had three or more subtypes. HPV prevalence is highest among women <25 years, 55–64 years and ≥ 65 years of age.

Conclusion: The prevalence of HPV in women in Yueyang city was 20.5%, with HR-HPV being dominant. As women aged <25 years, 55–64 years, and ≥ 65 years are at a relatively higher risk, more attention should be paid to them for prevention and control of HPV infections.

KEYWORDS

human papillomavirus, genotypes, prevalence, epidemiology, cervical lesions

Introduction

Cervical cancer is one of the most common cancers in women. In 2020, 604,127 new cases and 341,831 deaths from cervical cancer were reported worldwide (1). In China, 109,741 new cases and 59,060 deaths from cervical cancer were reported in 2020 (2); therefore, cervical cancer remains a serious health threat to women in China. Human papillomavirus (HPV) is a circular, non-enveloped deoxyribonucleic acid (DNA) virus that can cause various lesions on the skin and mucous membranes. HPV infection is a common sexually transmitted disease (3), and approximately 75% of sexually active people become infected with this virus (4). Although most HPV infections are short-lived and do not seriously cause disease, some of these infections that last for several years may have a high potential to lead to malignancies (5). Persistent high-risk HPV (HR-HPV) infection is a critical step in cervical carcinogenesis (6, 7). Cervical cancer can be prevented, detected, and treated early. As such, early prevention has become the focus of cervical cancer prevention. At present, three types of vaccines have been licensed internationally for HPV infections. Vaccination has been effective in preventing cervical cancer in developed countries. However, only a minority of women in China have been vaccinated (8). Many studies suggest that the prevalence and genotype distribution of HPV vary by climate, region, ethnicity, and country (9, 10). Determining the distribution of HPV genotypes in Yueyang city will aid in cervical screening and vaccination and provide a scientific basis for the prevention and treatment of cervical cancer in the city.

Therefore, this study aimed to investigate the epidemiological characteristics of HPV genotypes in women from Yueyang city.

Materials and methods

Research subjects

In this cross-sectional study, participants were 125,604 women who underwent HPV typing tests at eight hospitals in Yueyang city from September 2019 to September 2022 (Yueyang People's Hospital, Yueyang Central Hospital, Miluo People's Hospital, Huarong County People's Hospital, Pingjiang County People's Hospital, Pingjiang County Maternal and Child Health Hospital, Yueyang County People's Hospital, and Linxiang People's Hospital). The age range of the participants was from 11 to 97 years, the average age is 43.42 ± 11.078 years old. The sampling criteria were as follows: non-menstrual period, no sexual activity within 24 h, no vaginal medication within 3 days, no vaginal irrigation, and no prior application of acetic acid or iodine solution. This study was approved by the ethics committee of the hospitals mentioned above.

This study focused on HPV infection prevalence as the main research index, which is known to be 15.5% based on the results of similar published literature (11). Using the following formula, $\alpha = 0.05$ (two-sided test) was selected, the allowable error $\delta = \pm 0.22\%$, $Z_{1-\alpha/2} = 1.96$, and $p = 15.5\%$, $n = (Z_{1-\alpha/2}^2 / \delta^2) / p(1-p)$ calculated with HPV infection prevalence yields 103,958 cases to be investigated. Considering that the rate of lost visits is 10 to 20%, the study included a total of 125,604 people, which can ensure the accuracy and scientific validity of the results of the study.

HPV testing

The HPV genotyping test kits applied by Yueyang People's Hospital, Yueyang Central Hospital, Miluo People's Hospital, Miluo Maternal and Child Health Hospital were provided by 21 HPV GenoArray Diagnostic Kit (HBGA-21PKG) (HybriBio, China, Guangdong), the HPV test kits applied by Pingjiang People's Hospital, Pingjiang Maternal and Child Health Hospital, Hualong People's Hospital were provided by 23 HPV Genotyping Panel kit from (CFDA: 20193401918) (Yanengbio, China, Shenzhen), and the HPV test kits applied by Linxiang People's Hospital were provided by 26 HPV Genotyping Panel kit from (CFDA: 20173404697) (TELLGEN Life Science, China, Shanghai). The sensitivity and specificity of these kits were higher than 95% compared to FDA approved kit.

In this study, 21 HPV genotypes were detected: 15 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, and 68) and 6 LR-HPV genotypes (6, 11, 42, 43, 44, and 81). Testing for the different HPV genotypes was conducted to identify the distribution and genotype of HR-HPV and low-risk HPV (LR-HPV) subtypes. Positive and negative controls were used for each test, and the test kit instructions were strictly followed.

Statistical analysis

SPSS Statistics 23.0 software was used for data analysis. Categorical variables were expressed as frequencies (proportions). Categorical variables were compared using Chi-square or Fisher's exact probability tests. Pairwise comparison between groups was performed using the Bonferroni method, adjusting for the level of p -values. p -values < 0.05 (two-sided) were considered statistically significant.

Results

Prevalence of HPV detection and subtype distribution

In this study, 125,604 women from eight hospitals in Yueyang city met the selection criteria (Figure 1). The age of the participants was from 11 to 97 years, and the average age was 43.42 ± 11.078 years. Among these participants, 25,714 showed positive results for HPV, and the prevalence of HPV detection was 20.5% (95%CI: 20.2–20.7%). Overall, 21,946 HR-HPV cases were noted, and the prevalence of HR-HPV and LR-HPV was 17.5% (95%CI: 17.3–17.7%) and 5.0% (95%CI: 4.9–5.1%), respectively.

Infection and distribution characteristics of different HPV genotypes

The top five subtypes of HR-HPV detected in order of prevalence (from the highest to the lowest) were as follows: HPV 52 (6,413 cases, OR = 5.1%; 95%CI, 5.0–5.2%), HPV 16 (3,410 cases, OR = 2.7%; 95%CI, 2.6–2.8%), HPV 58 (3,208 cases, OR = 2.6%; 95%CI, 2.5–2.6%), HPV 53 (3,004 cases, OR = 2.4%; 95%CI, 2.3–2.5%), and HPV 51 (2,093 cases, OR = 1.7%; 95%CI, 1.6–1.7%). The top three

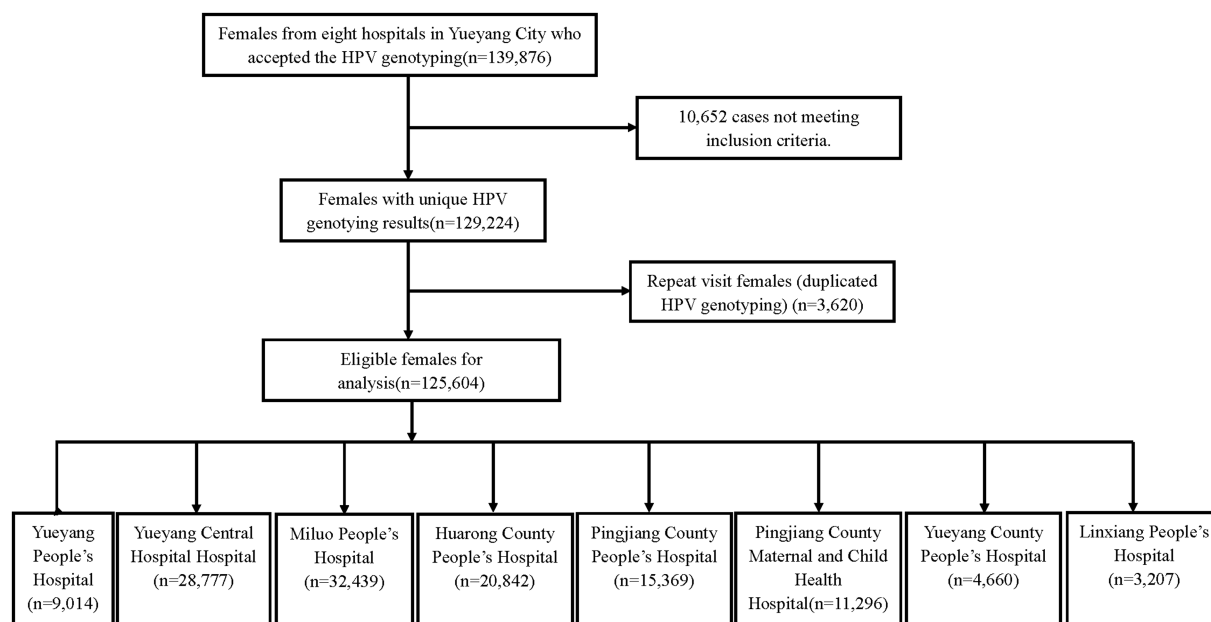


FIGURE 1
Study population.

subtypes of LR-HPV (from the highest to the lowest) were as follows: HPV 81 (2,576 cases, OR=2.1%; 95%CI, 2.0–2.1%), HPV 42 (1,303 cases, OR=1.0%; 95%CI, 1.0–1.1%), and HPV 43 (978 cases, OR=0.8%; 95%CI, 0.7–0.8%) (Table 1; Figure 2). Single subtype infections were the most common and accounted for 19,293 cases or 15.3% (95%CI: 15.2–15.6%) of the group. Dual subtype infections accounted for 4,673 cases or 3.7% (95%CI: 3.6–3.8%). Multiple infections (≥ 3) accounted for 1,957 cases or 1.6% (95%CI, 1.5–1.6%) ($p < 0.001$, Table 2).

HPV infections in women across different age groups

Table 3 shows the prevalence of HPV infections in different age groups. Of the 125,604 testing samples, they were classified into six age groups as follows: <25 years, 25–34 years, 35–44 years, 45–54 years, 55–64 years, and ≥ 65 years. The ≥ 65 -year-old group had the highest prevalence of 32.3% (95%CI: 30.9–33.6%) ($p < 0.001$).

There were two peaks in HPV prevalence. The first peak was for women aged <25 (OR=26.7%; 95%CI, 25.2–28.2%) and the second was for women aged 55 and over (OR=29.9%; 95%CI, 29.2–30.5%) (Bonferroni correction, $p < 0.001$) (Table 3, Figure 3). Any HPV infections and HR-HPV infections also exhibited these two peaks in the same age groups (Bonferroni correction, $p < 0.001$, respectively) (Table 3, Figure 3B), same trend for double HPV infection, and multiple HPV infection groups (Figure 3C). These two HPV prevalence peaks were also observed in the prevalence of HPV52, HPV16, and HPV58 infection (Bonferroni correction, $p < 0.001$, respectively) (Table 3, Figure 3A).

However, when we compared the prevalence of LR-HPV infection, single HPV infection, HPV53 and HPV81 infection in different age

TABLE 1 Single and multiple type infection rates different HPV subtypes in Yueyang.

HPV subtype	Positive, <i>n</i> (%)	95%CI for positive (%)	Single-type infection, <i>n</i> (%)	Multiple-type infection, <i>n</i> (%)
HPV52	6,413 (5.11)	4.98–5.23	4,054 (3.23)	2,359 (1.88)
HPV16	3,410 (2.71)	2.63–2.80	2,049 (1.63)	1,361 (1.08)
HPV58	3,208 (2.55)	2.47–2.64	1,824 (1.45)	1,384 (1.10)
HPV53	3,004 (2.39)	2.31–2.48	1,626 (1.29)	1,378 (1.10)
HPV81	2,576 (2.05)	1.97–2.13	1,343 (1.07)	1,233 (0.98)
HPV51	2,093 (1.67)	1.60–1.74	1,044 (0.83)	1,049 (0.84)
HPV68	1,645 (1.31)	1.25–1.37	826 (0.66)	819 (0.65)
HPV33	1,397 (1.11)	1.05–1.17	746 (0.59)	651 (0.52)
HPV39	1,334 (1.06)	1.01–1.12	694 (0.55)	640 (0.51)
HPV42	1,303 (1.04)	0.98–1.09	623 (0.50)	680 (0.54)
HPV56	1,210 (0.96)	0.91–1.02	579 (0.46)	631 (0.50)
HPV18	1,177 (0.94)	0.88–0.99	624 (0.50)	553 (0.44)
HPV43	978 (0.78)	0.73–0.83	450 (0.36)	528 (0.42)
HPV66	915 (0.73)	0.68–0.78	437 (0.35)	478 (0.38)
HPV59	895 (0.71)	0.67–0.76	415 (0.33)	480 (0.38)
HPV31	815 (0.65)	0.60–0.69	391 (0.31)	424 (0.34)
HPV6	840 (0.67)	0.62–0.71	399 (0.32)	441 (0.35)
HPV44	569 (0.45)	0.42–0.49	371 (0.30)	198 (0.16)
HPV35	502 (0.40)	0.36–0.43	208 (0.17)	294 (0.23)
HPV11	451 (0.36)	0.33–0.39	216 (0.17)	235 (0.19)
HPV45	360 (0.29)	0.26–0.32	162 (0.13)	198 (0.16)

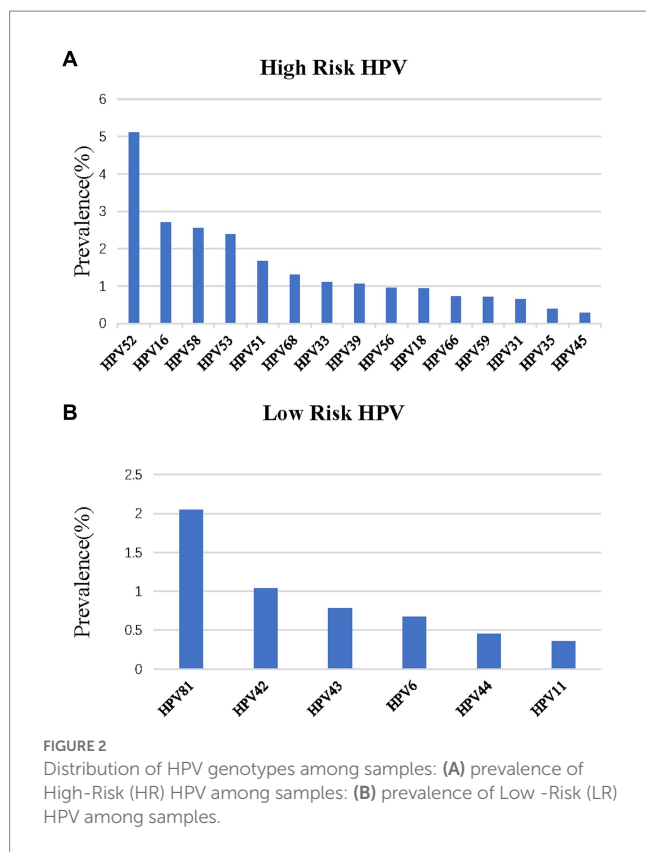


TABLE 2 Prevalence of single and multiple HPV infection (N = 25,833).

Genotype of HPV infection	Number of cases	Prevalence, %
1 HPV subtype ^a	19,203	15.29
2 HPV subtype	4,673	3.72
3 HPV subtype	1,364	1.09
4 HPV subtype	401	0.32
5 HPV subtype	119	0.09
6 HPV subtype	41	0.03
7 HPV subtype	20	0.02
8 HPV subtype	7	0.01
9 HPV subtype	5	0.004

^aHigher prevalence of single than multiple HPV infections. $p < 0.001$.

groups, the second peak in the group aged 55–64 (Table 3, Figure 3) (Bonferroni correction, $p < 0.001$, respectively).

Among the 21 HPV subtypes, the prevalence of HPV 52 subtype was the highest in all the age groups in addition to the ≥ 65 years old group, with the prevalence of 8.4% (95%CI: 7.6–9.2%) ($p < 0.001$). Of the six LR-HPV subtypes, HPV 81 had the highest prevalence ($p < 0.001$). The subtype prevalence differed for each age group. For the two age groups with larger sample sizes, namely the 35–44-year-old group ($n = 34,346$) and the 45–54-year-old group ($n = 41,748$), the top five subtypes were HPV 52, HPV 16, HPV 58, HPV 53, and HPV 81 and HPV 52, HPV 53, HPV 16, HPV 58, and HPV 81, respectively. For the < 25 -year-old group ($n = 3,343$), which had the fewest number

of cases, the top five subtypes were HPV 52, HPV 16, HPV 51, HPV 58, and HPV 81 (Table 3; Figure 3A).

Discussion

The primary objective of this study was to investigate the prevalence and genotype distribution of HPV infections in Yueyang city. The results of this study showed that the total HPV the prevalence in this region was 20.47%, which was higher than that reported in previous studies in other parts of China, such as Huzhou (15.5%), Tianjin (14.71%), Xinjiang (14.0%), and Shanghai (17.92 and 18.98%) (8, 11–14). However, the HPV prevalence in Yueyang was lower than that in Shandong (28.4%), Changsha (26.5%), Chongqing (26.2%), and Beijing (21.06%) (14–17). This indicates that the HPV prevalence differ according to regions. In the present study, the prevalence of HR-HPV was 17.47%, which was higher than that of LR-HPV (4.97%). This indicates that the Yueyang region is dominated by HR-HPV infection, an observation that is consistent with the findings of most previous studies in China (17–20). Persistent HR-HPV infection can cause cervical abnormalities and lead to cervical cancer (21, 22). Therefore, there is an urgent need to enhance HPV infection screening and genotype monitoring in Yueyang city.

Epidemiological studies have shown that the five most common genotypes worldwide are HPV 16, HPV 18, HPV 52, HPV 31, and HPV 58 (23) and that the most common genotypes in Asia are HPV 16, HPV 52, HPV 58, HPV 18, and HPV 56 (24). The present study showed that HPV 52 (5.11%), HPV 16 (2.71%), HPV 58 (2.55%), HPV 53 (2.39%), and HPV 81 (2.05%) are the dominant subtypes in Yueyang, which are the same as those reported by Yurong Zhu in Huzhou (11), but different from those dominant in Zhejiang (52, 58, 16, 51), Xinjiang A(16, 52, 58, 53, 31), and Shanghai (16, 58, 52, 51, 54) (8, 14, 25). This means that the prevalence of different HPV subtypes differs depending on the region, socioeconomic levels, resource availability, and ethnicity.

In the present study, the most common HPV genotype was HPV 52. This finding is the same as that in Beijing, Jiangsu, Zhejiang, and Guangdong (17, 25–27). Many studies have also concluded that HPV 16 is the most common genotype, as seen in Xinjiang, Tianjin, Shanghai, and Shandong (8, 12, 14, 28). That said, bivalent vaccines, which can prevent HPV 16 and HPV 18 infections, and quadrivalent vaccines, which can prevent HPV 6, HPV 11, HPV 16, and HPV 18 infections, do not prevent HPV 52 and HPV 58 infections. Currently, only 9-valent vaccines can prevent HPV 52, HPV 16, and HPV 58 infections, which have high prevalence in Yueyang, as well as prevent HPV 6, HPV 11, HPV 18, HPV 31, HPV 33, and HPV 45 infections. However, women who have received 9-valent vaccinations are a minority in Yueyang. Therefore, it is critical to enhance efforts in increasing vaccination rates among women in Yueyang. In addition, the present study found that HPV 53 and HPV 81 are relatively more prevalent in Yueyang. These two subtypes are not covered by the 9-valent vaccines; therefore, more research is required to determine whether it is necessary to develop and distribute vaccines in Yueyang to target HPV 53 and 81 specifically.

Numerous studies have shown that HPV infection is significantly age-specific. The data in the present study supports previous findings of a bimodal distribution of the HPV prevalence in China (11, 19,

TABLE 3 Prevalence of HPV infection by age group (N = 125,604).

Prevalence of HPV infection (n/%)							
HPV genotype	<25 (n = 3,343)	25–34 (n = 27,695)	35–44 (n = 34,346)	45–54 (n = 41,748)	55–64 (n = 13,863)	≥65 (n = 4,609)	p value ^a
Any HPV Infection ^b	892 (26.68)	4,735 (17.10)	6,055 (17.63)	8,519 (20.41)	4,025 (29.03)	1,488 (32.28)	<0.001
High-risk HPV							
Any high-risk HPV ^b	583 (17.44)	4,068 (14.69)	5,176 (15.07)	7,235 (17.33)	3,520 (25.39)	1,364 (29.59)	<0.001
52 ^b	194 (5.80)	1,285 (4.64)	1,499 (4.36)	2,075 (4.97)	1,019 (7.35)	389 (8.44)	<0.001
16 ^b	170 (5.09)	683 (2.47)	742 (2.16)	1,041 (2.49)	524 (3.78)	275 (5.97)	<0.001
58 ^b	93 (2.78)	576 (2.08)	666 (1.94)	1,039 (2.49)	582 (4.20)	276 (5.99)	<0.001
53 ^b	80 (2.39)	475 (1.72)	643 (1.87)	1,051 (2.52)	556 (4.01)	207 (4.49)	<0.001
51	96 (2.87)	430 (1.55)	471 (1.37)	633 (1.52)	331 (2.39)	145 (3.15)	-
68	51 (1.53)	297 (1.07)	381 (1.11)	524 (1.26)	292 (2.11)	111 (2.41)	-
33	62 (1.85)	201 (0.73)	288 (0.84)	425 (1.02)	277 (2.00)	151 (3.28)	-
39	45 (1.35)	251 (0.91)	319 (0.93)	402 (0.96)	202 (1.46)	105 (2.28)	-
56	51 (1.53)	203 (0.73)	224 (0.65)	392 (0.94)	248 (1.79)	106 (2.30)	-
18	48 (1.44)	221 (0.80)	290 (0.84)	365 (0.87)	186 (1.34)	73 (1.58)	-
66	39 (1.17)	160 (0.58)	213 (0.62)	281 (0.67)	164 (1.18)	66 (1.43)	-
59	49 (1.47)	182 (0.66)	192 (0.56)	257 (0.62)	151 (1.09)	68 (1.48)	-
31	27 (0.81)	142 (0.51)	183 (0.53)	235 (0.56)	151 (1.09)	87 (1.89)	-
35	14 (0.42)	84 (0.30)	105 (0.31)	156 (0.37)	103 (0.74)	44 (0.95)	-
45	17 (0.51)	60 (0.22)	64 (0.19)	133 (0.32)	58 (0.42)	30 (0.65)	-
Low-risk HPV							
Any low-risk HPV ^b	269 (8.05)	1,060 (3.83)	1,318 (3.84)	2,118 (5.07)	1,099 (7.93)	382 (8.29)	<0.001
81 ^b	87 (2.60)	383 (1.38)	538 (1.57)	935 (2.24)	495 (3.57)	149 (3.23)	<0.001
42	55 (1.65)	222 (0.80)	246 (0.72)	404 (0.97)	261 (1.88)	125 (2.71)	-
43	55 (1.65)	169 (0.61)	196 (0.57)	355 (0.85)	152 (1.10)	59 (1.28)	-
6	58 (1.73)	181 (0.65)	186 (0.54)	223 (0.53)	133 (0.96)	55 (1.19)	-
44	9 (0.27)	81 (0.29)	148 (0.43)	208 (0.50)	92 (0.66)	32 (0.69)	-
11	50 (1.50)	89 (0.32)	86 (0.25)	134 (0.32)	70 (0.50)	25 (0.54)	-
1 HPV infection ^b	599 (17.92)	3,659 (13.21)	4,834 (14.07)	6,470 (15.50)	2,654 (19.14)	867 (18.81)	-
2 HPV infection ^b	183 (5.47)	816 (2.95)	926 (2.70)	1,510 (3.62)	902 (6.15)	337 (7.31)	-
Multiple HPV infection ^b	110 (3.29)	260 (0.94)	295 (0.86)	539 (1.29)	469 (3.38)	284 (6.16)	-

^aOverall differences in the prevalence of HPV infection between age groups were analyzed using the Chi-square test.

^bPairwise comparisons of HPV prevalence in different age groups were performed using the Bonferroni correction.

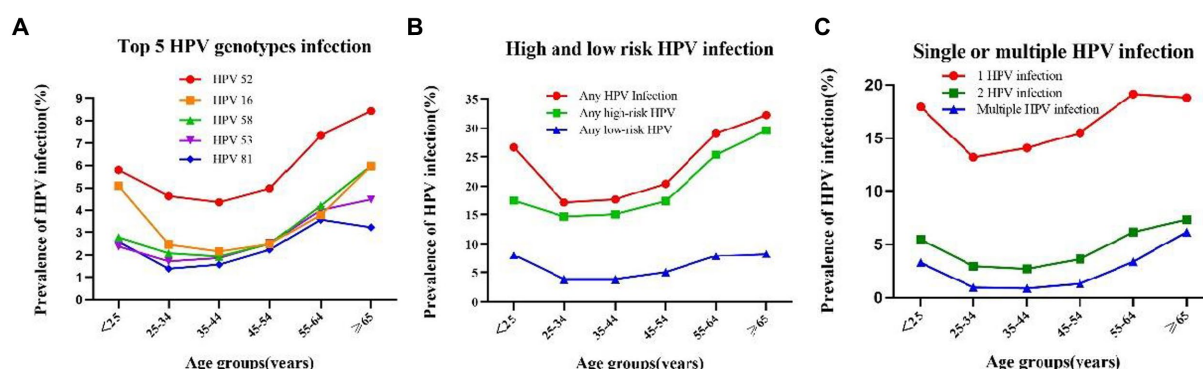


FIGURE 3

Prevalence and of HPV grouped by age: (A) Prevalence and of HPV 52,26,58,53 and 81 in each age group; (B) prevalence of High-Risk (HR), Low -Risk (LR), and HPV infection in each age group; (C) Prevalence and single and multiple infection of HPV in each age group.

29–31), as our results showed incidence peaks for women aged <25 and ≥55 years. The incidence peak for young women aged <25 years could perhaps be attributed to their insufficient understanding of sex and their immature immune systems. For women aged ≥55 years, they may have been affected by physiological and immune disorders during menopausal transition. Therefore, we recommend enhancing HPV vaccination programs and lowering the age of cervical cancer screening for young women in Yueyang.

This study has some limitations. First, since the screening samples were taken from various hospitals, we cannot rule out operational mistakes and errors in the results. Second, the sample also included patients who actively sought HPV testing because they were experiencing symptoms, which may have led to higher rates of HPV infection. Finally, because not all women had the opportunity to undergo free cervical cancer screening, data from women with a lower socioeconomic status and cervical cancer awareness would have been left out from the study. Hence, such data could not be deemed as fully representative of the women in Yueyang. We recommend that enhancing efforts to promote and increase cervical cancer screening and to make free screenings available to more women should be continued, which will increase the quantity and comprehensiveness of data for future research.

In conclusion, the data from this multi-center study have shown that the HPV prevalence of women in Yueyang was 20.47%. HR-HPV was the dominant subtype, and the five most common genotypes were HPV 52, HPV 16, HPV 58, HPV 53, and HPV 81. Based on age groups, incidence peaks were observed for women aged <25 years, 55–64 years, and ≥65 years. We believe that this study will provide significant value to future cervical cancer screening and HPV vaccine development strategies in Yueyang.

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 scheme was reviewed and approved by the Ethics Committee of Yueyang People's Hospital, approval number: 2019003. The patients/participants provided their written informed consent to

participate in this study. The research was conducted ethically in accordance with the World Medical Association Declaration of Helsinki.

Author contributions

YG and XZ conceived and designed the study and took responsibility for the content of the manuscript. JH and MZ analyzed the data, took responsibility for the accuracy of the data analysis, and wrote the first draft of the manuscript. YG and XZ reviewed the paper. CL, BX, YL, LW, LZ, ML, and ZZ were responsible for clinical data collection. 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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A call for switching to a 1-dose 9vHPV national vaccination program in Ethiopia

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Background

Cervical cancer (CC), caused by a group of human papillomaviruses (HPV), is primarily a sexually transmitted disease. In Ethiopia, CC is the second most common cancer with estimated annual new cases and deaths of 7,450 and 5,300, respectively (1). CC and other high-risk (HR), probable-risk (PR), and low-risk (LR) carcinogenic genotypes-associated diseases are vaccine-preventable. Currently, six commercial prophylactic HPV vaccines are available: three bivalent (HPV 16 and 18) vaccines (2vHPV), two quadrivalent (HPV 6, 11, 16, and 18) vaccines (4vHPV), and a nonavalent (HPV 6, 11, 16, 18, 31, 33, 45, 52, and 58) vaccine (9vHPV) (2). All these vaccines were originally licensed as a three-dose (3-dose) schedule over six months intervals, but a two-dose (2-dose) schedule was approved in girls younger than 15 years in 2016 after post-license efficacy evidence became available (3, 4). Subsequently, Ethiopia launched the HPV vaccination program with 2-dose Gardasil®4 (4vHPV vaccine) in December 2018 with the support of Gavi, the Global Vaccine Alliance.

Like many Gavi-eligible countries, Ethiopia had to choose a single-age cohort primarily targeting schoolgirls aged 14 years old because of the global vaccine supply constraint. The supply constraint is projected to continue for several years with many Gavi-eligible countries planning the introduction of HPV national immunization programs (5). Importantly, WHO set a goal of achieving CC elimination by 2030 worldwide. A dose-reduction vaccination approach is urgently needed if the WHO goal of CC elimination by 2030 is to be met. High-quality large-scale observational studies in Costa Rica and India demonstrated that a one-dose (1-dose) HPV vaccine regimen induces similar long-term protection to that of multidose (3, 4). On 7th April 2022, based on these efficacy data, the WHO's Strategic Advisory Group of Experts on Immunization (SAGE) recommended that each country could decide to implement alternative, off-label regimens, including a 2-dose schedule in all age groups or a 1-dose schedule for individuals aged 9–20 years (6). Before the implementation of a 1-dose HPV vaccine regime, however, the following key policy questions should be answered based on the currently available evidence in the Ethiopian context: (i) which 1-dose vaccine type regime (1-dose 4vHPV versus 1-dose 9vHPV) is optimal; (ii) does Ethiopia need locally generated evidence to make a policy switch to a 1-dose 9vHPV vaccine regime; (iii) will 1-dose 9vHPV vaccine be sufficient to offer protection to HIV-infected girls; and (iv) is the HPV vaccine uptake optimal? In this opinion paper, we provide our insights related to these key policy questions.

Single-dose 9vHPV vaccine will offer protection to more commonly circulating HR-HPV genotypes

According to the recent systematic review, HPV 16 is the most dominant HPV genotype among HPV-positive women in Ethiopia, accounting for over 37% of HR-HPV infections while HPV18 is responsible for 4.4% of infections (Table 1) (7) with a combined HPV16/18 infection prevalence of 54%. The seven common non-HPV16/18 HR-HPV genotypes detected in Ethiopia were [HPV-31 (3.8%), 33 (1.7%), 35 (4.8%), 39 (1.9%), 45 (3.5%), 52 (6.8%), 58 (3.1%), and 68 (2.8%)] (7). The data implies that 46% of women's infections with HR-HPV genotypes in Ethiopia are related to non-HPV 16/18 genotypes. A very recent study found that HPV16 (31.8%), 31 (19.1%), 52 (11.8%), 58(10.9%), and 35 (10%) were the most frequently detected HR-HPV genotypes while HPV18 was detected in only 2.7% of HPV-positive women (Table 1) (8). These data altogether suggest that vaccinating girls with the 4vHPV vaccine would protect only 54 % of infections with HR-HPV genotypes, leaving 46% of vaccines vulnerable to infections with non-4vHPV vaccine HR-HPV genotypes. By contrast, the 9vHPV vaccine is highly efficacious in offering protection to five additional HR-HPV genotypes (9). Thus, if Ethiopia switches to the 9vHPV vaccine, over 90% of vaccinated women will likely be protected from the 9vHPV vaccine HR-HPV genotypes-related persistent infections and diseases (Table 1).

Evidence for the efficacy of 1-dose 9vHPV vaccine in east Africa in HIV-negative cohorts

Given obtaining efficacy data based on virological or cervical dysplasia (disease) endpoints will take several years when vaccination given to girls before first sexual debut, the efficacy of the 1-dose 9vHPV vaccine can be assessed through immunobridging trials (10). An immunobridging trial is a non-inferiority comparison of geometric mean concentrations (GMCs) of anti-HPV antibodies for specific HPV genotypes in a new population group with those in a population group in whom efficacy had been established in randomized clinical trial (RCT) with virological or disease endpoints. It assumes if the GMCs of antibodies between two groups are comparable, the efficacy between the two groups is comparable too. Accordingly, immunobridging RCTs on a 1-dose 9vHPV vaccine schedule have been conducted in sub-Saharan African countries where the 90% of the global CC burden occurs and dose reduction is a critical strategy for increasing HPV vaccine coverage (10). Recent randomized multi-center, double-blind, controlled trials with clinical endpoints at 35 months in Tanzania (11) and at 18 months in Kenya (12) indicate 96.0% and 91.4% efficacies, respectively in the HPV 16/18/31/33/45/52/58, substantiating the evidence obtained from immunobridging RCT in Tanzania. These results from RCTs conducted in Tanzania and Kenya, where like Ethiopia their populations have similar additional comorbidities (e.g., HIV, parasites, and malnutrition) that may compromise the quality and durability of vaccine-induced immune responses, proved the

efficacy of 1-dose 9vHPV vaccine in preventing CC associated with infections with HPV-16, 18, 31, 33, 45, 52, and 58. Based on these established efficacy results along with the diversity of HR-HPV genotypes distribution data in Ethiopia, we are advocating a policy switch to a 1-dose 9vHPV vaccine schedule. Nevertheless, if the Ministry of Health needs prior local evidence to make the in-demand decision, conducting an immunobridging RCT study that aims to compare antibody GMC specific to HPV genotypes with the Tanzanian 1-dose 9vHPV vaccine immunobridging cohort (10) can be considered instead of waiting for several years until local efficacy data based on virological or disease endpoints will be generated.

Will 1-dose 9vHPV vaccine be sufficient for HIV-infected girls?

In sub-Saharan Africa, including Ethiopia, the burden of HIV infection is also high. In 2023 alone, >620,000 new HIV infections are estimated to occur in Ethiopia with women being disproportionately infected (13). Approximately 1 in 5 CCs occur in women living with HIV (WLWH). Additionally, WLWH are more likely to be infected with more HR-HPV genotypes not covered by the 4vHPV vaccine (14). Considering this, WLWH will more likely benefit from the introduction of the 9vHPV vaccine. Despite accumulated evidence on immunogenicity and tolerability of 3-dose of both 4vHPV and 9vHPV vaccines in HIV-infected children and women (15), however, there is no RCT regarding the efficacy of 1-dose 9vHPV vaccine in HIV-positive individuals so far. An immunobridging study would be the fastest strategy to get approval for the use of a 1-dose 9vHPV vaccine in WLWH. Until results from immunobridging studies are publicly available, we dither advocating the use of 1-dose 9vHPV in HIV-infected girls. Instead, we recommend at least 2-dose 9vHPV vaccination over the ongoing 2-dose 4vHPV given the 9vHPV vaccine's potential to offer protection to more non-4vHPV vaccine HR-HPV genotypes (9).

Economic benefits of 1-dose 9vHPV vaccine

Besides the additional public health benefits, a mathematical model-based study suggests the cost-effectiveness of the 9vHPV vaccine in Ethiopia compared to the current 2-dose 4vHPV vaccination program (16). Another modeling analysis showed similar economic beneficial impact of a 1-dose of 9vHPV vaccination schedule in Tanzania (17), where like Ethiopia, high diversity of HR HPV genotypes has been detected in women with abnormal cervical lesions. The minimum price per dose for Gardasil 4vHPV and 9vHPV vaccines are \$4.50 and \$5.18, respectively (16). In Ethiopia, the estimated number of girls of age 14 years who are eligible to take the current 2-dose 4vHPV vaccine in 2023 alone is above 1.5 million (13). If Ethiopia adopts a 1-dose 9vHPV vaccine as its national HPV immunization schedule, it will save a minimum of \$5.73 million per year and a total of \$40.11 million by end of 2030, assuming that the vaccine uptake and coverage is 100% and the number of HPV vaccine eligible girls remain the same. In addition, it will save the costs

TABLE 1 Relative distribution of 9vHPV vaccine HPV genotypes in women with and without abnormal cervical dysplasia in Ethiopia and potential protection offered by 9vHPV and 4vHPV vaccination.

9vHPV vaccine HPV genotype	Proportion (%) of 9vHPV vaccine genotypes [Derbie et al. (7) ^a]	Potential protection offered by 9vHPV* vaccine	Potential protection offered by 4vHPV [§] vaccine	Proportion (%) of 9vHPV vaccine genotypes [Seyoum et al. (8) ^b]	Potential protection offered by 9vHPV vaccine	Potential protection offered by 4vHPV* vaccine
HR-HPV						
HPV 16	37.0	+	+	31.8	+	+
HPV 18	4.4	+	+	2.7	+	+
HPV 31	3.8	+	–	19.1	+	–
HPV 33	1.7	+	–	2.7	+	–
HPV 45	3.5	+	–	6.4	+	–
HPV 52	6.8	+	–	11.8	+	–
HPV 58	3.1	+	–	10.9	+	–
LR-HPV						
HPV 6	2.0	+	+	UK	+	+
HPV 11	2.7	+	+	UK	+	+

^asystematic review; ^ba hospital-based cross-sectional study; *proposed Gardasil 9vHPV vaccine; [§]currently in-use Gardasil 4v HPV vaccine; + offers protection; – offers little or no protection; UK, unknown; HR-HPV, high-risk oncogenic HPV genotypes; low-risk HPV genotypes which can cause anogenital warts.

required for second dose injection needle and delivery, including personnel, transportation and storage costs. And this saved money can be stretched to support vaccine delivery or building other healthcare services.

Vaccine uptake and barriers to reaching the optimal level

Despite the excellent safety profile of all HPV vaccines, its uptake in Ethiopia is suboptimal as low as 44.4% (18). In addition, a low disparity between uptake of 1-dose and 2-dose was observed (19), suggesting that switching to a 1-dose schedule alone may not necessarily increase HPV vaccination coverage to an optimal level. The main barriers to vaccine uptake were concerns about side effects (primarily fear of injection and pain at the site of injection), a misconception that the vaccine is given as anti-fertility, and a lack of adequate information on the benefit of the HPV vaccine before the vaccination schedule and lack of regular vaccination program (18, 19). However, barriers related to vaccine side effects can be circumvented by dose reduction as girls will prefer a 1-dose over 2-dose schedule to avoid pain caused by the second shot. Future country-wide study that involves all stakeholders is urgently needed to identify the main barriers to single-dose vaccine uptake and mitigation strategies.

Conclusions and recommendations

Until the global vaccine shortage is circumvented and becomes affordable to low and middle-income countries (LMICs), including Ethiopia, we suggest switching to

a 1-dose 9vHPV vaccination program to accelerate the elimination of CC in Ethiopia by 2030. For HIV-infected girls, however, we recommend the use of at least a 2-dose 9vHPV vaccination schedule until evidence regarding the efficacy of 1-dose 9vHPV vaccination in WLWH are available. Switching to a 1-dose vaccination schedule can also partially circumvent the current suboptimal HPV vaccine uptake in Ethiopia.

Despite its health benefits, the 9vHPV vaccine is not yet included in the Gavi support list for low-income countries (LICs). Thus, high CC burden LICs, including Ethiopia with a high prevalence of non-4vHPV vaccine HR-HPV genotypes should request Gavi to consider the 9vHPV vaccine in its support list or seek other global international support. If Gavi requires local evidence to support switching to a 1-dose 9vHPV vaccine schedule, Ethiopia may consider conducting a pilot immunobridging RCT instead of clinical efficacy data.

Author contributions

Conceptualization: TG and AMu. Writing of the initial draft of the manuscript: TG. Editing and reviewing the last draft: TG, LW, AMi, and AMu.

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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HPV prevalence and genotype distribution in 2,306 patients with cervical squamous cell carcinoma in central and eastern China

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HPV-positive cervical intraepithelial neoplasia and guiding them in implementing appropriate interventions. Furthermore, HPV genotyping is helpful for doctors to follow up HR-HPV-positive women and to evaluate the protective effect of HPV vaccine.

KEYWORDS

human papillomavirus, cervical squamous cell carcinoma, high-risk genotypes, prevalence, China

1. Introduction

Cervical cancer is the most common malignancy of the female reproductive system and ranks second after breast cancer in developing countries (1). In China, there are approximately 110,000 new cases of cervical cancer and 60,000 deaths each year (2). Human papillomavirus (HPV) infection is closely associated the development of cervical cancer (3). So far, more than 200 different Human papillomavirus (HPV) genotypes have been found in nature (4), of which more than 40 can infect the male and female reproductive tracts, including the skin of the penis, vulva, anus, vagina, cervix, and rectum (4, 5).

These HPV genotypes are further classified into low-risk HPV (LR-HPV) and high-risk HPV (HR-HPV) based on their carcinogenicity. HR-HPVs including HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, -59, are mainly responsible for the development of cervical cancer (6–8). Of which, HPV-16 and HPV-18 are the primary HR-HPV genotypes related to 71% cervical cancer worldwide (9, 10). There are significant differences in the prevalence of different HPV genotypes in cervical cancer (11–13). At present, there is no systematic evaluation of age distribution, HPV genotype distribution and HPV infection rate in patients with cervical squamous cell carcinoma (CSCC) in a large sample in central and eastern China. It is also unclear whether there is significant difference in HPV genotype distribution and infection rate between CSCC patients and cervical cancer screening individuals.

To answer the questions above, we collected tissue samples from 2,306 CSCC patients who underwent surgery, as well as the exfoliated cervical cells from 10,245 cervical cancer screening population. We systematically investigated the prevalence and genotype distribution of HPV in both groups. Our results can provide new clues for further understanding of the pathogenesis of CSCC and the prevention strategies for CSCC.

2. Materials and methods

2.1. Collection of tissue samples from CSCC patients

From January 2010 to October 2022, we collected 2,306 clinically diagnosed CSCC cases from 48 provincial and county-level hospitals in 30 cities across 6 provinces in central and eastern China (Supplementary Table S1). The patients' average age was 53 ± 11.89 (ranging from 19–90) years old. All patients underwent radical total hysterectomy, and their surgically excised tissues were pathologically

examined. Two experienced pathologists reviewed the slides of paraffin-embedded CSCC tissues and clinical pathological data of the CSCC samples according to the classification criteria of gynecological tumors in WHO (14). This study was approved by the Ethics Committee of Nanjing Traditional Chinese Medicine Hospital (approval no. 2012NJL008).

2.2. Specimen collection of exfoliated cervical cells from the healthy women undergoing cervical cancer screening

In this study, a total of 10,245 healthy women were recruited, who aged between 35–64 years old and underwent cervical cancer screening in the community of Jiangning District, Nanjing City, Jiangsu Province in 2022. Exfoliated cervical cells were collected from each participant by experienced professional gynecologists using a plastic sampling brush. The brush was inserted into the cervix and rotated five times in the same direction before slowly being withdrawn. The brush head was then broken off and placed vertically in a labeled sampling tube containing 95% ethanol fixative. The bottle cap was tightly sealed and shaken 5–8 times and stored at 4°C for HPV DNA genotype testing.

2.3. DNA extraction from paraffin-embedded tissue and exfoliated cervical cells

Firstly, the excess paraffin around each paraffin-embedded tissue was removed and each paraffin-embedded tissue was cut into 4 μ m thick slices, yielding for 3–5 slices. These slices were then placed in a 1.5 mL centrifuge tube and 150 μ L lysis buffer was added. Then mix thoroughly and centrifuge instantaneously. The tubes were heated in a metal bath at 100°C for 10 min. Immediately centrifuge at 13,000 rpm for 10 min. The middle layer of the DNA solution was collected for further use. According to the instructions of DNA extraction kit (catalog number DP340, Tiangen biochemical technology Co., Ltd., Beijing, China), the total DNA of exfoliated cervical cells in cervical cancer screening group was extracted.

2.4. HPV genotyping test

All DNA samples were tested using the Human Papillomavirus Genotyping kit for 23 Types (HPV 23 full-Genotyping) (catalog

number QX202203011) provided by Yaneng Bioscience (Shenzhen) Co., Ltd. This HPV genotyping kit detects 23 genotypes, including six LR-HPV genotypes (HPV-6, -11, -42, -43, -81 and -83) and 17 HR-HPV genotypes (HPV-16, -18, -58, -52, -31, -33, -59, -45, -51, -56, -73, -66, -39, -53, -68, -82, and -35). PCR was conducted on ABI7500 fluorescent detector produced by Life Technology (United States) Co., Ltd. Then, after each specimen was fluorescently labeled by PCR, the presence or absence of hybridization signals at various sites on the membrane chip were then used to determine the results. PCR amplification, hybridization, incubation, and color development were performed according to the instructions.

2.5. Statistical analysis

The statistical analysis in this study was conducted using the SPSS 26.0 software (IBM, United States). The data were processed and visualized using GraphPad Prism 8 (GraphPad software, San Diego, United States). Chi-square test was used to analyze the difference in prevalence or attribution between different HPV types. $p < 0.05$ was regarded as statistical significance.

3. Results

3.1. Introduction of the source of cervical squamous cell carcinoma tissue samples

A total of 2,306 cervical squamous cell carcinoma (CSCC) tissue samples were collected from 48 hospitals in six provinces in central and eastern China, including Jiangsu, Anhui, Zhejiang, Henan, Jiangxi, and Shandong. Among them, Jiangsu province contributed the highest number of samples, covering 38 hospitals and 1,688 cases, accounting for 73.20% of the total samples (Figure 1A). The samples from Jiangsu province were collected from 11 prefecture-level cities, of which Nanjing accounted for 22.22%, Huai'an for 14.69%, Yancheng for 14.63%, Suzhou for 14.04%, Zhenjiang for 12.74%, Taizhou for 9.48%, and Xuzhou for 8.12%, respectively (Figure 1B).

3.2. Overall prevalence of HPV infection in CSCC patients

Among the 2,306 cases of CSCC, a total of 2,138 cases were HPV-positive, accounting for 92.71% (2,138/2,306), while 168 cases were HPV-negative, accounting for 7.29% (168/2,306) (Figure 2A). Among HPV-positive patients, the youngest patient was 19 years old and the oldest was 90 years old. There was an obvious HPV infection peak in the 46–50 age group of HPV-positive CSCC, with a positivity rate of 18.73% (432/2,306) (Figure 2B). Furthermore, patients under the age of 34 accounted for 4.86%, those over 66 years old accounted for 16.35%, and those over 80 years old still accounted for 1.95% (Figure 2B). We compared the age distribution pattern between the HPV-positive and HPV-negative groups of CSCC samples. The age distribution curves of the two groups were very similar, with no significant statistical differences ($p > 0.05$, Figure 2C). These findings suggested that HPV-negative CSCC should also be included in control measures to identify early detection methods and prevent misdiagnosis due to missed examination.

3.3. HPV genotype distribution in HPV-positive CSCC

In this study, HPV DNA genotype testing was performed using a Human Papillomavirus Genotyping kit for 23 Types (please see the Methods section for more details). Twenty-two different HPV genotypes were identified in CSCC tissues successfully, except HPV-83 genotype (Table 1). Among the 2,138 cases of HPV-positive CSCC, there were a total of 121 different combinations of HPV infection genotypes. Among them, there were 17 forms of single-type HPV infection, including all 17 HR-HPV genotypes, while 104 combinations of multiple-type HPV infections (data not shown). Our data showed that HR-HPV genotypes were not only presented in the form of single-type HPV infection in CSCC but also in the form of multiple-type mixed HPV infection. However, the five detected LR-HPV genotypes were only presented in multiple-type HPV mixed infection in CSCC tissues (Table 1).

The results indicated that the top eight high-frequency (greater than 1%) HR-HPV genotypes were HPV-16, -18, -58, -52, -31, -33,

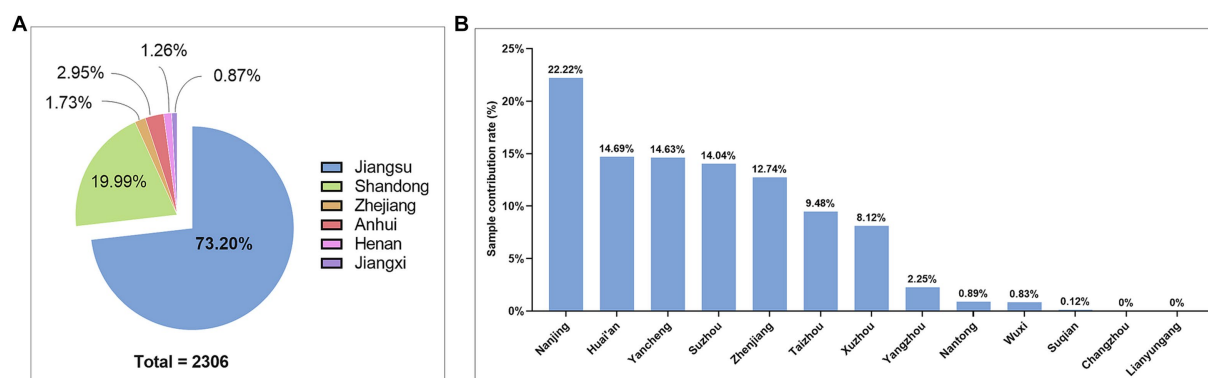


FIGURE 1
Sample source and geographical distribution in this study. (A) Pie chart of sample attribution rate by province; (B) Histogram of the sample attribution by city-level hospitals in Jiangsu Province.

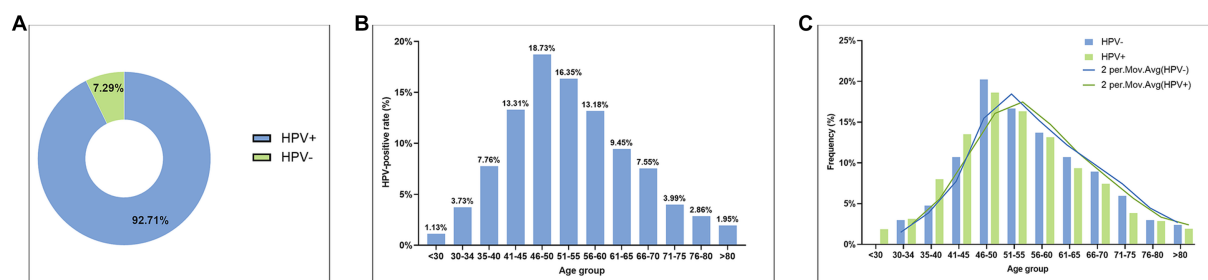


FIGURE 2

The overall prevalence of HPV infection in CSCC samples. (A) Pie chart of HPV-positive and HPV-negative rates in CSCC; (B) Histogram of age distribution of HPV infection in HPV-positive CSCC; and (C) Comparison of age distribution between HPV-positive CSCC and HPV-negative CSCC.

-59, and -45 in Chinese women with CSCC. Among them, HPV-16 was the most frequent one in both single-type and multiple-type HPV infection, accounting for 71.61% and 77.85%, respectively, followed by HPV-18 (7.19% and 23.18%), HPV-58 (5.19% and 21.45%) and HPV-52 (2.97% and 17.30%). Meanwhile, in the CSCC cases with LR-HPV positive, HPV-11 with multiple-type mixed infection had the highest frequency, accounting for 8.65% of cases (Table 1).

Of the 2,138 cases of HPV-positive CSCC, 86.48% (1849/2138) were found infected with single-type HPV, while 13.52% (289/2138) were identified with multiple-type HPV after de-redundancy. It should be noted that the cumulative total number of cases with multiple-types HPV infection in our study, would exceed 289 when calculating the frequency for each HPV genotype (Table 1). Specifically, 82.35% (238/289) of cases were infected with two types of HPV; 13.84% (40/289) of cases were infected with three types of HPV; while 3.81% (11/289) of cases were infected with four or more types of HPV (Figure 3A) with one case each infected with seven and nine types of HPV simultaneously.

Notably, among the 11 cases featuring four or more types of HPV infection, HPV-16 infection was detected in 90.9% (10/11) of cases. Meanwhile, HPV-16 infection was detected in 77.5% (31/40) of cases with three types of HPV mixed infection, 77.3% (184/238) of cases with two types of HPV mixed infection, and 71.6% (1,324/1849) of cases with single-type HPV infection (Figure 3B). Thus, our findings provide support for the assertion that HPV-16 is the most significant causative factor in the onset and progression of cervical cancer. Consequently, the timely detection and treatment of HPV-16 infection is crucial measure for preventing cervical cancer.

3.4. Attribution of common HR-HPV genotypes targeted by nine-valent HPV vaccine in cervical cancer

The HPV vaccine represents a significant preventative measure against HPV infection and serves as a crucial method for reducing the incidence of HPV-related cervical cancer. Among the HPV genotypes prevented by the nine-valent HPV vaccine, HPV-16, -18, -31, -33, -45, -52, and -58 are HR-HPV, while HPV-6 and HPV-11 are LR-HPV. In order to assess the efficacy of the nine-valent HPV vaccine in reducing the incidence of cervical cancer, we analyzed the cumulative attribution rates of the seven HR-HPV genotypes protected by the

vaccine in CSCC with HPV infection. The results showed that the positive rate of single-type HPV-16 infection in CSCC was 61.93% (1,324/2,138); the cumulative positivity rate of HPV-16 single-type or multiple-type infection was 72.45% (1,549/2,138); while the cumulative positivity rate of both HPV-16 and HPV-18 infection was 79.61% (1,702/2,138). Additionally, the cumulative positivity rate of the seven HR-HPV types related to nine-valent HPV vaccine in CSCC reached 95.09% (2,033/2,138). Considering that HPV-59 is also a common HR-HPV genotype in central and eastern Chinese women (Table 1), we further calculated the cumulative positivity rate for the eight HR-HPV types (HPV16/18/31/33/45/52/58/59) in CSCC which was found to be 96.91% (2,072/2,138) (Figure 4). These findings suggest that effective prevention measures targeting these eight HPV types could potentially reduce the incidence of CSCC by approximately 97%.

3.5. Genotype distribution of HPV infection in various age groups in CSCC

In this study, HPV-16 was identified as the most frequent detected HPV genotype across all age groups (Figure 5A). Notable, the peak of HPV infection was observed among HPV-positive CSCC cases aged 46–50 years old (Figure 5A), and the trend of HPV16 positivity rate was found to be similar to that of overall HPV infection (Figure 2B). In addition, the number of HPV infection genotypes in the eligible female population increases gradually with age (Figure 5B). At the same time, the positivity rate of each HPV genotype also increased gradually with age (Figure 5C). Among the age group, the highest prevalence and number of HPV infections was among women aged 40–60 years old, with a gradual decline observed in the group aged over 60 years. Notably, multiple HPV genotype infections were identified among individuals aged over 80 years (Figure 5C).

3.6. Comparison of the prevalence and genotype of HR-HPV between cervical cancer and healthy women undergoing cervical cancer screening

To compare the prevalence and genotype distribution of HR-HPV between cervical cancer patients and healthy individuals

TABLE 1 Genotype distribution of HPV infection in HPV-positive cervical squamous cell carcinoma.

HPV type	Genotype		Amount of single-type infection	Amount of multiple-type infection	Amount of two-type infection	Amount of three-type infections	Amount of four or more types infection	Amount of HPV-positive cases	Frequency of single-type infection	Frequency of multiple-type infection	HPV-positive rate
HR-HPV	1	HPV-16	1,324	225	184	31	10	1,549	71.61%	77.85%	72.45%
	2	HPV-18	133	67	53	11	3	200	7.19%	23.18%	9.35%
	3	HPV-58	96	62	45	13	4	158	5.19%	21.45%	7.39%
	4	HPV-52	55	50	36	8	6	105	2.97%	17.30%	4.91%
	5	HPV-31	69	33	25	6	2	102	3.73%	11.42%	4.77%
	6	HPV-33	53	39	29	6	4	92	2.87%	13.49%	4.30%
	7	HPV-59	37	20	10	8	2	57	2.00%	6.92%	2.67%
	8	HPV-45	20	14	9	2	3	34	1.08%	4.84%	1.59%
	9	HPV-51	7	12	6	5	1	19	0.38%	4.15%	0.89%
	10	HPV-56	7	12	9	1	2	19	0.38%	4.15%	0.89%
	11	HPV-73	8	11	7	2	2	19	0.43%	3.81%	0.89%
	12	HPV-66	9	9	4	1	4	18	0.49%	3.11%	0.84%
	13	HPV-39	11	5	4	1	0	16	0.59%	1.73%	0.75%
	14	HPV-53	5	11	8	3	0	16	0.27%	3.81%	0.75%
	15	HPV-68	4	10	7	2	1	14	0.22%	3.46%	0.65%
	16	HPV-82	7	3	1	2	0	10	0.38%	1.04%	0.47%
	17	HPV-35	4	4	2	2	0	8	0.22%	1.38%	0.37%
LR-HPV	18	HPV-11	0	25	12	7	6	25	0.00%	8.65%	1.17%
	19	HPV-6	0	20	11	5	4	20	0.00%	6.92%	0.94%
	20	HPV-81	0	8	3	3	2	8	0.00%	2.77%	0.37%
	21	HPV-42	0	7	7	0	0	7	0.00%	2.42%	0.33%
	22	HPV-43	0	7	4	1	2	7	0.00%	2.42%	0.33%
Total			1849	289	238	40	11	2,138	100.00%	100.00%	100.00%

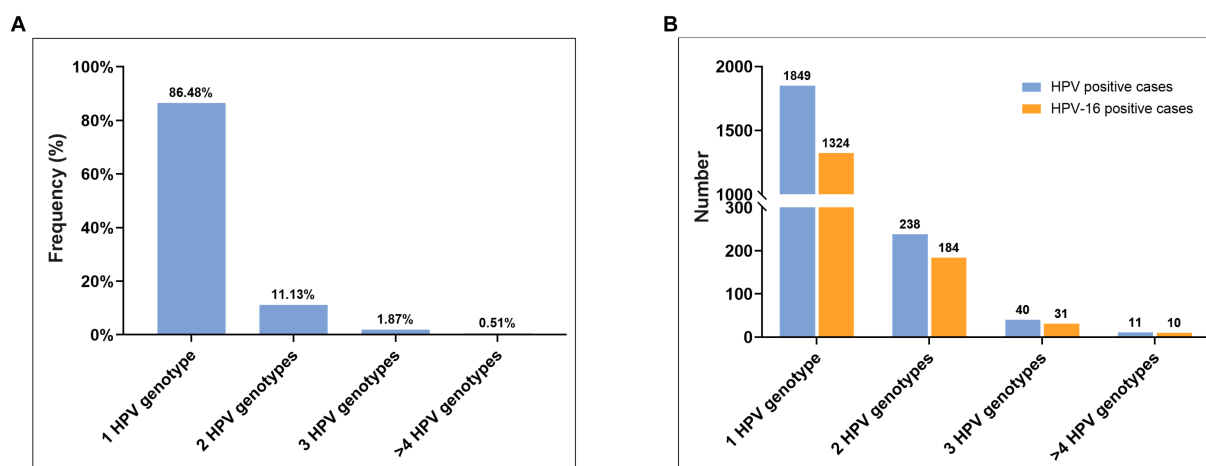


FIGURE 3

HPV genotype distribution in HPV-positive CSCC. (A) The prevalence of single-type HPV infection, two-type HPV mixed infection, three-type HPV mixed infection, and four or more types of HPV mixed infection. (B) The HPV-16 infection rate in different HPV infection form.

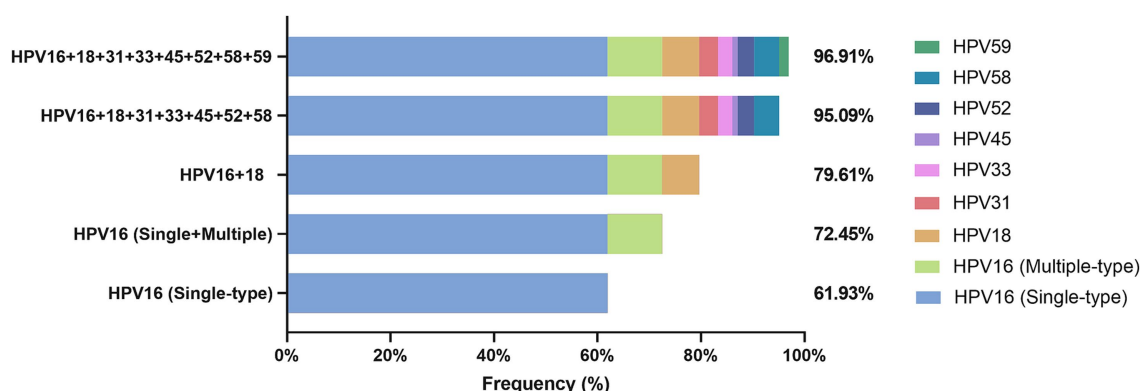


FIGURE 4

Cumulative attribution rates of common HR-HPV genotypes in HPV-positive CSCC cancer.

undergoing cervical cancer screening (screening population), we analyzed the HPV genotyping results of 10,245 individuals in the screening population in Jiangning District, Nanjing City, Jiangsu Province in 2022 (Table 2). Our results demonstrated a marked difference in the prevalence of common HR-HPV genotypes when comparing the screening population to cervical cancer patients. Specifically, the overall HPV positivity rate in screening population was 10.7% (1,097/10,245). Among them, the most common HPV genotypes were HPV-52 (2.25%), HPV-58 (1.60%), HPV-16 (1.31%), HPV-53 (1.22%), HPV-68 (0.93%), HPV-39 (0.92%), HPV-51 (0.78%), and HPV56 (0.74%). Among the eight common HR-HPV genotypes found in both cervical cancer patients and screening population, HPV-16 (ranked first in CSCC), HPV-58 (ranked third in CSCC), and HPV-52 (ranked fourth in CSCC) were found to be the same HPV genotypes. It was worth mentioning that the positivity rate of HPV-18 ranked only 14th in the screening population. Therefore, early detection and treatment of common HR-HPV genotypes associated with cervical cancer is critical for prevention and control of cervical cancer in China.

4. Discussion

4.1. The infection rate of HR-HPV In CSCC patients is relatively high worldwide

HPV infection is closely associated the development of cervical cancer. The analysis of HPV infection rate is of great significance for developing prevention and control strategy of cervical cancer. A Meta-analysis of 5,081 cervical cancer found that the infection rates of HR-HPV were 90.6%, 87.8%, and 89.8% in North China, Northeast China, and East China, respectively (15). The prevalence of HPV infection was 95.9% of 339 CSCC patients in Hong Kong, China (16). Another meta-analysis of 79 studies with 5,954 Asian cervical cancer patients found the HPV infection rate to be 85.9% (17). De Sanjose et al. conducted a retrospective analysis on 10,575 cervical cancer patients worldwide and found that 85% were HPV-positive in their paraffin tissue (18). The HPV infection rates were 88%, 87%, 91%, 82%, 95%, and 79% in Asia, Europe, North America, South America, Oceania, and Africa, respectively (18). In our study, the infection rate

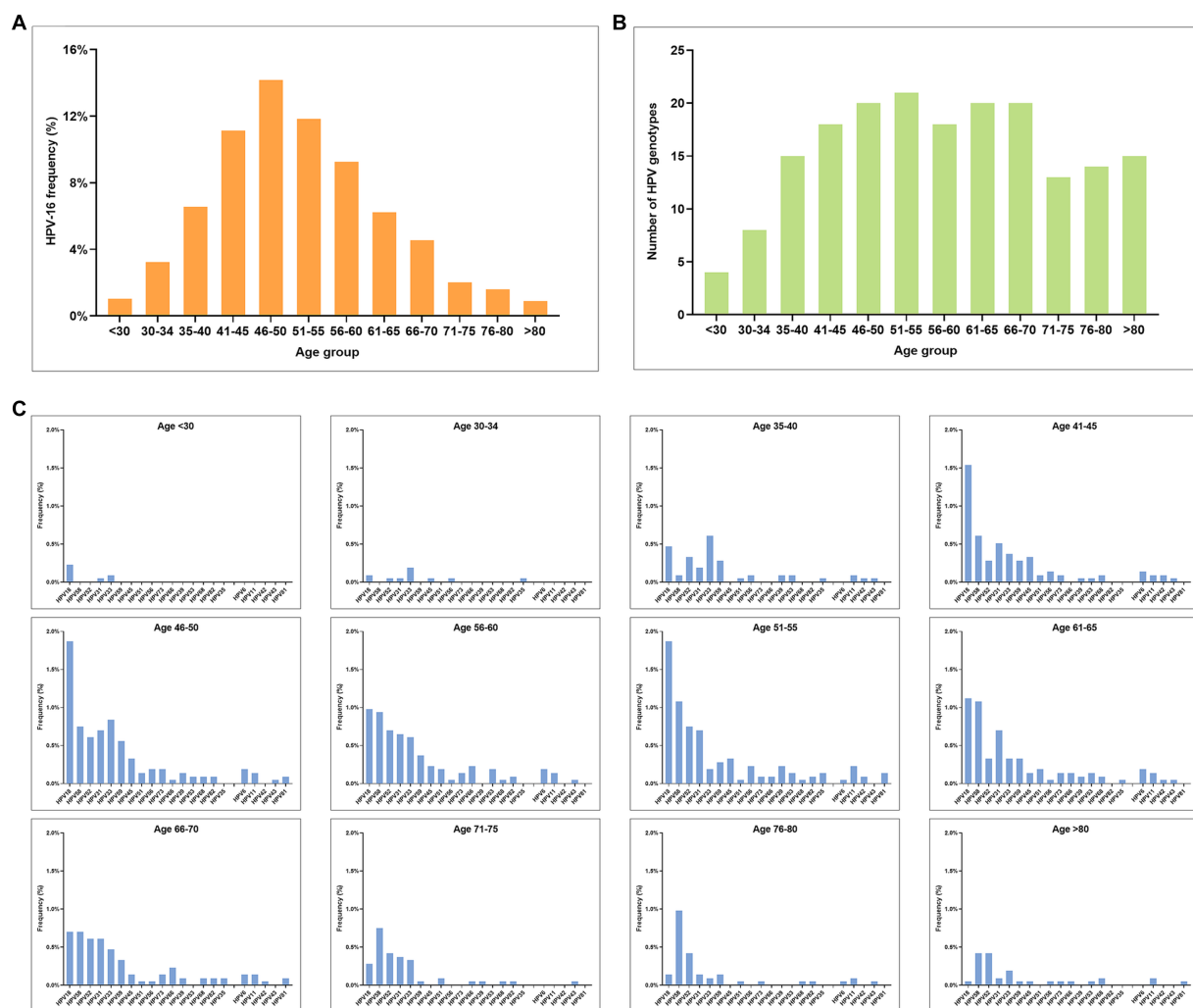


FIGURE 5

Prevalence of HPV infection in different age groups among HPV-positive CSCC. (A) Age distribution of HPV-16 infection. (B) Number of HPV infection genotypes in different age groups. (C) Genotype distribution and prevalence rate of HPV infection in different age groups (excluding HPV-16).

of HPV in CSCC patients was 92.71%, which was higher than the rates reported worldwide. The findings highlighted the urgency of preventing and controlling HPV-associated cervical cancer.

4.2. Women over 65 years old should be included in cervical cancer screening in China

Our study showed that the highest incidence of cervical cancer was observed in individuals aged 40–60 years, accounting for 61.57% of all CSCC cases. Of note, the incidence rate in the group over 66 years old was still 16.35%. Among them, the incidence rate of cervical cancer was 11.54% in the women aged 66–75 years old, 2.86% in those aged 76–80 years old, and 1.95% in those over 80 years old (Figure 2B).

Our data indicated that the HPV-16 infection rate was consistently high across all age groups of cervical cancer, accounting for 72.45% of CSCC cases (Figure 5A, Table 1). Previous study also found that HPV-16 was the most common HR-HPV type (72%) and

the most common persistence infection type (16%) in older adult women (19). Moreover, with increasing age, there was a gradual increase in the number of mixed infections with different HPV genotypes (Figure 5B), as well as the frequency of infection with each HPV genotype (Figure 5C). The peak of HPV infection is reached at 40–60 years old, and gradually decreases after 60 years old. Multiple HPV infections are still present in cases over 80 years old (Figure 5C). Zhao reported the prevalence of HPV infection in older adult women (≥ 60 years old) and the significance in older adult cervical lesions (20). Their results showed that the overall infection rate of HR-HPV was 16.73% (84/502), which was consistent with our results in the group aged over 65 years (16.3%). Their results also showed that of 39 cases who had undergone cervical biopsy by colposcopy, 5 cases were diagnosed with cervical cancer (5.62%), 10 cases were CINII+ (11.24%), and 5 cases were CINIII (5.62%). Previous studies showed HR-HPV prevalence of older adult women in Guangxi are higher than those of middle-aged women and caused higher CIN incidence rate (21, 22). Although the HPV infection rate decreases in older adult patients compared with young women, the pathogenicity of HPV does not decrease (20).

TABLE 2 Genotype distribution of HPV infection in healthy women undergoing cervical cancer screening.

HPV type	Genotype		Amount of single-type infection	Amount of two-type infection	Amount of three-type infections	Amount of four or more types infection	Amount of HPV-positive cases	Frequency in HPV-positive screening population	HPV-positive rate in screening population
HR-HPV	1	HPV-52	160	49	10	12	231	15.58%	2.25%
	2	HPV-58	105	39	11	9	164	11.06%	1.60%
	3	HPV-16	97	21	10	6	134	9.04%	1.31%
	4	HPV-53	63	40	16	6	125	8.43%	1.22%
	5	HPV-68	50	26	12	7	95	6.41%	0.93%
	6	HPV-39	53	22	12	7	94	6.34%	0.92%
	7	HPV-51	44	22	9	5	80	5.39%	0.78%
	8	HPV-56	39	18	13	6	76	5.12%	0.74%
	9	HPV-59	36	23	8	8	75	5.06%	0.73%
	10	HPV-33	37	19	9	3	68	4.59%	0.66%
	11	HPV-66	26	14	8	8	56	3.78%	0.55%
	12	HPV-35	20	12	5	7	44	2.97%	0.43%
	13	HPV-31	28	8	2	5	43	2.90%	0.42%
	14	HPV-18	23	10	2	4	39	2.63%	0.38%
	15	HPV-45	9	0	1	2	12	0.81%	0.12%
	16	HPV-73	4	2	1	0	7	0.47%	0.07%
	17	HPV-26	2	3	0	0	5	0.34%	0.05%
LR-HPV	18	HPV-81	7	17	9	5	38	2.56%	0.37%
	19	HPV-42	6	14	15	4	39	2.63%	0.38%
	20	HPV-43	1	12	6	4	23	1.55%	0.22%
	21	HPV-6	1	7	3	5	16	1.08%	0.16%
	22	HPV-40	1	7	3	4	15	1.01%	0.15%
	23	HPV-11	1	2	0	1	4	0.27%	0.04%
Total			813	387	165	118	1,483	100.00%	/

So, it should be given sufficient attention to older adult patients with HPV-positive infection. In addition, with the rapid development of China's economy, the average life expectancy of the Chinese population has significantly increased. A recent study indicated that life expectancy in China is expected to increase steadily from 77.7 years in 2019 to 79 years by 2030 and 81.3 years by 2035, with an average of 85.1 years for women and 78.1 years for men (23). In our study, the number of cervical cancer patients aged 66–80 years old represented 14.40% of all CSCC cases. Therefore, it is necessary to include the older adult population aged 66–80 years old in the cervical cancer screening and control strategy in China.

4.3. The order of HPV genotype prevalence in cervical cancer tissues varies between China and other countries

In this study, the total HPV detection rate was 92.71% (2,138/2306) in 2,306 CSCC tissues, including 86.48% (1849/2138) for single type HPV detection rate and 13.52% (289/2138) for multiple-type detection

rate. Among the cases with multiple genotypes infection, 238 cases (11.13%) had two HPV genotypes infection, 40 cases (1.87%) had three HPV genotypes infection, and only 11 cases (0.51%) had four or more HPV genotypes infection (Figure 3B). Additionally, our results suggest that LR-HPV genotypes (such as HPV-6, -11, -42, -43, -81, and -83) are rare to detect in CSCC tissues. When these LR-HPV genotypes are detected, they are often found in mixed infection with other HR-HPV genotypes. Nevertheless, HR-HPV genotypes, including HPV-16, -18, -31, -33, -35, -39, -45, -51, -52, -56, -58, and -59, are closely related to the development of CSCC, with HPV-16 and HPV-18 being the most common types in CSCC tissues.

The top eight HPV genotypes of cervical cancers in Chinese women are HPV-16, -18, -58, -52, -31, -33, -59, and -45, which differ from the commonly occurring HR-HPV genotypes in other countries. Literature reports suggested that HPV-35 and HPV-45 were common in cervical cancer tissues in West Africa (5, 24). Our data show that the positivity rate of HPV-59 was the seventh of the top eight HR-HPV genotypes in China, while HPV-35 has the lowest positivity rate (0.37%) among the commonly occurring HR-HPV genotypes in China (Table 1). This may be related to differences in

HPV detection techniques or HPV genotyping profiles, as well as differences in specimen types and quantities, regions, and ethnicities. de Sanjose reported 85% (8,977/10,575) cases had positive results of HPV DNA (18). Among these cases, 8,977 (85%) had positive results of HPV DNA. The most common HPV types were HPV-16, -18, -31, -33, -35, -45, -52, and -58, with a total global relative contribution rate of 8,196 (91%) of the 8,977 cases (18). This study shows that there's variability in the risk of cervical cancer due to different HR-HPV genotypes, and it is significant to study the distribution patterns of different HR-HPV genotypes in cervical cancer tissues (18). The WHO recommends that each country should establish its own HPV genotyping database for cervical carcinoma, which will be an important guide for the prevention and treatment of CSCC and the development of prophylactic HPV vaccines (25, 26).

Our study also found that the positivity rate of HPV-16 and HPV-18 were the highest among all genotypes detected in CSCC tissues, with a percentage of 79.61% for both genotypes, while HR-HPV genotypes, HPV-31 and HPV-33 ranked as the fifth and sixth most prevalent genotypes (9.07% for both genotypes), which is consistent with the low carcinogenic risk of them. In China, it is important to pay attention to the HR-HPV genotypes HPV-52 and HPV-58 regional ranked third and fourth regionally (detection frequency of both was 12.3%), as well as HPV-59 and HPV-45 (the detection frequency was 2.67% and 1.59%, respectively). Therefore, HR-HPV genotyping testing for cervical cancer in women is an important clinical reference index of the need for colposcopy and close follow-up in women over 30 years old.

4.4. Rational HPV genotyping profile can improve the efficiency and effectiveness of cervical cancer screening

In our study, the overall incidence rates of HR-HPV and LR-HPV in central and eastern China were 13.16% and 1.32% in cervical cancer screening individuals, respectively (Table 2). Of which the common HPV genotypes in healthy women were HPV-52, -58, -16, -53, -68, -39, -51, and -56, with positive rates of 2.25%, 1.60%, 1.31%, 1.22%, 0.93%, 0.92%, 0.78%, and 0.74%, respectively (Table 2). Our results also identified that the cases of HPV infection genotype and frequency were significantly lower in people under 30 than in other age groups, which is supported by the study of Fatima Mijit et al. (27). This suggests that the lower positivity rate of HPV in women under 30 may be due to their higher immune levels (28, 29). However, cases of advanced cervical cancer have also been reported in women under 30 years old in recent years (30). Our results showed that there were 41 cases of CSCC in women under 30 years old, with the youngest diagnosis for a 19-year-old patient (Figure 2B). Therefore, it is an important way to prevent cervical cancer, through taking personal protective measures, reducing the chances of HPV infection, and improving immunity.

On July 6, 2021, the World Health Organization (WHO) and the United Nations Population Fund (UNFPA) released the second edition of the "Cervical Cancer Screening and Treatment Guidelines for Precancerous Lesions," which clearly recommended HPV-DNA testing as the preferred screening method for cervical cancer over the current cytology or Pap smear (25). HPV DNA testing is more cost effective in terms of sensitivity and specificity of the test

technology. Currently, many companies tend to develop HPV testing kits that can detect as many HPV genotypes as possible (31). However, excessive HPV genotypes will inevitably increase the complexity of multiple PCR technologies and the cost of reagents, resulting in high terminal price and increased difficulty in promotion. This study found that the most common HR-HPV genotypes in Chinese CSCC were HPV-16, -18, -58, -52, -31, -33, -59, and -45, with positive rates of 72.45%, 9.35%, 7.39%, 4.91%, 4.77%, 4.30%, 2.67%, and 1.59%, respectively. These eight HR-HPV genotypes can be detected in 96.93% of CSCC cases (Figure 4). The detection rate of the other nine HPV types was only 3.07%. Considering that the WHO recommends HPV DNA testing as the preferred screening method for cervical cancer (25), and based on our results, we suggest that the eight common high-frequency HPV genotypes, HPV-16, -18, -58, -52, -31, -33, -59, and -45 in China, should be prioritized as targets for multiple nucleic acid testing to reduce technical complexity and reagent costs and lay the foundation for community or home-based promotion.

Our current findings suggest that including the L1 protein of HPV-59 in the protective profile of HPV prophylactic vaccine could improve the protection rate of HPV vaccines. However, it is important to note that this conclusion should be further confirmed by large size real-world studies.

We also strongly recommend that Chinese women should receive polyvalent (such as nine-valent) vaccines whenever possible, which can achieve maximum vaccine protection through one vaccination program (32).

In conclusion, HPV is a common sexually transmitted infection worldwide that disrupts normal social life and has deadly consequences. With few exceptions, the burden of HPV infection and related diseases remains high in developing countries. Factors contributing to these high rates include poor living conditions, co-infection with other pathogens, poor health care facilities, and the high cost of vaccines. China is one of the countries with a high incidence of HPV infection and cervical cancer. Therefore, it is of great significance to study HPV infection patterns in patients with cervical squamous cell carcinoma and healthy individuals, and to develop new vaccines against HPV and new technologies for the rapid detection of HPV based on the high incidence of HPV genotypes in the Chinese population. This will accelerate the elimination of cervical cancer worldwide.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethics Committee of Nanjing Traditional Chinese Medicine Hospital. 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

JG, ZW, and JH were responsible for study concept and design. CH, MY, JiL, JC, JJ, HW, LX, JuL, DL, JG, ZW, and JH were responsible for specimens sampling. CH, WH, and JG were responsible for the experiments in this study. JG, WH, RZ, and JH were responsible for the acquisition, analysis, and interpretation of data. WH, RZ, and JH were responsible for drafting the manuscript. 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.2023.1225652/full#supplementary-material>

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Vaccine efficacy against SARS-CoV-2 for Pfizer BioNTech, Moderna, and AstraZeneca vaccines: a systematic review

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The purpose of this systematic review was to report on the vaccine efficacy (VE) of three SARS-CoV-2 vaccines approved by Health Canada: Pfizer BioNTech, Moderna, and AstraZeneca. Four databases were searched for primary publications on population-level VE. Ninety-two publications matched the inclusion criteria, and the extracted data were separated by vaccine type: mRNA vaccines (Pfizer and Moderna) and the AstraZeneca vaccine. The median VE for PCR-positive patients and various levels of clinical disease was determined for the first and second doses of both vaccine types against multiple SARS-CoV-2 variants. The median VE for PCR-positive infections against unidentified variants from an mRNA vaccine was 64.5 and 89%, respectively, after one or two doses. The median VE for PCR-positive infections against unidentified variants from the AstraZeneca vaccine was 53.4 and 69.6%, respectively, after one or two doses. The median VE for two doses of mRNA for asymptomatic, symptomatic, and severe infection against unidentified variants was 85.5, 93.2, and 92.2%, respectively. The median VE for two doses of AstraZeneca for asymptomatic, symptomatic, and severe infection against unidentified variants was 69.7, 71, and 90.2%, respectively. Vaccine efficacy numerically increased from the first to the second dose, increased from the first 2 weeks to the second 2 weeks post-vaccination for both doses, but decreased after 4 months from the second dose. Vaccine efficacy did not differ by person's age.

KEYWORDS

SARS-CoV-2, COVID-19, vaccine efficacy, systematic review, mRNA vaccines, AstraZeneca vaccine

Introduction

As of 14 April 2023, SARS-CoV-2 has infected over 762 million people worldwide, causing approximately 6.8 million deaths (1). Several vaccines have been developed to protect people from illness due to this virus. In December 2020, the first SARS-CoV-2 vaccine, Pfizer BioNTech, was approved by the FDA for emergency use (2). Health Canada has approved the use of three SARS-CoV-2 vaccines: Pfizer, Moderna, and AstraZeneca on 9 December 2020, 23 December 2020, and 26 February 2021, respectively (3) (Table 1). Each company conducted a series of phase 3 randomized controlled trials to determine the VE. In November 2020, Pfizer determined that the VE against infection seven or more days after the second dose was 95% (4). In December 2020, Moderna determined its VE was 94.1% against SARS-CoV-2 infection 14 or more days after the second dose (5). In March 2021, AstraZeneca determined its VE was 79% against symptomatic infection 15 or more days after the second dose (6). All three vaccines required two doses to maximize efficacy. Nevertheless,

TABLE 1 Names of companies, research names, and vaccine types for vaccines included in this systematic review.

Company	Research name	Vaccine type
Pfizer BioNTech	BNT162b2	mRNA
Moderna	mRNA-1273	mRNA
AstraZeneca	AZD1222 (ChAdOx1)	Non-replicating viral vector

these efficacy figures may not reflect the efficacy in real-world settings due to factors such as age distributions and other demographics, as well as the increased prevalence of different SARS-CoV-2 variants.

After the completion of initial publications that determined the safety and efficacy of randomized controlled trials on smaller samples of individuals, further research was completed on larger, population-level settings. The purpose of this systematic review was to determine the efficacy of the first and second doses of the mRNA and AstraZeneca vaccines against SARS-CoV-2 infections in real-world, population-level settings, between December 2020 and May 2022. These peer-reviewed studies determined the efficacy of SARS-CoV-2 vaccines based on a positive PCR test and against a range of clinical presentations, including asymptomatic, symptomatic, severe/critical infection, hospitalization, or death. Key variants of concern in this review were the original/China variant, B.1.1.7/Alpha/UK variant, B.1.351/Beta/South African variant, and B.1.617.2/Delta/Indian variant (Table 2) (7).

Materials and methods

Search strategy

Four databases, including PubMed, Medline OVID, Web of Science, and CAB Direct, were searched systematically in June 2021, September 2021, and May 2022 using the search string described in Table 3. Searches were completed to identify all available peer-reviewed literature published on the efficacy of SARS-CoV-2 vaccines developed by Pfizer, Moderna, and AstraZeneca. In June 2021, 621 publications were identified, 323 of which were duplicates, leaving 298 distinct publications to be further screened. In September 2021, 1,228 publications were identified, 970 of which were duplicates, leaving an additional 258 distinct publications to be further screened. In May 2022, 4,511 publications were identified, 3,153 of which were duplicates, leaving an additional 1,358 publications to be screened.

Selection criteria and data extraction

To be included in this review, publications were required to meet all inclusion criteria and none of the exclusion criteria (Table 4). Two authors (LR and GA) independently reviewed titles and abstracts (step 1) and full-text publications (step 2) for inclusion in this review. Reviewers met to settle conflicts collaboratively. When publications were reported on both the mRNA vaccine(s) and the AstraZeneca vaccine, these publications were recorded as separate studies, resulting in a discrepancy

TABLE 2 Names of the variants of the SARS-CoV-2 virus included in selected articles.

Country (Area) of Origin	WHO label (Variant Name)	Pango Lineage
China	(Original variant)	
United Kingdom	Alpha (UK variant)	B.1.1.7
South Africa	Beta (South African variant)	B.1.351
India	Delta (India variant)	B.1.617.2
Brazil	Gamma (Brazil variant)	P.1, P.1.1, P.1.2, B.1.1.28
South Africa	Omicron	B.1.1.529
United States of America	Epsilon	B.1.427, B.1.429
United States of America	Iota	B.1.526, B.1.526.1, B.1.526.2
Colombia	Mu	B.1.621, B.1.621.1
Brazil	Zeta	P.2, B.1.1.33, B.1.1.28

TABLE 3 Search string used to identify publications from each of the four databases used for potential inclusion in the study.

Search string
“vaccin* OR inoculat* OR immunization*” AND “Pfizer OR AstraZeneca OR Moderna OR pfizer-biontech OR pfizerbiontech OR bnt162b2 OR mrna-1273 OR ChAdOx1-S OR Oxford AstraZeneca” AND “efficacy OR effective*” AND “COVID-19 OR SARS-CoV-2 OR COVID19* OR COVID OR SARSCOV-2 OR SARSCOV2 OR Severe Acute Respiratory Syndrome Coronavirus 2 OR Severe Acute Respiratory Syndrome Corona Virus 2”.

between the number of publications and studies reported in the results section. Studies with fewer than 100 participants were not included in this review. For each study included, VE was extracted and described.

If a study determined VE over a range of days after the first or after the second dose, the VE at 14+ days post-vaccination was used when available, and if that was not available, we used the closest VE reported after 14 days. If that information was unavailable, we reported on VE at the latest time reported prior to 14 days after vaccination. This time interval was chosen as this was the most common reporting time for all studies on SARS-CoV-2 vaccines. The decline in VE over time was determined by comparing the 95% CI of VE of the first reported time interval to the 95% CI of VE at later intervals.

The VE for severe infection included those reported for severe/fatal/critical clinical disease, hospitalization, and death. To report on severe infection, the average VE was calculated for the separate values for various types of severe disease. The VE for mRNA vaccines that had separate results for Pfizer and Moderna was recorded as the average VE of these values. The variant in a study population was recorded as N/R for studies that did not specifically identify the variant infecting participants with a positive test. Studies that reported that variants were non-Delta, non-Alpha,

TABLE 4 Inclusion and exclusion criteria.

Inclusion criteria	• Peer-reviewed, human studies on a population level that included at least 100 participants • Accessible in English • Examining the efficacy of Pfizer, Moderna, or AstraZeneca vaccines against any level of infection or clinical signs and symptoms.
Exclusion criteria	• Systematic reviews, commentary articles, or gray literature, • Reporting on research on vaccines that were not Pfizer, Moderna, or AstraZeneca • Reporting on outcomes limited to antibody levels from blood tests/serology, safety of SARS-CoV-2 vaccines, side effects/adverse effects occurring from the vaccine, use of non-standard doses of vaccines, or VE determined without non-vaccinated controls. • Reporting on mathematical models, simulation, or emulation trials of VE • Studies limited to people with severe illness or conditions leading to reduced immune function

and non-Beta were also recorded as N/R. Studies that reported mild to moderate infections were recorded as symptomatic infections. If a study reported separate VE values for the three symptomatic outcomes (mild, moderate, and symptomatic), these VE values were averaged and reported as one value for the symptomatic outcome. If studies reported a VE for both unidentified variants and variants that were not listed as known variants of concern, these two VEs were averaged and reported as the VE for unidentified variants. Most studies reported VE for all age groups in addition to specific age ranges. However, for those studies that only reported VE for specific age ranges, the average VE across all ages was reported.

Clinical outcomes

PCR-positive infections were defined as any positive PCR test reported, regardless of symptoms. Asymptomatic COVID-19 infections were defined as a positive PCR test with no reported clinical signs or symptoms of COVID-19. Mild to moderate COVID-19 infections were defined by a positive PCR test and two or more of the following clinical signs and symptoms: fever, new-onset cough, new-onset rapid breathing, myalgia, new-onset shortness of breath with or without exertion, chills, sore throat, loss of taste, loss of smell, headache, nasal congestion, diarrhea, runny nose, tiredness, nausea or vomiting, loss of appetite, and any evidence of significant lower respiratory tract infection (Tachypnea: 20–29 breaths per minute at rest, oxygen saturation of 94% or less on room air, abnormal chest x-ray/CT consistent with pneumonia or lower respiratory tract infection, or adventitious sounds on lung auscultation). Symptomatic COVID-19 infections were defined by a positive PCR test and one or more of the following clinical signs and symptoms: fever (temperature $>37.6^{\circ}\text{C}$), cough, sore throat, shortness of breath, muscle pain/myalgia, loss of smell/anosmia or loss of taste/ageusia, loss of appetite, nasal congestion, headache, nausea and vomiting, anorexia, dizziness, agitation, weakness, seizures, or findings suggestive of stroke, including trouble with speech or vision, sensory loss, or problems with balance in standing or walking, chills, diarrhea, chest pain, hoarseness, runny nose, sneezing, stomach pain, sinus pain, sweating, swollen

glands, watery eyes, malaise/fatigue, or pneumonia. Severe/critical COVID-19 infections were defined by a positive PCR test and one or more of the following clinical signs and symptoms: clinical signs of pneumonia including fever, cough, dyspnea, fast breathing, and respiratory rate of 30 or more breaths per minute; heart rate at or exceeding 125 beats per minute; oxygen saturation at 93% or less, while the participant was breathing ambient air at sea level or a ratio of the partial pressure of oxygen to the fraction of inspired oxygen below 300 mm Hg; respiratory failure; acute respiratory distress syndrome; evidence of shock (systolic blood pressure <90 mm Hg, diastolic blood pressure <60 mm Hg, or a need for vasopressors); clinically significant acute renal, hepatic, or neurologic dysfunction; sepsis, septic shock, acute thrombosis, MIS-C, requiring mechanical ventilation, adventitious sounds on lung auscultation, admission to an intensive care unit, or death.

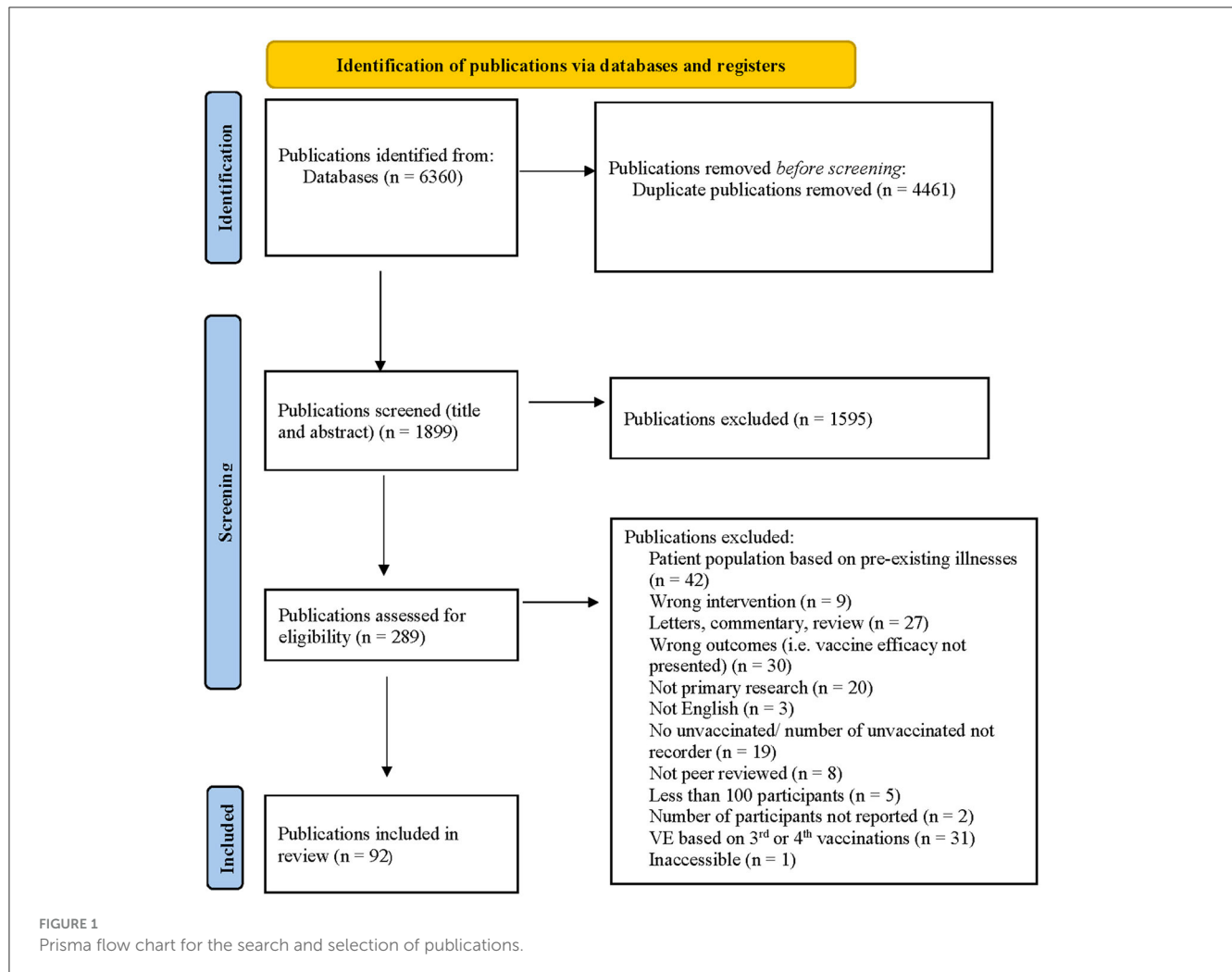
Results

Description of the publications

After applying the inclusion and exclusion criteria to the 298 unique publications identified in June 2021, 263 publications were excluded by title and abstract screening. Fourteen additional publications were excluded after full-text screening, leaving 21 publications for inclusion in the review. After applying the inclusion and exclusion criteria and screening procedures to the 258 unique publications identified in September, an additional 12 publications were included in the review. After applying the inclusion and exclusion criteria and screening procedures to the 1,358 new publications identified in May 2022, an additional 59 publications were included in the review (Figure 1).

In total, 92 publications were included in this systematic review, which included a total of 114 studies (Supplementary Tables 1, 2). Some included data from more than one country ($n = 4$) (67, 82, 87, 95). Most studies were conducted in the UK ($n = 33$) (10, 13, 14, 16, 18, 24, 42, 43, 45, 49, 50, 53, 54, 56, 65, 69, 71, 85, 94, 97) or the USA ($n = 34$) (9, 12, 17, 21, 22, 26, 28, 32, 33, 36, 37, 46, 48, 52, 55, 59, 60, 64, 66, 67, 70, 72, 73, 79–84, 87–89, 91, 95). Of the studies reporting in the UK, 14 were conducted specifically in England (10, 13, 14, 24, 42, 49, 50, 53), 5 in Scotland (54, 85, 97), and 3 in Wales ($n = 3$) (18, 65). Other studies were in Israel ($n = 9$) (15, 29, 30, 39–41, 57, 74, 92), Italy ($n = 8$) (11, 19, 20, 34, 35, 38, 63)), Brazil ($n = 6$) (23, 67, 82, 93, 96), Malaysia ($n = 4$) (44, 76, 77), Spain ($n = 4$) (31, 61, 87), Finland ($n = 3$) (68, 87), South Africa ($n = 3$) (67, 82, 98), Canada ($n = 3$) (27, 75, 90), France ($n = 2$) (62), Argentina ($n = 2$) (67, 82), Germany ($n = 2$) (67, 82), Qatar ($n = 2$) (25, 78), Sweden ($n = 2$) (58), Hungary ($n = 2$) (86), Turkey ($n = 2$) (67, 82), Kuwait ($n = 2$) (8), South Korea ($n = 1$) (47), Poland ($n = 1$) (87), Thailand ($n = 1$) (99), Peru ($n = 1$) (95), Chile ($n = 1$) (95), and Japan ($n = 1$) (51).

Data collection for the studies occurred between May 2020 and February 2022. Most studies investigated the VE of one of the mRNA vaccines ($n = 85$) (8–79, 81–92), whereas 29 (8, 10, 13, 14, 23, 24, 31, 35, 43, 49, 50, 54, 56, 58, 62, 65, 68, 69, 71, 76, 85, 86, 93–99) examined the AstraZeneca vaccine (Supplementary Tables 1, 2). In total, 13 (8, 16, 31, 40, 45, 49, 53, 56, 62, 75, 85) studies determined VE after the first dose only,



36 (11, 15, 24, 28, 32, 35, 36, 44, 48, 52, 54, 55, 57, 58, 60, 63, 65, 70, 72–74, 77, 80, 86–89, 93–95, 99) determined VE after the second dose only, and 65 (8–10, 12–14, 17–23, 25–27, 29–31, 33–35, 37–39, 41–43, 46, 47, 49–51, 59, 61, 62, 64, 66–69, 71, 76, 78, 79, 81–84, 90–92, 96–98) determined VE after both the first and second doses.

Supplementary Tables 1, 2. Many studies ($n = 54$) (10, 12–14, 18–20, 22, 25–27, 29–31, 34, 37–39, 41–43, 46, 49, 50, 53, 54, 61, 64, 66–69, 71, 79, 80, 82, 85, 86, 90, 92, 96, 97) determined VE at multiple time intervals.

Time post-vaccination

The timing of follow-up post-vaccination differed; some authors ($n = 30$) (15, 19, 25–27, 29–31, 34, 37, 38, 41, 42, 46, 61, 64–67, 71, 79, 80, 82, 86, 87, 90, 92) determined VE beginning 7 days post-vaccination after the first or second dose, whereas others ($n = 62$) (8–10, 16, 17, 20, 21, 23, 24, 28, 32, 33, 35, 36, 39, 43–45, 47, 48, 50–52, 54, 55, 57–60, 62, 63, 68–70, 72, 73, 76–78, 81, 83–85, 88, 91, 94, 96, 98, 99) followed participants beginning 14 or more days after receiving the first or second dose of the vaccine. Twenty-two studies (8, 11–14, 18, 22, 31, 40, 49, 53, 56, 74, 75, 89, 93, 95, 97) used an alternative follow-up period starting from 0 days to 10 months after vaccination. If VE was reported 0–7 days or 0–14 days after vaccination, these results were not recorded in

Type of study

The study designs included randomized controlled trials, observational studies of prospective and retrospective cohorts, and case-control studies. Twelve studies used a blinded, randomized, controlled clinical trial (9, 17, 33, 37, 38, 67, 82, 87, 93–95, 98). Most studies ($n = 58$) used an observational cohort approach that followed vaccinated and unvaccinated people over the same time period. Cohort studies were divided between prospective ($n = 32$) (12, 18, 28, 29, 31, 36, 39–43, 53, 55–57, 62, 68, 69, 71, 72, 85, 86, 90) and retrospective ($n = 26$) (8, 11, 15, 16, 19, 20, 26, 30, 34, 35, 47, 58, 63–65, 73, 74, 76, 77, 79, 92) cohort study designs. In case-control studies ($n = 44$) (10, 13, 14, 21–25, 27, 32, 44–46, 48–52, 54, 59–61, 66, 70, 75, 78, 80, 81, 83, 84, 88, 89, 91, 96, 97, 99), patients with a positive PCR test with or without clinical signs, and symptoms of COVID-19 were matched by age with people with a negative PCR test during the same time period.

Populations included in the studies

The number of participants in the randomized controlled trials ranged from 1,882 to 46,077, with a median of 9,636. Studies were conducted on populations of different age ranges, including 12+ ($n = 1$) (82), 16+ ($n = 1$) (67), 18–65 ($n = 1$) (98), and 18+ ($n = 6$) (17, 33, 38, 93–95). Three of these studies were limited to youth: 5–11 years (87), 12–15 years (37), and 12–17 years (9), respectively. One study was on individuals aged 12+ (82), one was 16+ (67), one was 18–65 (98), and six were 18+ (17, 33, 38, 93–95). Most studies ($n = 10$) (9, 17, 33, 37, 67, 82, 87, 93, 95, 98) included participants randomly selected from the population, whereas two (38, 94) only included healthcare workers.

The number of participants in the prospective cohort studies ranged from 1,023 to 6,538,911 with a median sample size of 407,994. The source population or source of data for these observational studies included data extracted by a local health provider ($n = 5$) (12, 31, 55, 72), local health database ($n = 1$) (36), national healthcare provider ($n = 1$) (29), national health database ($n = 8$) (39–41, 53, 85, 86), healthcare workers ($n = 9$) (8, 18, 43, 62, 68, 90), a self-reported survey ($n = 7$) (42, 56, 57, 69, 71), and veterans' health administration ($n = 1$) (28).

The number of participants in the retrospective cohort studies ranged from 1,610 to 5,693,624 with a median sample size of 398,593. The source population or source of data for these observational studies included local health databases ($n = 3$) (64, 73, 79), local healthcare providers ($n = 2$) (30, 74), national health databases ($n = 11$) (11, 35, 58, 65, 76, 77, 92), healthcare workers ($n = 8$) (8, 15, 16, 19, 20, 34, 63), prison healthcare records ($n = 1$) (26), and ministry of education health and immunization records ($n = 1$) (47).

The number of participants in the case-control studies ranged from 404 to 6,647,733 with a median sample size of 96,726. The source population or source of data for these observational studies included local healthcare providers ($n = 1$) (78), local health databases ($n = 8$) (21, 27, 59, 61, 75, 80, 84, 88), national healthcare providers ($n = 3$) (24, 45), national health databases ($n = 7$) (23, 25, 54, 96, 97), healthcare workers ($n = 1$) (66), military personnel health records ($n = 1$) (32), hospital records ($n = 9$) (48, 51, 52, 60, 70, 81, 83, 89, 99), national immunization system ($n = 10$) (10, 13, 14, 49, 50), ministry of education health and immunization records ($n = 1$) (44), and veterans' health administration records ($n = 3$) (22, 46, 91). Amirthalingam (2021) reported multiple accounts of VE after the second dose, depending on various time intervals between doses 1 and 2. [Supplementary Tables 1, 2](#) include the authorized interval between doses 1 and 2 as reported by Public Health Canada (100).

Outcomes of interest

Reported outcomes included asymptomatic infection ($n = 12$) (9, 12, 15, 29, 32, 33, 41, 59, 69, 78, 94), PCR-positive tests ($n = 77$) (10–12, 16, 18–22, 24–36, 38–44, 46, 47, 53, 55–59, 61–66, 68, 69, 71–75, 77–79, 82, 84, 86–88, 90–92, 94, 95, 98, 99), symptomatic infection ($n = 43$) (8, 9, 12–15, 17, 20, 23, 24, 27, 29, 30, 32–35, 37, 38, 41, 49–51, 55, 59, 67, 69, 78, 91, 93–98),

mild/moderate symptomatic infection ($n = 1$) (98), severe/critical disease ($n = 15$) (11, 17, 25, 29, 33, 39, 41, 54, 67, 70, 78, 82, 93, 95), hospitalization ($n = 47$) (11, 14, 21, 23, 27, 29, 30, 32, 36, 39, 41, 45, 48, 52–54, 60, 61, 64, 65, 68, 70, 72–74, 76, 77, 79–81, 83–85, 88, 89, 91, 93, 95–97), and death ($n = 25$) (11, 14, 23, 25, 27, 29, 33, 35, 36, 39–41, 46, 61, 74, 76–78, 86, 91, 96, 97) ([Table 4](#) and [Supplementary Table 1](#)). Commonly, severe illness, hospitalization, and death were included together. Fifty-four studies (13, 14, 21, 23, 25, 27–30, 32, 35, 36, 41, 43, 48–50, 55, 59, 62, 65, 68–73, 75, 77–82, 84, 86, 90, 93, 94, 96–99) reported VE against specific variants ([Table 4](#) and [Supplementary Table 1](#)) and 99 (8–12, 14–24, 26, 28–41, 43–47, 49, 51–68, 71–77, 79, 81–83, 85–99) used the term 'unknown' (N/A) if additional laboratory tests were not done or did not identify a known variant.

Vaccine efficacy

The median VE for all vaccines was numerically highest for the clinical outcomes such as severe illness, hospitalization or death, and symptomatic infection measured at approximately 14 days after a second dose of the vaccine ([Table 5](#)). [Supplementary Table 3](#) describes the VE by age. Thirty-six studies (10, 14, 21–23, 27–29, 33, 36, 39, 46, 48, 60, 61, 66, 67, 69, 75, 77–82, 85, 86, 91, 95, 97) reported VE for various age groups. [Supplementary Table 4](#) describes the VE over time. Thirty-four studies (13, 14, 18, 20, 21, 23, 27, 36, 43, 48, 50, 51, 54, 56, 58, 59, 68–70, 75, 77, 79, 80, 82, 84, 85, 89, 97) reported VE over multiple time intervals. This table includes any additional time intervals not reported in [Supplementary Tables 1, 2](#).

Vaccines were less efficacious against asymptomatic cases, PCR-positive cases, or mild to moderate symptoms than against severe infections ([Table 5](#)). The median VE against unidentified variants for PCR-positive infections, asymptomatic infections, symptomatic infections, and severe infections after two doses of an mRNA vaccine were 89, 85.5, 93.2, and 92.2%, respectively. The median VE against unidentified variants for PCR-positive infections, asymptomatic infections, symptomatic infections, and severe infections after two doses of the AstraZeneca vaccine was 69.6, 69.7, 71, and 90.2%, respectively.

Of the 36 (10, 12, 18–22, 25, 26, 29–31, 34, 35, 38, 39, 41–43, 46, 47, 59, 61, 62, 64, 66, 68, 69, 71, 78, 79, 82, 84, 90–92) studies that reported VE for PCR-positive infection after the first and second doses of mRNA vaccines, 24 found an increased VE after the second dose ([Supplementary Tables 1, 2](#)) (10, 19, 21, 22, 25, 26, 29–31, 39, 41, 43, 46, 47, 61, 64, 66, 68, 69, 78, 79, 90–92). Of the 6 (10, 43, 68, 69, 71, 98) studies that reported VE for PCR-positive infection after the first and second doses of AstraZeneca, 3 (10, 68, 69) found increased VE after the second dose ([Supplementary Tables 1, 2](#)). The median VE for all vaccines was numerically lower against unidentified variants after the first dose than after the second dose ([Table 5](#)). The median VE for PCR-positive infections from an mRNA vaccine was 64.5 and 89% after one or two doses against unidentified variants, respectively. The median VE for PCR-positive infections from the AstraZeneca vaccine was 53.4 and 69.6%, respectively, after one or two doses against unidentified variants. Vaccine efficacy <14 days after the

TABLE 5 Distribution of vaccine efficacy against SARS-CoV-2 by vaccine type, outcome, and variant.

Outcome	Variant	One Dose mRNA				Two doses mRNA			
		n ^a	Min	Median	Max	n ^a	Min	Median	Max
PCR positive	ALPHA	7	30	67	90.1	6	76.4	91.7	100
	BETA	1	-	47.9	-	1	-	96.4	-
	DELTA	6	56	62.3	77	12	50.7	74.7	86.7
	EPLISON	1	-	76.3	-	1	-	97.6	-
	IOTA	-	-	-	-	1	-	95.7	-
	MU	-	-	-	-	1	-	90.4	-
	N/R	36	31	64.5	97.7	51	50.1	89	99.1
	OMICRON	1	-	20.4	-	1	-	13.9	-
	ORIGIN	1	-	53	-	1	-	94.9	-
	P.1/P.1.1/P.1.2	2	61	-	74.2	1	-	95.5	-
Symptomatic	ALPHA	3	47.5	52	73	3	93.7	94.9	97
	DELTA	5	35.6	64.9	68	6	49.2	87	92.7
	N/R	17	45	66	99.2	23	58	93.2	100
	OMICRON	1	-	31.7	-	1	-	70.3	-
Asymptomatic	DELTA	2	47	-	62.5	3	45.9	71.5	74
	N/R	3	29	52	59.5	8	39.2	85.5	93.8
Severe, hospitalization, and death	ALPHA	2	67.7	-	87.2	4	85	92.9	96.7
	DELTA	4	71.2	85.1	100	12	68.5	94.2	100
	N/R	19	41.2	69.3	100	36	73.8	92.2	100
	OMICRON	-	-	-	-	4	54.5	69.3	84.5
Outcome	Variant	One Dose AZ				Two doses AZ			
		n ^a	Min	Median	Max	n ^a	Min	Median	Max
PCR positive	ALPHA	2	56	-	63	2	61.7	-	79
	BETA	1	-	30	-	1	-	19.2	-
	DELTA	2	43	-	100	2	67	-	88
	N/R	8	7	53.4	86.2	12	24.9	69.6	95
	ORIGIN	1	-	91	-	-	-	-	-
Symptomatic	ALPHA	3	45.1	48.7	73	4	70.4	78.5	97
	BETA	1	-	36.8	-	1	-	9.6	-
	DELTA	4	30	39.5	42.9	4	67	68.8	82.8
	N/R	6	17.8	48.8	75.4	8	45.5	71	100
	OMICRON	1	-	17.7	-	1	-	48.9	-
	P.1	-	-	-	-	1	-	63.6	-
	P.2	-	-	-	-	1	-	68.7	-
	B.1.1.28	-	-	-	-	1	-	72.6	-
	B.1.1.33	-	-	-	-	1	-	88.2	-
Symptomatic	ALPHA	-	-	-	-	1	-	28.9	-
	DELTA	1	-	50	-	1	-	57	-
	N/R	-	-	-	-	1	-	69.7	-
Severe, hospitalization, and death	ALPHA	1	-	85.5	-	1	-	95.1	-
	DELTA	2	83.8	-	100	2	95.1	-	100
	N/R	6	-15	53.1	73	10	81.4	90.2	100

^an represents the number of studies reporting results for this vaccine–outcome–variant combination. mRNA, Pfizer BioNTech or Moderna; AZ, Astra Zeneca.

first dose of an mRNA vaccine was lower than VE determined at least 14 days after the first dose (26, 66) (Supplementary Tables 1, 2, 4).

Most studies that identified variants determined VE for either the Alpha or Delta variants (Table 5) (13, 14, 21, 25, 27–30, 32, 35, 36, 41, 42, 48–50, 55, 59, 62, 65, 68–73, 75, 77–81, 84, 86, 90, 94, 97, 99). The median VE after the second dose of an mRNA vaccine was numerically lower for Delta (VE = 74.7%) compared to Alpha (VE = 91.7%) for PCR-positive cases. The median VE after the second dose of an mRNA vaccine was numerically lower for Delta (VE = 87%) compared to Alpha (VE = 94.9%) for symptomatic infections. The median VE after the second dose of an mRNA was numerically higher for Delta (VE = 94.2%) compared to Alpha (VE = 92.9%) for severe infections. The median VE against the Omicron variant after two doses of the mRNA vaccines against severe infections was 69.3% (13, 48, 70, 80, 84). This was numerically lower than the Delta and Alpha efficacy for this outcome, as reported above. After the second dose of the AstraZeneca vaccine, the median VE for positive PCR infections against the Delta variant was 77.5% compared to 70.4% against the Alpha variant. The median VE after the second dose of the AstraZeneca vaccine for severe infection against the Delta variant was 97.6 and 95.1% against the Alpha variant.

Many studies ($n = 30$) reported the VE across various age ranges separated within the study population (10, 14, 21–23, 27–29, 33, 36, 39, 46, 48, 60, 61, 66, 67, 69, 75, 77–82, 85, 86, 91, 95, 97). Most of these studies reported VE 14 days after either dose. The VE did not differ by the age of the person in any of the included studies (Supplementary Table 4).

A few studies ($n = 6$) compared the VE by previous exposure to SARS-CoV-2 as measured by persons who were previously infected or not previously infected (17, 42, 43, 69, 98) (Supplementary Tables 1, 2). The results differed by study. In three studies, VE did not differ due to previous infections (17, 42, 98), whereas three other studies reported that prior infections led to an increased VE for the first and second doses of either vaccine (43, 69).

Vaccine efficacy was highest between 2 and 10 weeks after the second dose of the vaccine (Supplementary Tables 1, 2, 4). Vaccine efficacy decreased over time following this initial period. The median time when VE began to decline for mRNA vaccines was 10–14 weeks (13, 14, 69), 13–18 weeks (21, 36, 43, 58, 68, 77, 79, 82, 84), and 13–16 weeks (14, 77, 80, 89) for symptomatic, PCR-positive, and severe illness, respectively. The median time when VE declined for the AstraZeneca vaccine was 10–14 weeks (13, 14), 8–17 weeks (58), and 10–14 weeks (14, 97) for symptomatic, PCR-positive, and severe illness, respectively.

Discussion

Studies included in this systematic review indicated that the Pfizer, Moderna, and AstraZeneca vaccines were efficacious against asymptomatic, mild, moderate, and severe COVID-19, especially following two doses. Vaccine efficacy was highest against severe infections, including hospitalization and death. Reported VE was dependent on the variant of concern during the time of the study, the number of doses included during the reporting period (i.e., one vs. two doses), and the specific outcomes measured.

Vaccine efficacy by the outcome

For all vaccines, VE was higher against severe infections than mild or moderate symptomatic infections and asymptomatic infections. Studies reported on several clinical outcomes, including any PCR-positive infection, asymptomatic infections, symptomatic mild-moderate infections, severe/critical infections, hospitalizations, and deaths. Vaccine efficacy against severe infection in this study included severe infection, hospitalization, and death. Vaccine efficacy differed by clinical outcome. The median VE reported in the current study for two doses of mRNA and AstraZeneca was 85.5 and 69.7%, respectively, for asymptomatic infections, and 92.2 and 90.2% for severe infections, respectively.

SARS-CoV-2 variants

Vaccine efficacy is affected by the dominant variant of SARS-CoV-2 in the region and time period in which a study is conducted. The Alpha variant is 40–80% more transmissible than the origin strain of SARS-CoV-2, leading to higher infection rates (101). Furthermore, the Alpha variant led to more severe clinical signs and symptoms, including a 55% increase in mortality compared to previous variants (101). The VE for AstraZeneca is lower for the Beta variant than for the origin strain (101). Furthermore, serum from individuals vaccinated with either mRNA vaccine had lower neutralizing antibody titers against the Beta variant than against the origin strains (101). The Beta variant elicited improved entry and binding to target cells in the body over the origin strain, thus resulting in a higher infection rate (102). The P.1 variant recorded similar mutations as the Beta variant, thus resulting in an assumed similar VE (101). Regarding the Delta variant, studies reported a 2.5-fold decrease in neutralization using serum from individuals vaccinated with Pfizer, compared to the original SARS-CoV-2 variant, while neutralization by serum from the AstraZeneca vaccine was 4.3-fold lower compared to the original SARS-CoV-2 variant (103). This reduced neutralization illustrates a possible reason for the reduced VE against the Delta variant and may explain the increased breakthrough infections that have been reported in several countries when the Delta variant became most dominant (103). The VE for PCR-positive infection after two doses of Moderna was reported as 98.4% for Alpha and 86.7% for Delta (21). The VE for symptomatic infection after two doses of Pfizer was reported as 94.9% for Alpha and 83.3% for Delta (14). The VE for symptomatic infection after two doses of AstraZeneca was reported as 82.1% for Alpha and 64.2% for Delta (14). Thus, the results demonstrate how the VE of these vaccines changes depending on the variant circulating in the study population.

Time after vaccination

Vaccine efficacy is dependent on the time after vaccine administration. In many studies within this review, VE was determined at least 14 days after the first dose (8–10, 16, 17, 19–21, 23, 25–27, 29–31, 33–35, 38, 41, 45–47, 51, 59, 61, 62,

64, 66, 76, 78, 79, 81, 83–85, 90–92, 96–98). However, various studies have suggested that the vaccine effect of one dose can numerically increase for up to 21 days (19, 25, 29–31, 43, 50, 53, 56, 68, 69, 71, 75, 85) or 28 days after the first dose (8, 10, 14, 46, 49, 85, 96). Additionally, many studies reported VE at least 7 days after the second dose (14, 15, 19, 22, 25, 27, 29–31, 34, 37, 38, 41, 42, 46, 61, 64–67, 71, 79, 80, 82, 86, 87, 90, 92). Although various studies suggested that vaccine effects can numerically increase for up to 14 days (8–10, 13, 14, 17, 18, 20, 21, 23–26, 28, 31–33, 35, 36, 39, 41, 43, 44, 47–52, 54, 55, 57–64, 68–70, 72, 73, 76–78, 81, 83, 84, 86, 88, 91, 94, 96–99), or 28 days after second vaccination this is not substantiated by studies measuring antibodies (86). Antibody studies showed that among individuals who had no previous infections, IgG antibody levels were maximized approximately 4 weeks after the first dose and 2 weeks after the second dose for mRNA vaccines (104–106). Similarly, neutralizing antibodies were maximized approximately 28 days after the first dose and 14 days after the second dose of the AstraZeneca vaccine (107, 108). Therefore, in any future, efficacy research focused on these vaccines, outcomes should be recorded during this period to determine the maximum possible VE.

Over a longer period after the second dose, VE declined. The median time when VE declined after two doses of the mRNA vaccines and AstraZeneca was 13–18 weeks (21, 36, 43, 58, 68, 77, 79, 82, 84) and 8–17 weeks (58) for PCR-positive infections, respectively.

Limitations

Many studies controlled for several co-variables such as age, sex, socio-economic status, geographical source, and pre-existing illnesses. However, other studies did not control for any co-variables or only controlled for a few co-variables, such as age and sex. These analytical inconsistencies may have reduced the comparability of VE and increased the variability of VE reported across the studies.

Publications were included in this report if they reported population-level data within specific age groups and geographic areas. This review excluded publications that focused on specific pre-existing immunocompromised-related conditions such as multiple sclerosis and cancer. Immunocompromised individuals were included in the broad population, yet vaccine efficacy specific to these cohorts was not determined. Therefore, the vaccine efficacy results cannot be generalized to individuals who are immunocompromised.

Many individuals across Canada have received more than two doses of a SARS-CoV-2 vaccine (109). Research on VE in populations receiving a third or fourth dose of the vaccine was beyond the scope of this systematic review. Additionally, many Canadians were vaccinated with a combination of available vaccines (109). Similarly, the VE has been determined for persons receiving a combination of different vaccines for their first and second SARS-CoV-2 vaccinations. For example, the initial vaccine may have been AstraZeneca, followed by a second dose using an mRNA vaccine. The VE for heterologous vaccination protocols was not included in the current study. Therefore, the findings of the current study may not apply to either third booster doses or VE for heterologous vaccination programs, and further research is required to determine the VE of heterologous and booster vaccines.

The results of this study cannot be generalized to new and emerging variants of SARS-CoV-2. In May 2021, the Delta variant was determined to be the variant of concern within North America, yet by November 2021, the Omicron variant was identified and was still the variant of concern in North America 1 year later (110). The database searches were completed in May 2022, and consequently only included six studies reporting on the efficacy of the Omicron variant. Therefore, we cannot conclude the efficacy of vaccines against Omicron or any future variants. Nonetheless, because of emerging new variants of concern such as Omicron, ongoing research is required to determine the effectiveness of mRNA and AstraZeneca vaccines. More recently, bivalent mRNA vaccines specifically targeted against the Omicron variants have been approved for use in Canada (111, 112). This review does not include VE for these bivalent vaccines.

The results of this study present estimates of the efficacy of several SARS-CoV-2 vaccines, including Pfizer, Moderna, and AstraZeneca vaccines, based on population-level studies. After 14 days, a second dose of any of the vaccines represented in this study provided highly efficacious protection against severe infections, hospitalization, or death due to SARS-CoV-2 infections. VE still provided good protection against other outcomes such as mild-moderate symptomatic infection, asymptomatic infection, and any PCR-positive infection, suggesting that these vaccines can protect against any severity of infection due to SARS-CoV-2. In both mRNA and AstraZeneca vaccines, efficacy was highest after the second dose, against the original strains of SARS-CoV-2, and when the time reporting clinical signs and symptoms was at least 14 days after vaccination. As strains continued to evolve from the origin, VE decreased. Furthermore, these results illustrated that VE wanes over time after the second dose, suggesting that although two doses provide adequate protection, this does not remain indefinitely. Nonetheless, whether reporting on PCR-positive infection, mild, moderate, or asymptomatic infection, all the vaccines included in this systematic review provided good protection against SARS-CoV-2, while these same vaccines provided excellent protection against severe illness, hospitalization, and death.

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

LR conducted the literature search, identified manuscripts for inclusion, and wrote the first draft and edited versions of the manuscript. CD is the senior author who contributed to the concept and design of the study, mentored the first author, and wrote sections of the manuscript. GA was the second reviewer of the manuscripts for inclusion. ML contributed to the design of the manuscript, critically evaluated the analysis, and provided clarity to the final draft. All authors contributed to manuscript revision, read, 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.2023.1229716/full#supplementary-material>

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A review of knowledge, attitudes and practices regarding mosquitoes and mosquito-borne infectious diseases in nonendemic regions

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Mosquito-borne infectious diseases (MBIDs) present significant public health risks within tropical and subtropical regions. However, the rapid spread of MBIDs from these areas to temperate regions increase the risk of their emergence in nonendemic regions, i.e., regions where diseases are still sporadic and not sustained in the population. Raising awareness about preventive measures and protective behaviors is of primary importance to face the risks of vector-borne diseases. In this context, the number of studies on knowledge, attitude, and practice (KAP) about mosquitoes and MBIDs has grown rapidly in response to the need to identify knowledge and practices in nonendemic countries to fight mosquito proliferation. Building upon the recent developments in this field, we conducted the first-ever literature review to examine KAP studies conducted in nonendemic regions. Our aim was to identify the community's knowledge and attitudes that shape practices concerning the prevention of MBIDs. We used specific keywords regarding the scope of this review and then selected studies that were performed in nonendemic regions for MBIDs, including regions located in European countries, the USA or Asia. We identified 32 KAP studies, the oldest from 2003. The findings in the reviewed studies show that survey participants generally possessed a rather good understanding of mosquito breeding sites. However, there were notable variations in knowledge and perception of MBIDs, primarily linked to the geographic location of the survey and the prevalence of infectious outbreaks related to mosquito transmission. These findings highlight the significant influence of knowledge and awareness in fostering effective mosquito control practices. Moreover, socioeconomic status, particularly educational attainment, and respondents' gender emerged as key determinants in explaining the variability of appropriate practices. The survey results thus show the crucial role of knowledge, emphasizing the need for widespread awareness and information campaigns, encompassing both appropriate practices and efficient mosquito control methods. Understanding the interaction between these factors could provide good guidelines for implementing awareness plans and ultimately motivate the population to actively fight against mosquito proliferation and MBIDs development.

KEYWORDS

knowledge, attitude, practice, mosquitoes, mosquito-borne infectious diseases (MBIDs), nonendemic regions, preventive methods

1 Introduction

Global climate change has intensified concerns regarding mosquito-borne infectious diseases (MBIDs) due to the expanding global distribution of arboviruses transmitted by mosquitoes in recent years (1). Nonendemic regions, referring to areas where diseases remain sporadic and are not sustained within the population, are increasingly grappling with this problem, as seen in various European countries and the United States, for instance. The expansion of mosquito vector species in these areas is further compounded by poor mosquito prevention and control practices (2). MBIDs are transmitted to people through the bite of an infected female mosquito, which lays its eggs on water surfaces in various habitats, such as salt marshes, lakes or ponds, polluted water retention systems, or any other location where water accumulates. While all mosquitoes require standing water to reproduce, different mosquito species thrive in different habitats, including temporary water habitats or water sources that remain for long periods (3). For instance, some *Aedes* mosquito species have evolved to lay their eggs in artificial containers. This is why urban areas are becoming suitable environments for the development of these species by providing mosquitoes with a multitude of man-made breeding sites, such as flowerpots, empty containers, and rain gutters, leading to their rapid colonization in cities (4).

Considering the absence of prophylactic measures or vaccines for the majority of MBIDs, effective control of the mosquito vector stands as a crucial step in curtailing the proliferation of both mosquitoes and these diseases (5). Vector control measures rely on the use of different individual or collective practices. Among them, chemical insecticides are widely used. However, their adverse impacts on nontarget species and their secondary effects due to resistance in mosquito populations have made chemical insecticides a controversial social issue (6). On the other hand, environmental measures such as draining standing water, proper disposal of empty containers or the use of bed nets on standing water containers are considered sustainable methods to control the spread of *Aedes* and *Anopheles* mosquitoes in urban areas (7). To be effective, these methods must be used in conjunction with other control methods and involve the local community. Additionally, public health risk awareness, ovitrap surveillance, and door-to-door control measures contribute to reducing the risks of spreading MBIDs (8). The choice between different existing and emerging vector control methods or combinations of techniques must be driven by the epidemiological, environmental, and socioeconomic context (9). One of the most significant explanations for the invasion and proliferation of mosquito vectors in urban areas is the practices of city residents (10). Knowledge, attitude and practices (KAP) questionnaires are frequently used to assess which parameters influence good or bad practices as well as knowledge and attitudes regarding mosquitoes and MBIDs. These surveys focus mainly on the mosquito life cycle, mosquito ecology, mosquito identification, biting times, control methods, and transmitted diseases. These surveys are essential to identifying inappropriate population behaviors and targeting the lack of awareness of a disease and/or vector as well as finding efficient strategies that involve the community in vector control. Given the importance of this issue, a review of studies based on KAP surveys can provide decision-makers with new information to better manage mosquito spreading and health risks. This study is the first to synthesize current knowledge on 32 published KAP studies conducted in nonendemic regions.

2 Materials and methods

Searches were carried out on Google Scholar in February and March 2023 from peer-reviewed journal articles and reviews written in English, without period restriction. We first used a list of specific keywords that matched with the scope of our study. This first process of selection allowed us to identify 903 articles. Then, we kept articles that only concerned studies that were performed in countries that are considered as nonendemic regions (or at least partially, like Vietnam) for MBIDs, i.e., regions where diseases are still sporadic and not sustained in the population. It includes studies conducted across various European countries, such as regions in France, Spain, Germany, Greece, and Italy, as well as a diverse array of states in the USA, including California, Florida, Louisiana, Utah, Alabama, and Arizona. Additionally, it encompasses research from regions in Asian countries such as Korea, Vietnam, Bangladesh, India, Malaysia, and Turkey. This second selection process led to the selection of 76 articles. Finally, after removing duplicates or articles that did not match our inclusion criteria (no KAP survey, type of article), our search yielded a total of 32 studies that matched our inclusion criteria (Table 1). These studies differed according to several factors such as the country/city, the mosquito species, MBIDs and targeted populations (Table 2).

3 Results

3.1 Knowledge, attitude and practices regarding mosquito development and associated health risks

3.1.1 Knowledge of larval breeding sites and MBIDs

The reviewed studies revealed that a major part of the participants in the surveys had very good knowledge of the role of standing water as potential mosquito breeding sites (11–18). For instance, results from campaign prospecting door-to-door visits and interviews in Sant Cugat, Spain, showed that 84.9% of the surveyed persons had good knowledge about *Ae. albopictus* aquatic habitats (13). Similarly, Tuiten et al. (12) found that 99% of the respondents in New York (United States) knew that *Culex* mosquitoes breed in standing water (12). Morse et al. (15) obtained similar results in a survey in Alabama (United States) where 98% of the inhabitants identified standing water as sites that are suitable for mosquito breeding (15). In Lyon (France) a survey carried out amongst people participating in community gardens showed that 94.7% of the respondents knew that the tiger mosquito (*Aedes albopictus*) breed in small or medium standing water (18). Thus, overall, respondents in KAP studies recognized standing water containers as potential egg-laying sites.

However, these studies also showed that a significant proportion of the surveyed persons had only a partial knowledge of the type of containers used as breeding sites, or identified other locations that are not suitable as breeding sites (15, 18–23). For instance, in Lyon, 81.6% of the respondents believed that tiger mosquitoes breed in vegetation that in fact is suitable resting sites for adults, but cannot serve for larval development (18). Morse et al. (15) showed that empty containers or rain gutters were not associated with potential breeding sites even though they represent highly suitable larval habitats for

TABLE 1 Selection criteria used for studies considered for the review.

Key words	Number of articles	Filter 1: studies in nonendemic countries	Filter 2: study types
attitude <i>Aedes</i>	13	1	1
attitude <i>Culex</i>	0	0	0
attitude <i>Anopheles</i>	0	0	0
attitude mosquito	45	1	1
attitudes mosquito	26	8	3
education mosquito	32	4	0
KAP mosquito	3	0	0
KAP mosquitoes	3	0	0
survey mosquito	344	8	8
knowledge mosquito	157	20	14
perception <i>Aedes</i>	9	2	2
perception <i>Anopheles</i>	3	0	0
perception <i>Culex</i>	1	0	0
perception mosquito	35	3	1
perceptions mosquito	42	15	13
practice mosquito	42	0	0
practices mosquito	111	9	1
protective behaviors mosquito	2	2	2
questionnaire mosquito	6	2	1
socioeconomic mosquito	21	2	1
additional records identified through other sources	8	8	8
Total with duplicates	903	85	56
Total without duplicates	-	-	32

mosquito colonization (empty containers and rain gutters were not identified by 49 and 39%, respectively). Caputo et al. (21) also revealed low knowledge about the breeding site characteristics of the Asian tiger mosquito *Aedes albopictus*; only 21.3% of the respondents had correct answers regarding knowledge on places where tiger mosquitoes lay eggs and larvae develop. In Greece, a minority of respondents was able to identify correctly the place where mosquitoes lay their eggs and larvae grow (20, 22, 23). Similar observations were made from a German study where only 11.6% of respondents identified tree holes as potential water-holding larval habitats in their gardens (19).

Although these KAP studies were conducted in nonendemic areas where MBIDs are still sporadic, they reveal different levels of knowledge about these infectious diseases according to places and targeted population in the surveys. Firstly, a good knowledge was evidenced in surveys performed in the South of the United States, where local transmissions occur, i.e., mosquitoes in the area have been infected with the virus and are spreading it to people (12, 14, 15, 17, 24–26). For instance, Bohmann et al. (17) demonstrated that survey participants in South Texas had a high level of knowledge on different diseases transmitted by mosquitoes: 100, 98 and 79% of respondents having previously heard of West Nile virus (WNV), Zika virus (ZIKV) and Dengue virus (DENV), respectively (17). By contrast, in Europe, MBIDs are still sporadic and epidemic events are mostly dependent on imported cases (i.e., when diseases are contracted abroad). Several

studies found that knowledge of diseases transmitted by mosquitoes was limited and that people are generally unaware of the health risks (21, 22, 27–30). Mouchtouri et al. (28) found that only 9.6% of the interviewed Greek women answered correctly at all questions concerning Zika virus (28). Constant et al. (31) found that the use of entomological surveillance of the main mosquito arbovirus vectors in anticipation of the emergence risk of MBIDs generates a significant increase in the population's knowledge of these diseases. Only 51.1% of the respondents residing in French Mediterranean regions knew that tiger mosquitoes could transmit chikungunya in 2012 compared to 88.3% in 2014 (31). In metropolitan France, health risks related to mosquito proliferation have increased significantly since 2011 when only 9 departments, out of 96, were colonized by the tiger mosquito compared to 81 in 2023 (84% of the departments). During the same period, the total number of imported cases of dengue, Zika and chikungunya increased from 15 in 2013 to approximately 1,126 cases in 2023 (of which 1,099 cases of dengue). This evolution is probably increasing more anxiety in the population toward these emerging diseases (32). For example, Duval et al. (18) found that a total of 88.5% of community gardeners in Lyon, France, knew that mosquitoes could infect humans and animals (18). In comparison, Caputo et al. (21) showed that 49% of the Italian residents knew that mosquitoes can transmit diseases but did not know which ones. Only 1% had already heard about dengue, chikungunya or yellow fever (21). Similarly, Stefopoulou et al. (22) highlighted that only one respondent correctly

TABLE 2 Characteristics of selected studies (n = 32).

Continent	Country	City	Mosquito species and/or MBIDs	Participants	Women (%)	Men (%)	Focus group and/or	References	
America	USA	NA	<i>Aedes</i> sp. / Zika	59	100	0	Gynecology patients	[25]	Curry et al. (2017)
		Key West/ Tucson	<i>Aedes aegypti</i>	775	49	51	Randomly selected participants	[53]	Haenchen et al. (2016)
		Provo (Utah)	Malaria, Dengue, Zika, Chikungunya and West Nile	1,043	68	32	Participants that traveled outside USA	[63]	Omodior et al. (2018)
		Louisiana	<i>Culex</i> sp. and <i>Aedes</i> sp.	260	64	30	Randomly selected participants	[37]	Moise et al. (2022)
		six states in the Northeast	<i>Culex</i> sp.	922	44	56	Randomly selected participants	[38]	Herrington et al. (2003)
		St. Johns County (Florida)	All mosquito species	24	NA	NA	Randomly selected participants in neighborhoods	[14]	Davidson et al. (2016)
		Region of southwestern Virginia	<i>Culex</i> sp. and <i>Aedes</i> sp.	188	NA	NA	Randomly selected participants	[34]	Butterworth et al. (2010)
		Tennessee/Texas	La Crosse encephalitis	70	69	30	Randomly selected participants	[33]	Barnes et al. (2022)
		Miami	<i>Aedes</i> sp.	44	25	75	Outdoor construction workers	[42]	Moore et al. (2017)
		Alabama	Malaria, Dengue, Zika, Chikungunya and West Nile	126	NA	NA	Randomly selected participants	[15]	Morse et al. (2019)
		New York	<i>Culex</i> sp.	97	68	32	Randomly selected participants in two neighborhoods (residential area/ suburban area)	[12]	Tuiten et al. (2009)
		Tucson (Arizona)	<i>Aedes aegypti</i>	355	NA	NA	Randomly selected participants	[48]	Walker et al. (2018)
		Washington	<i>Culex</i> sp. and <i>Aedes</i> sp.	184	NA	NA	Randomly selected participants in five neighborhoods	[41]	Dowling et al. (2013)
		San Antonio (South Texas)	<i>Aedes</i> sp.	31	90	65	Randomly selected participants in five neighborhoods	[17]	Bohmann et al. (2022)
		Three cities in North Carolina	All mosquito species	415	49	50	Randomly selected participants	[43]	Richards et al. (2017)
		Florida	<i>Aedes</i> sp.	413	NA	NA	Physicians	[24]	Doblecki-Lewis et al. (2016)
Asia	Korea	Seoul, Gyeonggi-do, and Incheon	<i>Aedes</i> sp.	249	88	12	Nursing students	[46]	Choi et al. (2018)

(Continued)

TABLE 2 (Continued)

Continent	Country	City	Mosquito species and/or MBIDs	Participants	Women (%)	Men (%)	Focus group	References	
	Vietnam	Hanoi	Dengue and Japanese encephalitis	513	30	70	Participants in 3 livestock households and 3 non-livestock households	[16]	Nguyen-Tien et al. (2021)
Asia/Europe	Bangladesh, India, Malaysia, and Turkey	NA	<i>Aedes</i> sp.	223	53	47	Physicians	[30]	Koonisetty et al. (2021)
Europe	Germany	NA	<i>Anopheles plumbeus</i>	118	31	64	Persons involved in citizen sciences project	[19]	Heym et al. (2017)
	Spain	Valldoreix	<i>Aedes albopictus</i>	309	60	40	Users of the Health Care Center	[11]	Curcó et al. (2008)
		Sant Cugat	<i>Aedes albopictus</i>	820	65	35	Randomly selected participants	[13]	Abramides et al. (2013)
	France	Lyon	<i>Aedes albopictus</i>	222	51	49	Community gardeners	[18]	Duval et al. (2022)
		South of France	<i>Aedes albopictus</i>	1,506	NA	NA	Randomly selected participants	[27]	Raude et al. (2012)
		Languedoc-Roussillon, Provence-Alpes-Côte d'Azur; Corsica	<i>Aedes albopictus</i>	574	55	45	Randomly selected participants	[31]	Constant et al. (2020)
		Camargue, petit camargue, Rhône alpes	All mosquito species	639	NA	NA	Randomly selected participants	[6]	Claeys et al. (2009)
	Greece	Athens	<i>Aedes</i> sp.	303	56	44	Participants in camp for migrants and in neighboring residential areas and urban areas	[23]	Kolimenakis et al. (2022)
		All administrative regions	<i>Aedes</i> sp.	573	100	0	Pregnant women	[28]	Mouchtouri et al. (2017)
		Vravrona	<i>Aedes albopictus</i>	40	50	48	Randomly selected participants	[22]	Stefopoulou et al. (2021)
	Italia	Lazio region	<i>Aedes albopictus</i>	1,579	55	45	Native populations and two resident communities originating from India	[21]	Caputo et al. (2020)
	Montenegro	Beci'ci,	<i>Aedes japonicus</i>	48	29	69	European participants of EMCA conference	[29]	Ibañez-Justicia et al. (2019)
	UK	NA	<i>Anopheles</i> sp.	125	60	44	Participants who plan to visit a malaria-endemic area	[45]	Goodyer et al. (2014)

identified the MBIDs among a list of 9 diseases (22). In 2018, 67.3% of the residents in Attica region in Greece were not able to list diseases that mosquitoes can transmit. Malaria was the most frequently answered response, followed by west Nile fever and dengue. It is clear that some viruses are less known by the populations in certain regions (22). For instance, a large part of the population did not know about la crosse encephalitis (LAC) virus while this is the most medically significant California encephalitis virus in the USA (33, 34). Only 16.3% of respondents were conscious of the existence of LAC (34).

Travelers and migrants coming from endemic countries could have an impact on the knowledge and practices regarding mosquitoes in nonendemic countries. For instance, in Italy, a questionnaire-based study was conducted to compare the KAP of native populations and two communities originating from the Indian subcontinent where MBIDs are a major public health concern. The results showed higher knowledge and concern about *Aedes*-transmitted diseases in Indian communities associated with a lower level of concern of nuisance caused by mosquitoes (21). This suggests that people who have been exposed to MBIDs are better prepared to face epidemics. More generally, it appears that populations from endemic-mosquito areas have a higher awareness of MBIDs; therefore, it could be expected that migration to nonendemic countries could improve local population knowledge (35, 36). To a lesser extent, temporary visitors such as tourists may also affect knowledge of local populations. Interestingly, pregnant women from Greece who traveled abroad (not exclusively in endemic countries) had better knowledge about the risks of MBIDs than women who had not traveled abroad (OR = 10.47, p value = 0.04) (28). The authors suggested that this difference could be explained by a higher awareness among individuals who have traveled to endemic areas.

To improve knowledge about mosquitoes and MBIDs, educational campaigns are implemented by public services. Among different information sources, participants of KAP surveys are generally informed by mass media (TV, radio, websites and newspaper) (12, 18, 23, 25, 28, 37). Tuiten et al. (12) reported that 70% ($n = 97$) of the respondents mentioned that newspaper was the main information source about WNV followed by television (36%), radio (26%) and websites (20%) (12). Similar observations were made in the Lyon study in which 85.1% of the respondents were informed about the Asian tiger mosquito by media and internet (18). Surprisingly, information leading to awareness through health care workers seem to be rare. Two studies found only 8–9% of the respondents were informed about mosquitoes and MBIDs by physicians (28, 38). This contrasts with endemic countries where between approximately 50 to 80% of the respondents are aware by health professionals that seems to play an important role in prevention about MBIDs and mosquitoes (39, 40).

3.1.2 Attitudes toward nuisance and health risk

Studies on MBIDs and nuisances caused by mosquito bites have shown that most respondents are more concerned about the negative impact on their well-being than the related health risks. In the reviewed papers, between 44 and 80% of the respondents considered mosquito bites to be a serious nuisance during their activity periods (14, 18, 19, 21, 41, 42). For instance, Davidson (14) found that in St Johns County (Florida, USA) 80% of the respondents were bothered daily or several days per week (14). In Lyon (France) the survey

amongst people participating in community gardens showed that the majority (81.3%) of the respondents were highly or moderately concerned by the presence of mosquitoes, with most of them being more preoccupied by nuisance and impacts on their quality of life than by disease transmission (18). An Italian study compared the attitudes of three groups of people: the native population and Italians originating from two endemic regions of India (21). In the group of native Italians 58.3% of the respondents were disturbed by mosquito bites whereas only 0.6 and 14.3% of the Malayalis and Punjabis, respectively, stated that they were concerned with mosquito bites. Some papers show that nuisance due to mosquito bites leads to changing activities, mostly by reducing time outdoors. For instance, in Washington, a large majority of respondents (61%) reported a change in activities (41).

Among other studies that have focused on the health impact of MBIDs and mosquito bites, some were conducted in the south of the USA, where MBIDs are present without being endemic. These studies demonstrated a great variety of risk perception according to the type of groups investigated. For instance, 95.5% of surveyed households in New Orleans stated that mosquitoes represented a major risk of transmitting diseases (37). Curry et al. (25) found that almost one woman out of three interviewed in the gynecology department was concerned about the spread of ZIKV, particularly when pregnant. This concern was amplified among women who had given birth at least once (82.6%) (25). In contrast, only 23% of surveyed workers in Miami said they were moderately or extremely concerned about contracting a disease transmitted by mosquitoes (42). Similarly, respondents in South Texas had, on average, moderate fear of mosquitoes and the diseases they transmit, although they perceived MBIDs as a serious problem (17). In Greece, Kolimenaskis et al. (2022) showed that 83% of the respondents consider mosquito as a problem for nuisance and health (23).

In contrast to the southern United States, studies conducted in Europe showed relatively lower levels of concern regarding mosquito-borne infectious diseases (MBIDs). In France, vulnerability to MBIDs was found to be low or moderate depending on the populations studied (ranging from 21.7 to 53.1% for random respondents in south of France and 27.7% for gardeners of community gardens) (18, 31). In the Lazio region (Italy), 51% of respondents reported that they felt never or rarely worried about *Aedes*-transmitted diseases compared to 29% who considered themselves very or extremely worried (21). Caputo et al. (21) found a large difference in attitudes between the investigated categories. Only 15.2% of native Italians reported being worried about diseases transmitted by *Ae. albopictus*, while among Malayali and Punjabi respondents, the proportions were 28.8 and 26.7%, respectively (21).

Overall, these results show that despite concern about MBIDs in nonendemic regions, perceived risks of epidemic-prone diseases are generally rather moderate, and seem to be related mainly to the characteristics of the surveyed group.

3.1.3 Mosquito mitigation practices and personal protection

The respondents in the analyzed surveys adopted different practices to avoid mosquito proliferation or to protect themselves against mosquitoes. Regarding these practices, questions in KAP studies addressed the use of personal protective measures and the management of potential breeding sites.

The majority of respondents in southern Texas (80.5%) declared that they correctly eliminated standing water (17). In a Spanish survey, the researchers observed that 76% of local population in Saint Cugat del Vallès, north of Barcelona, managed standing water to avoid potential breeding sites (13). Richards et al. (43) reported that 67% of respondents in north Carolina reported removing empty containers such as flowerpots, birdbaths or tires (43). In France, 82.1% of gardeners in community gardens responded that they actively fought against mosquitoes by eliminating standing water containers (18). Despite the efficiency of this mechanical method, some surveys show that it is not systematically used by local residents. Only 40% of respondents in southwest Virginia and 50% in New York reported regularly or occasionally removing standing water containers (12, 34). A survey conducted in southern France found that only 17.7% of respondents eliminated standing water and approximately one out of two stated that they did not follow any of the practices recommended by public health authorities (27). Even though construction workers are considered a population at risk for many MBIDs, 80% of surveyed construction industry employees in Miami did not use preventive measures such as removing standing water (42, 44).

Several studies also analyzed personal protective measures against mosquitoes. Although personal protective measures are not intended to prevent mosquito proliferation, they contribute to limiting the number of bites and thus slowing the propagation of MBIDs. Personal protection takes many forms such as the use of repellents, wearing covering clothing, using door or bed nets, and limiting outdoor activities especially at hours when mosquitoes are active. In general, the use of repellents was the most frequently cited method by KAP respondents, probably because they are easy to apply and decrease contact between mosquitoes and humans. Overall, between 60 and 90% of the respondents reported using repellents to protect themselves (18, 28, 37, 38, 45). However, in the study conducted by Caputo et al. (21), the majority of surveyed Italians (53%) reported that they used nothing to avoid *Aedes* bites, only 23% used environmental repellents at home, and 15% used personal repellents (21). Some studies have shown that bed nets or covering clothes that offer the advantage of being environmentally friendly and have no impact on human health are commonly used for personal protection (18, 22).

3.2 Explanations of urban citizens' practices in the eradication of breeding sites

3.2.1 Influence of knowledge and concern about mosquitoes and MBIDs on mosquito control practices

People's level of knowledge and concern about mosquitoes and MBIDs clearly have an impact on their control practices according to the previous publications that have addressed this issue (10 of the reviewed articles). Some KAP surveys show that a high level of knowledge, for example, of the mosquito life cycle is essential to identify potential larval habitats and implement more appropriate practices (17, 18, 21, 27, 41, 46). Caputo et al. (21) found that a low level of Italian citizens' knowledge (21% of correct answers) was associated with a low level of practices to reduce or remove breeding sites (0.7%) (21). Raude et al. (27) found in their study in French Mediterranean regions that a high ability to identify the *Aedes* species

was highly associated with protective behavior by the respondents ($p < 0.001$) and resulted in the elimination of standing water (27). In Washington (USA), Dowling et al. (41) demonstrated that approximately half of surveyed respondents who recognized breeding sites reported that they removed standing water (41). To our knowledge, only two studies have shown an opposite effect. Duval et al. (18) revealed no significant relationship between the level of knowledge and type of actions taken against mosquitoes and their breeding sites (18). In this survey focused on the community gardeners in Lyon (France), attitudes, more than knowledge, appear to be an explanatory factor. Gardeners who were very concerned about mosquitoes were overrepresented in the group of gardeners who modified all or some practices because of mosquitoes (76 and 62% of the respondents, respectively). Only 27.9% of respondents who had not changed their habits in the garden due to the presence of mosquitoes were concerned about the presence of mosquitoes. This finding suggests that the more concerned community gardeners are about the presence of mosquitoes in their gardens, the more likely they are to take action against this species (18). Moise et al. (37) observed in New Orleans (USA) that 20.5% of respondents had good practices regarding mosquito control despite a lack of knowledge about potential breeding sites (37).

3.2.2 Impact of socioeconomic status on mosquito management practices

Most studies show that knowledge and practices depend on the socioeconomic status of the respondents, such as employment status, educational level or income (12, 17, 27, 28, 37, 41, 47, 48).

Regarding household income, Walker et al. (48) found that the odds of finding containers colonized by *Ae. aegypti* in outdoor premises were positively associated with low-income households in Arizona (United States) (48). Moise et al. (37) observed that 31.6% of the New Orleans respondents in high-income households had negative practices against mosquito bites compared to 35.2% of respondents from low-income households. They also showed that employed individuals had significantly higher knowledge regarding mosquito control and practices to prevent mosquito proliferation and mosquito bites than unemployed individuals (OR: 1.00; 95% CI [0.000–0.006]) (37).

Educational level seems to be another key factor that explains good mosquito knowledge and appropriate practices (12, 17, 27, 28, 41, 47, 49). In Washington, respondents without a college degree had significantly more containers that could receive rainwater at their homes than respondents with a college degree (41). However, several studies found no link between the socioeconomic status of respondents and their level of knowledge and practices regarding mosquitoes and MBIDs (18, 19, 31, 34, 37). In a study of nonprofessional gardeners in France, Duval et al. (18) found no correlation between knowledge, practices and educational level (18). The absence of a significant relationship is probably because the respondents in the survey were all participating in community gardens, where awareness of mosquitoes is generally high. An American study in southwestern Virginia concluded that education and income were not significant predictors of the level of knowledge and practice (34). This study focused on the population of central Appalachia, which is an economically deprived region where financial resources for public health are low. In a study in Germany, educational level did not appear to have a significant effect on knowledge of

Anopheles breeding sites (19). This could be explained by the fact that 54% of respondents claimed to have no mosquito breeding habitats close to their homes or had no knowledge of them. Following these examples, the lack of relationship between education and income with level of knowledge and practices could be the result of lower education levels in the target population.

Despite the lack of correlation observed in some studies, it seems that the socioeconomic level of residents explains their level of knowledge and behavior in regard to mosquitoes and their potential breeding sites. Numerous publications show that a high living standard and educational level are related to more efficient management of potential larval habitats and reduced frequency of breeding sites in a neighborhood. Similar observations are found in endemic countries (50–52).

3.2.3 Role of demographic factors on protective behavior against mosquitoes

In addition to socioeconomic characteristics, several studies have investigated the effect of demographic variables on knowledge and practices regarding mosquitoes (12–14, 17, 19, 21, 22, 27, 38, 43, 53–55). Gender appears to be a key factor explaining the knowledge and behavior of respondents (12, 27, 31, 34, 43, 49). Overall, with regard to mosquitoes and MBIDs, female respondents show better knowledge and more appropriate practices. Studies in France and China revealed that the percentage of women who adopted protective behavior against mosquito bites (i.e., the use of chemical repellents or covering clothes) was significantly higher than male respondents (27, 31). A survey conducted in New York demonstrated that women had overall better knowledge about WNV than men (12). Additionally, in south-western Virginia, women declared themselves to be better informed about MBIDs and more concerned about health risks linked with mosquito bites and contracted diseases than men (34). Higher knowledge and concern in women could be explained by the fact that women are more anxious about health problematics and, in general, exhibit more responsibility than men regarding ‘care’ (56–59). A few publications have reported gender differences in practices regarding mosquito management. For example, two studies revealed that women respondents declared that they adopted better management of breeding sites than men, such as removing standing water and outdoor measures (43, 49).

Several studies have revealed that age also influences knowledge and practices regarding mosquitoes. Although the age categories are not similar among studies, most studies have found that older respondents had better knowledge and practices regarding the management of breeding sites (12, 41, 47, 53). For instance, individuals aged 45+ years in Washington reported a better level of mosquito ecology knowledge and were more active regarding standing water management (41). Similarly, Maeda et al. (47) found that self-reported source reduction was more common for respondents over 50 years than for younger participants in Washington D.C. and Maryland (USA) (47). In Key West and Tucson (USA), respondents aged 35+ years reported that they cleared standing water in their property more often than younger groups (53). Different practices according to age groups could be explained by the fact that older persons are more likely to garden than younger residents, and thus are more familiar with mosquito larval habitats.

In regards to protective behavior, a few studies have revealed opposite results (31, 34, 49). Among respondents living in

Mediterranean France, Constant et al. (31) observed that older individuals (≥ 68 years) were negatively correlated with personal protection against mosquitoes (Odd Ratio = 0.34) (31). However, this result should be interpreted with caution because, most of these respondents were retired which could influence specific mosquito protective behavior. In China, younger age was found to be a good predictor of adopting effective outdoor protective measures (49).

3.3 Knowledge and attitudes regarding mosquito control methods

3.3.1 Knowledge of mosquito control

Many types of mosquito control methods exist including physical, chemical, biological, mechanical and environmental methods. Mosquito control implies the local residents have good knowledge of the mosquito life cycle and be aware of their role in prevention. We found only three studies that examined people's knowledge of mosquito control in nonendemic countries (6, 18, 55). Claeys et al. (9) investigated social knowledge and perception of mosquitoes and mosquito control methods in France regarding larvicide spraying in wetland (6). This study is based on field surveys conducted between 1995 and 2006 in the Rhone river delta (South of France) and in some Alpine valleys (East of France). In total, the population sample of 639 persons were interrogated via standardized questionnaires or semi-directive interviews. The results showed that in the delta of the Rhone River in France recurring campaigns of larvicide applications were poorly known by the local population (6). More than 40% of the respondents did not know who was responsible for the mosquito control campaigns in the region, although mosquito control has been controversial for more than 10 years. Another French survey in Lyon investigated the level of knowledge about biological control methods among nonprofessional gardeners in community gardens (18). It is interesting to highlight that only 20.5% of the respondents had ever heard about biological control methods. Among them, more than one-third were unable to describe the concept of biological control. Interestingly, only 3.4% of the gardeners were able to explain different biological methods like predation, use of *Bt* toxin, and 2.3% confused these methods with mechanical methods. Despite a general lack of knowledge, the gardeners wanted more information about alternative control methods, as revealed by the average score of 4.16 on a five-point Likert scale on this issue. This study also found that respondents who had already heard about biological control methods also had a better practice regarding mosquito management. Another study on public opinion regarding mosquito control in four locations in Florida, USA, showed low knowledge of mosquito control methods (26, 55). The respondents pointed a lack of notifications prior to spraying campaigns and declared they would like to know more on this control method. To our knowledge, no study has yet investigated participants' knowledge of the sterile insect technique (SIT) or the use of the bacterium *Wolbachia* which are both implemented in endemic areas (60). SIT consists in releasing sterilized males in a target area. The systematic and repeated release of unfertile males decreases mosquito population over time (60). Releasing mosquitoes with *Wolbachia* over several months can reduce the number of a specific mosquito species, such as *Ae. aegypti* or compete with viruses within mosquitoes (61).

Knowledge questions in KAP surveys are largely restricted to mosquitoes and MBIDs. To increase the implications for the local population of mosquito control in nonendemic countries, there is a need for both better information on existing control methods and more surveys dealing with this issue.

3.3.2 Attitudes toward mosquito control methods

Attitudes toward mosquito control methods have been addressed in a larger number of surveys than the level of knowledge regarding those methods (6, 15, 22, 27, 29, 45, 55, 62). Understanding the population's perception of mosquito control and its effectiveness is necessary to assess which practices, e.g., protective behaviors or actions against mosquito proliferation would potentially be well accepted and used by the population. Claeys et al. (9) found two extreme perceptions in two regions of France, with one part of the population in favor of mosquito control regardless of the method and the other part against *Bti* larvicide spreading (6). For instance, in Camargue, 82% of the respondents were favorable to mosquito control. Additionally, the majority of the respondents living in areas where mosquito control had been implemented for 10 years would not want to return to a situation without mosquito control interventions. However, even the respondents who were the most favorable to mosquito control had some reservations; nearly 60% of all respondents underlined the potential damage to the ecosystem (6, 63). A Greek study analyzed residents' perception of SIT and highlighted high acceptance by most residents (22). Among the participants, 92.5% thought that SIT was a good idea and on an effectiveness scale of 0 to 5, 70% answered between 4 and 5. In addition, 79.5% of the respondents considered that this method had more advantages than chemical methods. They also expressed concerns regarding the chemicals used and their potential environmental and human health impacts. Moreover, they stated having used most of the recommended at-home mosquito control mechanical methods such as keeping doors and windows shut and eliminating standing water reservoirs (55).

Overall, it is clearly important to understand the public perception and beliefs about mosquito control. Another question that is rarely investigated in the KAP surveys and should be further developed is the perception of individuals' role in mosquito control. To the best of our knowledge, only one KAP survey has analyzed the perception of 126 respondents impact of their own mosquito avoidance behavior may have on mosquito proliferation in their home and neighborhoods (15). More than half of the respondents (53%) considered that removing standing water from containers and flowerpots, have some impact on the local mosquito population. Only 14% thought that their action had a very low impact, while 33% believed their impact very significant around their home and neighborhoods. Convincing people of the impact of their actions represent an educational opportunity to motivate population to contribute to mosquito control.

3.3.3 Attitudes toward awareness plans and effectiveness of citizen sciences

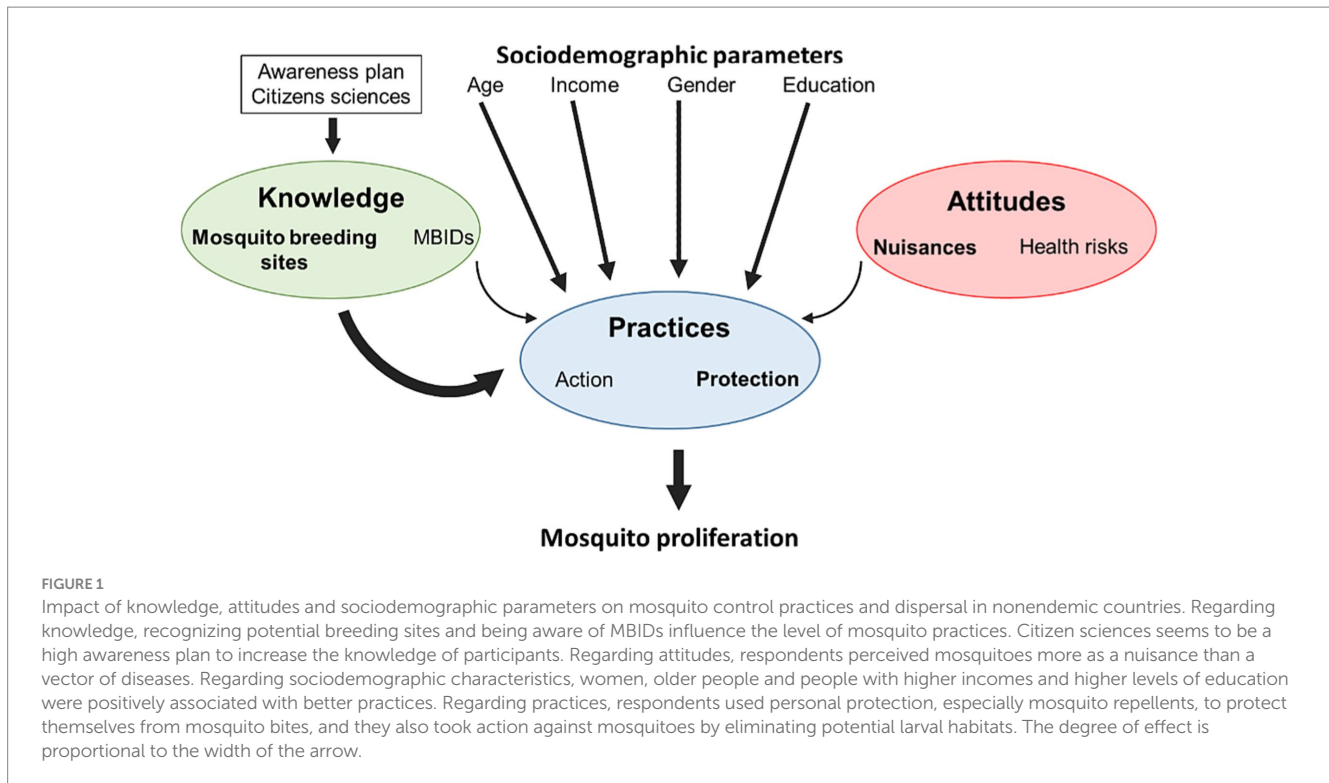
Another important factor that improves effectiveness of control and prevention measures is the willingness of the population to adapt their practices and routines. Awareness plans, information and education are necessary as a complement to vector control campaigns in order to limit mosquito proliferation thus vector-borne diseases. In Washington DC, scientists' interventions to control mosquitoes have

significantly reduced the number of breeding sites (41). According to a Greek study, raising awareness through printed educational materials alone has had no effect (20) or the opposite effect due to a decrease of MBID concern after the prevention campaign (64). Several studies highlighted that respondents perceived educational efforts by local authorities too low and asked for better strategies to enhance awareness about mosquito and MBIDs. For instance, Rampold et al. (55) revealed that all Florida respondents agreed that education regarding mosquito control efforts and their impact was needed (18, 55). Several studies also revealed that respondents identified local governments or public health organisms as responsible for this health issue (15, 18, 37). Despite this observation, people concerned with mosquitoes in their neighborhood may have other information channels. For instance, 73.2% of the nonprofessional gardeners in community gardens conducted their own research for information on Internet (18). The opposite was observed in Florida where most of the respondents had not research actively regarding mosquito control (55).

Citizen science is currently considered as a main driver to motivate and involve the public as citizen scientists. Volunteers are commonly involved in data collection, but can also be involved in initiating questions, designing projects, disseminating results, and interpreting data (UNEP 2019). With the aim to facilitate the public participation and collaboration in scientific research to increase scientific knowledge, citizen science could help for mosquito surveillance and mapping. Heym et al. (19) found that respondents that were involved in a citizen science project were able to recognize potential larval habitats and modified their practices regarding breeding site management (19). Some citizen science projects have already shown their effectiveness such as the "Mosquito Alert" project that aims to make an inventory, via the use of a smartphone app by volunteers, of places where of various mosquito species in Europe are observed. After validation and correction by entomologists, the collected information is used to create early alert for MBIDs risk through predictive models (65, 66).

4 Conclusion

This literature review sheds light on the disparities in knowledge, attitudes, and practices pertaining to mosquitoes and MBIDs in nonendemic regions. The understanding and behavior of the local populations have an impact on the proliferation of mosquitoes within urban areas. KAP questionnaires are commonly employed to evaluate the factors influencing both positive and negative behaviors, as well as the knowledge and attitudes concerning MBIDs. These surveys play a crucial role in identifying undesirable behaviors within the population and pinpointing gaps in awareness regarding diseases and vectors. Furthermore, they aid in the development of effective strategies that engage the community in vector control efforts. This review focused in particular on factors explaining what influences people's practices regarding mosquitoes, as raising awareness about preventive measures and protective behaviors is of primary importance to face the risks of vector-borne diseases. Different explanations were put forward in the surveys. Knowledge about mosquito breeding sites and awareness of mosquito's role in some MBIDs are key factors that explain more or less appropriate mosquito control practices on the individual level.



Moreover, socioeconomic and demographic status also explain the variability of observed practices (Figure 1). The reviewed studies revealed that a significant portion of survey participants possessed only partial knowledge of potential mosquito breeding sites. This is an information of utmost importance, as a very good knowledge of these breeding sites is crucial for individuals to adopt effective practices aimed at preventing the spread of mosquitoes. Another important result that we found in these studies is that there are varying levels of awareness and understanding regarding the infectious diseases transmitted by mosquitoes based on geographical locations. These variations are clearly dependent on the frequency and the scale of the sporadic outbreaks of MBIDs in the surveyed regions. Surveys conducted in continents with frequent outbreaks resulting from local transmissions of MBIDs, such as the Southern United States, consistently demonstrated a high level of knowledge. In contrast, in Europe, where MBIDs remain sporadic, and epidemic occurrences are largely reliant on imported cases, awareness regarding health risks associated with mosquitoes tended to be less widespread. Alongside these results on knowledge, several studies have also shown that most respondents in the surveys are more concerned about the negative impacts on their well-being than the related health risks. Despite a certain knowledge about MBIDs, the perceived risks of epidemic-prone diseases are generally rather moderate in nonendemic regions. We also noticed that multiple studies indicate that a strong understanding of potential mosquito larval habitats and heightened awareness of mosquito presence and MBIDs have a positive impact on the practices. Individuals who are both concerned and well-informed tend to adopt more appropriate measures in combating mosquitoes. There also seems to be a gender disparity, with female respondents that in general are better informed about mosquitoes and MBIDs, which tend to lead to the adoption of more effective

practices against mosquito proliferation. Many studies within this review analyze how knowledge and practices are influenced by the socioeconomic status of respondents, and the results show in particular that educational level seems to be a key factor that explains good mosquito knowledge and appropriate practices. This review has also highlighted a scarcity of KAP studies focusing on the population's awareness and perception of different kinds of mosquito control methods and their efficiency. To enhance local community engagement in mosquito control efforts in nonendemic regions, there is a pressing need for improved dissemination of information regarding existing control methods (both individual and control methods used by local authorities), coupled with an increase of the number of surveys addressing this topic. It is evident that further evaluation is necessary to identify sustainable or eco-friendly practices or measures against mosquito proliferation that could be readily embraced and adopted by the population.

All these findings show the crucial role of knowledge in promoting good practices, emphasizing the necessity for widespread awareness and information campaigns. Several studies indicated that respondents perceived local authorities' educational initiatives as insufficient and expressed the need for more effective strategies to enhance awareness regarding mosquitoes and MBIDs. However, there is uncertainty surrounding the definition of an effective awareness and information campaign, when it comes to content, medium and distribution method. Some of the reviewed studies revealed that under specific circumstances, informational and educational efforts may fail to achieve their intended outcomes and, in some cases, could even lead to unintended adverse effects on attitudes and practices concerning mosquitoes and MBIDs. Consequently, there is a great need for additional surveys that evaluate the impact of educational initiatives, information dissemination, and awareness campaigns.

Given the importance of the emerging health risk in nonendemic countries and regions due to mosquito proliferation, and in particular the tiger mosquito, this review of studies based on KAP surveys provide decision-makers with new information to better manage mosquito spreading and mosquito-borne diseases.

Author contributions

PD wrote the first draft of the manuscript. CA-L and CVM reviewed and edited the manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

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Pediatric central nervous system infections in the Amazon: clinical and laboratory profiles

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Background: Central nervous system (CNS) infections are important causes of mortality and morbidity in children, and they are related to severe problems such as hearing loss, neurological sequelae, and death. The objective was to describe clinical and laboratory exam profiles of children who were diagnosed with CNS infections.

Methods: We conducted a cross-sectional study based on medical records, which included pediatric patients aged from 3 months to 15 years, with a clinical suspicion of CNS infection between January 2014 to December 2019. The pathogens were confirmed in cerebrospinal fluid (CSF) samples using Gram staining, cell culture, molecular diagnostics (PCR and qPCR), and serology.

Results: Out of the 689 enrolled patients, 108 (15.6%) had laboratory-confirmed infections in CSF. The most common bacterial pathogens isolated from the culture were *Neisseria meningitidis* serogroup C in 19, *Streptococcus pneumoniae* in 11, and *Haemophilus influenzae* in seven samples. The viruses identified were *Enterovirus*, *Cytomegalovirus*, *Varicella-zoster virus*, *Epstein-Barr virus*, and *arbovirus*. No patient was found to be positive for *Herpes simplex virus 1 and 2*. Patients with viral infections showed altered levels of consciousness ($p = 0.001$) when compared to bacterial infections.

Conclusion: This study shows the presence of important vaccine-preventable pathogens, and different families of viruses causing CNS infections in the pediatric patients of Manaus.

KEYWORDS

cerebrospinal fluid, neurological infections, viral infection, adolescents, bacterial meningitis, Brazil

Introduction

Central nervous system (CNS) infections in children are considered a public health problem that causes high lethality rates, especially in developing countries (1). These infections may encompass some clinical conditions, such as meningitis, encephalitis, and meningoencephalitis, that require urgent attention and extensive investigative efforts. If CNS infection is not properly treated, it can have serious consequences in the pediatric population, leading to hearing loss, neurological sequelae, and death (2).

The epidemiology of CNS infections in children in developed countries is well documented, but in developing countries, such as Brazil, epidemiological and laboratory surveillance of these infections remains limited, leading to underestimation and underreporting of cases. Therefore, the incidence of CNS infections in developing countries is higher, with rates of 726 cases per 100,000 inhabitants, while in developed countries it is 11 per 100,000 inhabitants (3).

The main infection affecting the pediatric population is bacterial meningitis (BM) (4). The clinical and epidemiological characteristics of the infection are directly related to an interaction of socioeconomic, environmental, and infectious factors. Even though the introduction of conjugate vaccines has reduced the incidence and infant mortality rates due to BM, it remains lethal, especially in areas where vaccination coverage is limited (5). The main bacterial agents that can cause meningitis in children under 5 years of age are *Haemophilus influenzae* b, *Streptococcus pneumoniae*, and *Neisseria meningitidis* (6). In addition, Gram-negative bacteria of the Enterobacteriaceae family pose a risk to neonates (7).

CNS viral infections, including meningitis and encephalitis, contribute to the burden of pediatric neurological diseases (8). However, the burden of viral meningitis (VM) remains uncertain due to gaps in surveillance systems, especially in developing countries (4). Common viral pathogens associated with these conditions include viruses from the Picornaviridae, Herpesviridae, and Flaviviridae families (9).

Between 2014 and 2022, Brazil reported 127,215 cases of CNS infections in children with different etiologies. Among these, VM was the most prevalent with 58,872 of the cases. Although the numbers are alarming, epidemiological data on CNS infections in children in the Amazon region are scarce (10).

In this context, it is essential to understand the profile of neurological infections in the pediatric population. Therefore, active epidemiological and laboratory surveillance can contribute to the knowledge of circulating agents. Thus, this study aimed to describe the clinical and laboratory profiles of children from Manaus, Amazonas State, Brazil, who were diagnosed with CNS infections.

Methods

Study population

We conducted a cross-sectional study based on medical records. We studied pediatrics patients aged from 3 months to 15 years, admitted in 11 hospitals (S1 Appendix in Supplementary material) in Manaus from January 2014 to December 2019, with a clinical suspicion of CNS infection, who had presented with an acute neurological condition associated with a presence of one or more signs

or symptoms, such as neck stiffness, fever, rash, altered level of consciousness, muscle weakness. Lumbar puncture to get cerebrospinal fluid (CSF) sample was performed on all the patients on the admission day by the clinicians, and the CSF was forwarded for analysis at the Bacteriology laboratory at Fundação de Medicina Tropical Dr. Heitor Vieira Dourado (FMT-HVD). We identified patients using the laboratory database, we retrospectively identified 108 patients who had pathogens detected in CSF regardless of indication. We excluded patients who had undergone surgery, suffered any kind of trauma, or had CSF collected from an external ventricular shunt (EVS) or ventricle-peritoneal shunt (VPS).

Data collection and processing

Demographic, past medical history, admission history, examination, investigations, diagnosis, and management were obtained from electronic medical records. Laboratory tests were collected at the laboratory database. Children over 1 year with symptoms like fever, headache, vomiting, neck stiffness, seizures, or rash were considered as CNS infection cases. In children under 1 year, we also considered irritability, persistent crying, and the existence of fontanel bulging (11).

Case definition

Confirmed CNS infection were diagnosed based on the detection of bacteria pathogens in the CSF culture, latex agglutination test, as well as the presence of bacteria following Gram staining. Confirmed cases of viral infection were characterized by the etiological agent's confirmation through polymerase chain reaction (PCR) and the presence of dengue and/or Zika virus-specific IgM antibodies in the CSF.

Laboratory analysis

All cases were investigated through CSF analysis according to established laboratory protocols. Biochemical, cytological, and microbiological analyses of CSF were performed according to laboratory standards. This included the evaluation of CSF glucose, protein, and lactate levels, global and differential cell count, and staining with Gram, Ziehl-Neelsen and Nankin ink. Additionally, microbiological culture were performed.

Molecular diagnosis

For the viral investigation, 200 uL of CSF was extracted using the ReliaPrep™ (Promega) kit, following the manufacturer's instructions. PCR was employed to detect the following viruses: herpes simplex virus 1 and 2 (HSV-1 and 2), varicella zoster virus (VZV), Epstein-Barr virus (EBV), cytomegalovirus (CMV) and parvovirus B-19 (PVB-19). Reverse transcriptase PCR (RT-PCR) was used to investigate the enterovirus, dengue virus (DENV 1–4) and Zika virus, according to the protocols described by Brazil Ministry of Health (12), Lanciotti et al. (13, 14), Markoulatos et al. (15), and Oberste et al. (16). Each reaction contained CSF samples, a positive control, a negative control

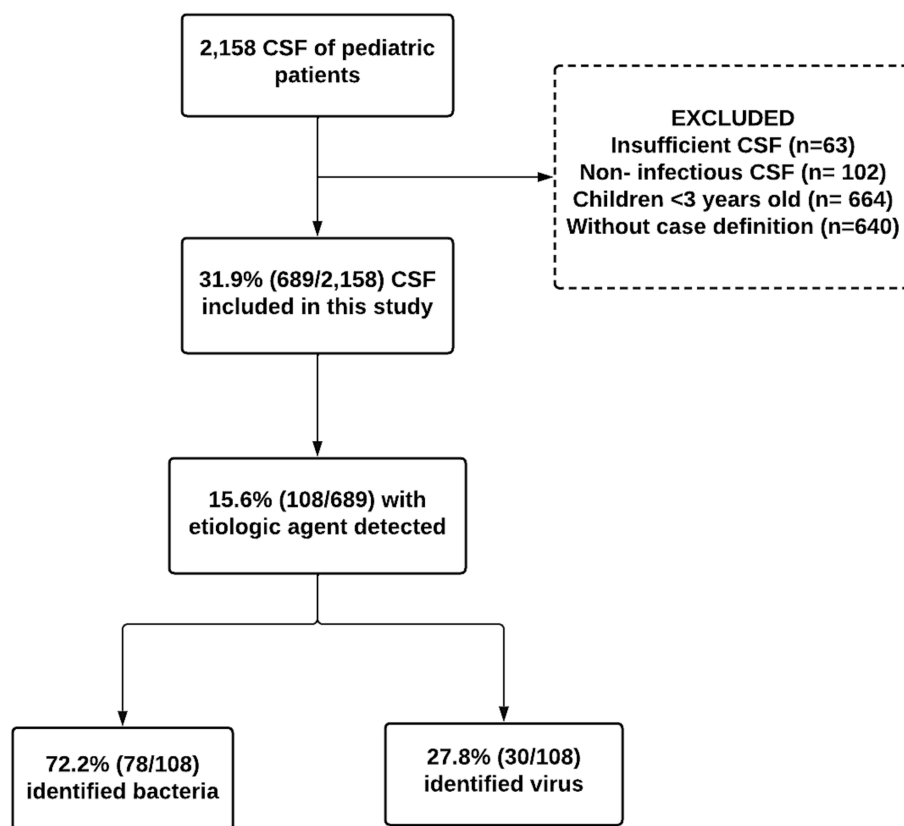


FIGURE 1

Flowchart of capture, inclusion, and exclusion according to the steps of this 108 research to compose the final sample of pediatric patients with etiological agents detected in the cerebrospinal fluid from Manaus, Amazonas, 2014–2019.

(water), and an internal control consisting of β -actin and RNase P (RP) amplification to validate the presence of nucleic acid.

Serological diagnosis

The CSF samples (diluted 1:5) were analyzed for presence of IgM antibodies to dengue virus (17), and IgM antibodies to Zika virus were measured using a recommended capture via enzyme-linked immunosorbent assays (ELISA) based on the Centers for Disease Control and Prevention (CDC) (CDC Fort Collins, CO, United States) (18).

Data analysis

We analyzed data statistically in BioEstat v.5.0. Categorical variables were presented as absolute and relative frequencies, while continuous variables were expressed as means and medians, considering standard deviation (SD) and range, respectively. To compare bacterial and viral groups, was used the chi-square test and odds-ratio comparisons, considering a value of p of 0.05 as statistically significant.

Software QGIS® 3.16.9-Hannover version for Windows® 10, was used to construct density maps to demonstrate the spatial distribution of cases with etiological agents detected in the CSF.

Ethics statement

This study was approved by the Ethics Review Board of the FMT-HVD under (CAAE: 12775119.5.0000.0005), following the guidelines and standards established in Resolution 466/12, of the National Health Council of the Brazilian Ministry of Health. A waiver of informed consent was granted due to the retrospective nature of the study, and patient-identifying data was anonymized.

Results

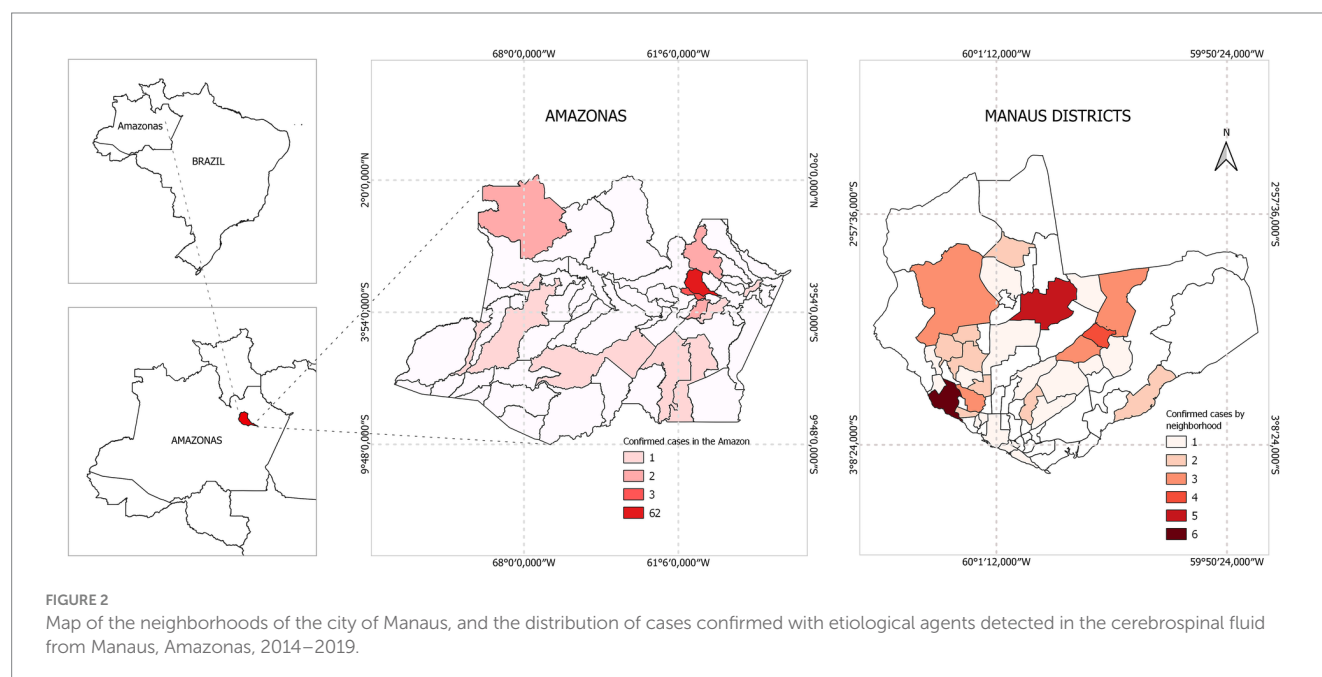
During the study period, 2,158 CSF samples from pediatric patients with suspected CNS infection were evaluated. Out of these, 1,469 samples were excluded for the following reasons: (1) insufficient samples quantity ($n=63$); (2) cases involving non-infectious CSF, external ventricular shunt, or ventricle-peritoneal shunt, and trauma ($n=102$); (3) children under 3 months of age without clinical information ($n=664$); (4) patient not meeting the defined case criteria ($n=640$). As a result, 689 samples were included in our analysis (Figure 1).

Demographics

Based on our case definition, 108 (15.6%) pediatric patients met our criteria, the mean age was 8 years [SD: 4.8]. Among them, 61.1%

TABLE 1 Baseline characteristics of patients with a central nervous system infection and laboratory-confirmed viral and bacterial infection in cerebrospinal fluid (2014–2019).

Characteristics	Total	Confirmed bacterial infection	Confirmed viral infection
	<i>n</i> = 108 (%)	<i>n</i> = 78 (%)	<i>n</i> = 30 (%)
Male	66 (61.1)	49 (62.8)	19 (63.3)
Female	42 (38.9)	29 (37.2)	13 (33.7)
Age group			
< 1 year	20 (18.0)	15 (19.1)	5 (16.6)
1–4 years	28 (26.0)	20 (25.6)	8 (26.6)
5–9 years	26 (24.0)	18 (23.0)	7 (23.5)
10–15 years	35 (32.0)	25 (32.3)	10 (33.3)



of the participants were male, and the most prevalent age group was 10 to 15 years (Table 1). Most of the patients (78.5%) were resident in urban area of Manaus, while 21.5% came from other municipalities of the Amazonas state (S1 Appendix in Supplementary material; Figure 2).

Clinical and laboratory data

Forty-five percent of the patients required hospitalization, with a median hospital stay of 9 days [range: 1–40 days]. Thirteen children required intensive care, and the average length of stay in the intensive care unit was nine days, which ranged from 1 to 17 days. Furthermore, 39.8% (43/108) of the patients had received prior treatment, with ceftriaxone and penicillin being the most administered antibiotics. Of the patients with a viral infection, nine were treated with both antibiotics and acyclovir. Common symptoms in both bacterial and viral infections included fever, vomiting, headache, and neck stiffness. Notably, altered levels of consciousness were significantly more prevalent (33.3%) in viral infections (Table 2). These data were confirmed by univariate analysis which showed that children with a

viral infection (OR: 7.23; CI 95%: 2.23–23.8; $p = 0.0009$) were more likely to have an altered level of consciousness. Death was only recorded in three cases of bacterial infection.

CSF diagnosis

Bacterial infections

Of the children with confirmed causes of meningitis, 72.2% (78/108) had BM. The CSF culture was positive in 57 patients (73%), and Gram staining identified pathogens in 27% of the CSF samples. Among the bacterial isolates, thirty-seven (64.9%) were vaccine-preventable. *N. meningitidis* serogroup C accounted for 24% of cases, primarily affecting children aged 10 to 15. *S. pneumoniae* constituted 14% of cases, primarily in children aged 1 to 4. *H. influenzae* type b represented 9% of cases, primarily affecting children under 1 year of age. In addition, *Staphylococcus* sp. and Enterobacterales were isolated from 11 and 7 CSF samples, respectively, distributed among different species, including *Serratia* sp. (3 cases), *Pseudomonas* sp. (2 cases), and *Klebsiella pneumoniae*, *Proteus vulgaris*, *P. mirabilis*, and *Acinetobacter* sp. (1 case each) (see Figure 3).

TABLE 2 Signs and symptoms of patients with a central nervous system infection and laboratory-confirmed viral and bacterial infection in cerebrospinal fluid 2014–2019.

Symptoms/Variables	Total	Bacterial	viral	p^*
	$n = 108$ (%)	$n = 78$ (%)	$n = 30$ (%)	
Fever	58 (53.7)	39 (50.0)	19 (63.3)	0.300
Vomiting	45 (41.6)	34 (43.5)	11 (36.6)	0.660
Headache	39 (36.1)	25 (32.0)	13 (43.3)	0.380
Neck stiffness	19 (17.5)	14 (17.9)	5 (16.6)	0.900
Altered level of consciousness	15 (13.8)	5 (6.4)	10 (33.3)	0.001
Seizures	9 (8.3)	4 (5.1)	5 (16.6)	0.060

*Chi-square test.

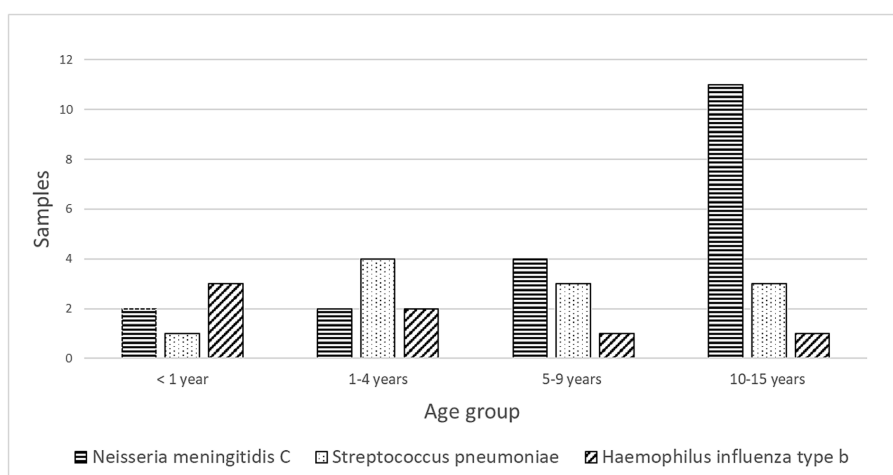


FIGURE 3

Distribution of Vaccine-preventable bacterial agents isolated in cerebrospinal fluid samples by age group of pediatric patients from Manaus, Amazonas, 2014–2019.

Viral infections

Different diagnostic methods identified viral agents in 27.8% (30/108) of the CSF samples, using both molecular and serological techniques (Table 3). Enteroviruses were the primary viral agents detected in 43.3% of the children, occurring in conjunction with meningitis in six patients and encephalitis in five. Only one enterovirus case was initially treated as unspecified BM with prior ceftriaxone treatment. In this patient, CSF protein, glucose, and lactate levels were normal, but cellularity was elevated (300 cells/mm³). Seven children with neurological symptoms were diagnosed with viruses from the Herpesviridae family. Two cases of encephalitis were caused by VZV and EBV, four cases of viral meningitis were caused by VZV, CMV and one case of meningoencephalitis was caused by EBV. In this group, the CSF was clear in four patients and turbid in three, with no evidence of a traumatic puncture. Median CSF parameters were 192 cells/mm³ (range: 0–906 cells/mm³), lymphocytes 98% (range: 5–100), protein 39 mg/dL (range: 0–1,394 mg/dL) and glucose 59 mg/dL (range: 28–83 mg/dL).

PVB-19 was detected in the CSF of 4 (13.3%) patients and was associated with one case of enterovirus. Two patients suffered status epilepticus. Three patients had anemia with hemoglobin levels below

TABLE 3 Frequency of viral agents identified in cerebrospinal fluid samples from pediatric patients from Manaus, Brazil (2014–2019).

Viral agents	N°	%
Enterovirus	13	45.0
Varicella zoster virus	2	7.0
Epstein–Barr virus	2	7.0
Cytomegalovirus	1	3.0
Dengue fever	4	13.0
Zika virus	3	10.0
Parvovirus B19	4	16.0
Total	30	100

N simple absolute frequency; %: relative frequency.

10g/dL. Five samples tested positive for dengue IgM in ELISA in serum and paired CSF. Two patients were diagnosed with ZIKV via qPCR. Dengue virus was associated with three cases of meningitis and two cases of encephalitis. Notably, one child with ascending acute flaccid paralysis and areflexia required neurological rehabilitation due to severe symptoms.

TABLE 4 Cellular and biochemical parameters of cerebrospinal fluid samples from pediatric patients with neurological infection diagnosed at a reference laboratory in Manaus, Brazil, from January 2014 to December 2019.

Biochemical parameters	Bacterial infection (Md)	Viral infection (Md)	<i>p</i>
Cytometry (Cell/mm ³)	850 (10–22,000)	95.5 (0–848)	<0.05
Glucose (mg/dL)	10 (10–91)	58 (17–83)	<0.05
Proteins (mg/dL)	193 (0–5,379)	62.5 (0–3,830)	<0.05
Lactate (mmol/L)	4.5 (0–9.2)	5.0 (0–100)	<0.05

Md = median.

CSF profile

Comparing bacterial and viral infections, we observed significant differences in CSF cellular counts and biochemical profiles ($p < 0.05$). Bacterial infections displayed an inflamed CSF profile with high cellular counts (median 850 cells/mm³, range 10–2,000), predominantly polymorphonuclear, elevated protein levels (median 193 mg/dL), and decreased glucose levels (median 10 mg/dL). In contrast, viral infections showed a moderately altered CSF profile, with a median cellular count of 95.5 cells/mm³, mainly lymphocytes (8–906), a median protein of 62.5 mg/dL, and glucose levels within the reference range (58 mg/dL) (Table 4).

Discussion

In this hospital-based study, we present data on the etiological agents responsible for CNS infections in pediatric patients hospitalized in Manaus, the capital of the state of Amazonas. Our findings reveal that 15.6% of the pediatric patients were diagnosed with a CNS infection, with *N. meningitidis* serogroup C being the most common bacterial pathogen, followed by *S. pneumoniae*. Among viral pathogens, enterovirus and herpesvirus were prevalent.

We observed that bacterial identification through Gram staining was possible in only 27% of cases, likely due to prior antibiotic use before hospital admission. This underscores the importance of combining molecular diagnostic methods with traditional techniques such as Gram staining and culture to enhance the identification of bacterial pathogens causing CNS infections.

While the use of molecular diagnosis improved the detection of viral pathogens in our study, a low detection rate was noted, consistent with findings in other studies (19). This may be attributed to prior medication usage, which might have eliminated the agent, resulting in undetectable nucleic acid, or due to infections by pathogens not covered by local diagnostic panels.

Our study also supports existing literature indicating that male children are more susceptible to neurological infections, with 61.1% of the children in our study being boys (20).

Clinical signs and symptoms for bacterial and viral infections overlapped, making it crucial to consider both clinical and laboratory findings for accurate diagnosis. Symptoms such as fever, vomiting, and headache were common in both types of infections, while altered consciousness was more pronounced in viral infections. Our findings are in line with previous reports attributing altered consciousness to viral etiologies (21, 22).

Vaccination has altered the epidemiological profile of BM over the past 25 years, reducing the prevalence of vaccine-preventable bacterial infections in pediatric patients. In the 1980s, pathogens like

H. influenzae type b, *N. meningitidis*, and *S. pneumoniae* were responsible for over 80% of childhood bacterial meningitis cases (23). The incidence of this condition varies by geographic region and is notably higher among pediatric populations (24). Recent data from the National Immunization Program (NIP) in Amazonas reveals vaccination coverage rates for meningococcal C conjugate, pneumococcal, and triple bacterial vaccines for children under 1 year falling below 80%. In our study, we found that 72.2% of CNS infections were bacterial in origin, which aligns with the existing literature's emphasis on the prevalence of vaccine-preventable bacterial infections in pediatric patients (25). Despite vaccination programs, our findings indicate ongoing circulation of vaccine-preventable bacteria causing CNS infections, highlighting insufficient vaccination coverage in the region, and contributing to the rise in identified cases. These findings underscore the importance of ongoing surveillance for nervous system infections and contribute to future assessments, the development of new diagnostic tests, and our understanding of the impact of vaccination in the pediatric population.

However, other bacteria, such as *Escherichia coli* and *K. pneumoniae*, have emerged as causes of BM, raising concerns about antimicrobial resistance (26, 27). Early antibiotic therapy is vital for treating CNS infections, and broad-spectrum cephalosporins are recommended until the causative agent and its susceptibility are determined (28). In our study, ceftriaxone was the most used antibiotic.

Molecular methods have advanced the diagnosis of neurological infections, increasing the detection of viral pathogens in CSF. Among the main etiological agents related to CNS infections are the enteroviruses, followed by viruses of the family Herpesviridae and the dengue virus (29). Enteroviruses are responsible for the majority cases of CNS infections, especially among the pediatric population (30). Although EV infections are usually asymptomatic, they can also be associated with a variety of clinical manifestations and severe CNS syndromes, such as encephalitis, meningitis, and acute flaccid paralysis (31). Our data indicates that Enteroviruses were the most prevalent viral pathogens, often associated with meningitis and encephalitis, which is consistent with previous studies (32, 33).

Antimicrobial treatments in cases of CNS infections are, in general, crucial for improving clinical outcomes, but their empirical application plays a crucial role in the evolution of resistance genes (34). In our study, we found one case of enterovirus meningitis, which was treated with antibiotic. Therefore, we emphasize the importance of a differential, rapid and assertive diagnosis in order to reduce the exposure of patients to unnecessary antimicrobial therapy.

PVB-19 has been linked to various syndromes, including erythematous or asymptomatic febrile infections (35, 36). However, the neurological manifestations associated with PVB-19 have not been extensively investigated. In our study, we detected PVB-19 in the CSF

of four children without skin manifestations. These children did not exhibit any signs of rash, but three of them had anemia and aplastic anemia. It is noteworthy that neurological PVB-19 infections without skin involvement have been well-documented in the literature (37, 38). Furthermore, it has also been suggested that this virus may be associated with pancytopenia and erythrocyte plasticity (39). With the growing number of reports highlighting neurological manifestations in regions where PVB19 is prevalent (40), it is crucial to emphasize the importance of considering this virus in investigations of neurological diseases.

Herpesviridae is a substantial family of DNA viruses that can potentially induce neurological diseases either during the primary infection or upon reactivation in immunosuppressive conditions (41). In our study, we identified herpesviruses, including VZV, CMV, and EBV, in 17% of CSF samples. These viruses were associated with a range of neurological manifestations such as meningitis, encephalitis, and meningoencephalitis. These findings align with those of Imamba and colleagues (42), who observed a similar pattern of herpesvirus-related meningitis and encephalitis as predominant conditions in children admitted to a hospital in Zambia.

Arboviruses, such as including dengue, Zika and chikungunya, are commonly found in Brazil, and are emerging pathogens known to cause both febrile and neurological syndromes in the general population (43). Consequently, the differential diagnosis of neurological syndromes has become increasingly vital in regions where these viruses are endemic (44). While Flaviviruses are typically associated with acute febrile infections characterized by skin rashes, they can also trigger neuroinflammation, leading to neurological disorders like meningitis, encephalitis, and meningoencephalitis (45). In our study, we identified Flaviviruses in seven cases (23%) of the suspected CNS viral infection. Notably, DENV was associated with cases of meningitis and encephalitis in pediatric patients. Our findings are consistent with those of Marinho et al. (46), who also reported a low prevalence of dengue in CNS samples from the pediatric population. In arbovirus-endemic regions like the Amazon, it is important laboratory surveillance for monitoring the diagnosis of encephalitogenic viruses.

The altered CSF profile provides evidence of a bacterial infection through the presence of a pleocytosis characterized by a predominance of neutrophils, low glucose levels, and high-protein levels (47). Viral infections, on the other hand, exhibit distinct presentations: mononuclear pleocytosis, moderate protein elevations, and normal glucose levels (48). Notably, our study reveals statistically significant differences in cellular and biochemical results within the CSF between bacterial and viral infections, indicating variations in cellularity, protein, and glucose levels. These findings align with the research conducted by Nazir et al. (49), emphasizing the greater alterations in CSF profiles for bacterial infections and underscoring the importance of measuring lactate levels in CSF as a crucial marker for distinguishing between bacterial and viral infections.

CSF examinations, being cost-effective and readily accessible, play a pivotal role in assessing both bacterial and viral infections. Particularly in resource-limited regions like the Amazon, where laboratory infrastructure is limited, reliance on field-testing is common. Establishing a marker such as lactate has the potential to significantly enhance clinical management.

This study has limitations due to a lack of information on risk factors and selection bias. Data analysis based on disease severity and

outcome for recovery or death was not possible due to this being a retrospective analysis of laboratory data and medical records. Despite the small number of samples between bacterial and viral infection groups, we cannot rule out the lack of power in comparisons between signs and symptoms. We were unable to obtain information on antibiotic therapy for all the patients and their treatment period. In addition, it was impossible to retrieve information about the vaccination status of children. As the bacteriology laboratory of FMT-HVD analyzed samples that were sent by other services, the total number of infections in the CNS of patients in the Manaus city may have been underestimated.

In conclusion, our study provides valuable insights into the frequency and etiology of CNS infections in pediatric patients in Manaus. This information can improve future diagnostic approaches, the impact of vaccination, and public health planning to prevent nervous system infections in this population. Despite vaccination efforts, vaccine-preventable bacteria continue to circulate, emphasizing the need for ongoing monitoring and prevention strategies.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Ethics Review Board of the Fundação de Medicina Tropical Heitor Vieira Dourado. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from primarily isolated as part of your previous study for which Ethical Approval was obtained. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

EM: Conceptualization, Investigation, Writing – original draft, Writing – review & editing. EF: Formal analysis, Writing – review & editing. CB: Investigation, Writing – review & editing. SM: Investigation, Methodology, Writing – review & editing. JC: Data curation, Formal analysis, Writing – review & editing. SP: Investigation, Writing – review & editing. RM: Investigation, Supervision, Visualization, Writing – review & editing. VSi: Investigation, Methodology, Writing – review & editing. YM: Investigation, Writing – review & editing. MR: Supervision, Validation, Writing – review & editing. SS-G: Supervision, Writing – review & editing. VSa: Supervision, Writing – review & editing. Data curation, Formal analysis. DC: Writing – review & editing, Supervision, Validation. PF: Methodology, Writing – review & editing, Investigation. LR: Supervision, Writing – review & editing, Methodology. LF: Funding acquisition, Supervision, Writing – review & editing, Validation. MB: Conceptualization,

Funding acquisition, 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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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2023.1329091/full#supplementary-material>

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Inequalities in health outcomes of SARS-CoV-2 infection by migration status in Barcelona, Spain

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Background: Migrants are a vulnerable population at risk of worse health outcomes due to legal status, language barriers, and socioeconomic and cultural factors. Considering the conflicting literature on the subject, it is important to further explore the extent and nature of these inequalities.

Objective: The aim of this study is to compare health outcomes associated with SARS-CoV-2 infection between Spanish native and migrant population living in Barcelona.

Methods: Observational retrospective cohort study including all adult cases of SARS-CoV-2 infection who visited a tertiary hospital in Barcelona between the 1st March 2020 and the 31st March 2022. We established the following five health outcomes: the presence of symptomatology, hospitalisation, intensive care unit admission, use of mechanical ventilation, and in-hospital 30-day mortality (IHM). Using Spanish natives as a reference, Odds Ratios (OR) with 95% confidence interval (95%CI) were calculated for migrants by multivariate logistic regression and adjusted by sociodemographic and clinical factors.

Results: Of 11,589 patients (46.8% females), 3,914 were born outside of Spain, although 34.8% of them had legal citizenship. Most migrants were born in the Americas Region (20.3%), followed by other countries in Europe (17.2%). Migrants were younger than natives (median 43 [IQR 33–55] years vs. 65 [49–78] years) and had a higher socioeconomic privation index, less comorbidities, and fewer vaccine doses. Adjusted models showed migrants were more likely to report SARS-CoV-2 symptomatology with an adjusted OR of 1.36 (95%CI 1.20–1.54), and more likely to be hospitalised (OR 1.11 [IC95% 1.00–1.23], $p < 0.05$), but less likely to experience IHM (OR 0.67 [IC95% 0.47–0.93], $p < 0.05$).

Conclusion: Characteristics of migrant and native population differ greatly, which could be translated into different needs and health priorities. Native population had higher odds of IHM, but migrants were more likely to present to care symptomatic and to be hospitalised. This could suggest disparities in healthcare access for migrant population. More research on health disparities beyond SARS-CoV-2 in migrant populations is necessary to identify gaps in healthcare access and health literacy.

KEYWORDS

SARS-CoV-2, population health, syndemic, emigrants and immigrants, health inequities, Spain

1 Introduction

The SARS-CoV-2 pandemic has been one of the greatest public health challenges in the era of globalisation. Its impact goes beyond health and across borders, affecting the economy, employment, education, and social interactions. The pandemic has also made more evident than ever before important inequalities through disproportionate health effects on some populations. The older adult, high-risk workers, people living in poverty, and displaced communities are among those most affected (1, 2).

Health inequalities have been harder to tackle during the pandemic due to its continuous detrimental effect on the social determinants of already vulnerable groups (3). This has given way to a syndemic, with the SARS-CoV-2 crisis converging with other adverse conditions to produce worse health outcomes in certain population groups, such as migrant communities (4). Migration and the displacement of people is one of the global priority challenges. The United Nations (UN) reported more than 244 millions of international migrants, including more than 20 million refugees (4).

During the different stages of the migratory process, people face precarious circumstances, such as food insecurity, poverty, violence, and issues with legalisation of their migratory status, that can gravely affect their physical and mental health (4). Migration is an important social determinant by itself, but it also interacts with other factor such as gender, labour, accommodation conditions, socioeconomic status, or ethnicity, to exacerbate health vulnerability, whether it is biological or due to health literacy, access to healthcare, or conflictive relations with state institutions. This could translate into worse health outcomes even though migrant population tends to be younger and healthier than autochthonous population (4, 5).

Despite being a developed country with universal access to quality healthcare, Spain was one of the first and most affected European countries to face the SARS-CoV-2 pandemic. According to the last situation update by the Spanish Ministry of Health, until the end of June, 2023, there have been almost 14 million reported cases of SARS-CoV-2 infection in Spain, as well as more than 121,000 deaths (6). Catalonia has been one of the Autonomous Communities most affected by the pandemic while having a significant proportion of migrant population. In Barcelona, Catalonia's largest and most cosmopolitan city, it is estimated that more than 350,000 people (20% of its population) were born outside of Spain, mainly in Latin America, Morocco, China, Pakistan, and other European countries (7).

European studies have described heterogeneous results; while some found no significant differences in health outcomes due to SARS-CoV-2 infection between native and immigrant population, others reported higher risk of infection and hospitalisation rates (8–19). There have been a few studies from Spain, but most were performed at the beginning of the pandemic and had also conflicting results; some concluded there were no significant differences in ICU admission or mortality, while others stated that some groups of immigrants had higher risk of ICU admission (20–24).

This inconsistency in literature, combined with a systematic neglect in registering variables such as country of birth or ethnicity, hinders adequate analysis of the impact migration status has on various health outcomes. Analysis of this data can enlighten us to better target policies and interventions to reinforce assistance during health crisis in an equitable way. The main objective of this study was

to compare health outcomes associated with SARS-CoV-2 infection between Spanish native and migrant population living in Barcelona.

2 Materials and methods

2.1 Study design and setting

This was an observational retrospective cohort study of all SARS-CoV-2 infections in adult patients who were visited at Hospital Clinic Barcelona, a large university tertiary hospital, between the 1st of March of 2020 and the 31st of March of 2022. The patients were seen at the Emergency Department, in outpatient clinics, during hospitalisation (hospital-onset infections), or they could have been diagnosed with SARS-CoV-2 incidentally, as part of pre-operative tests. Therefore, not all patients were symptomatic, and a SARS-CoV-2 infection was not necessarily the reason why patients were visited.

Infection by SARS-CoV-2 was confirmed either by Polymerase Chain Reaction or by Rapid Antigen Test, even if it was a self-administered test with a positive result reported by the patient. We excluded patients under 18 years of age, hospital workers, and patients whose relevant information could not be retrieved. Repeated positive SARS-CoV-2 tests in the same patient were only considered as reinfection if 90 days or more had passed since the first positive test. Since patients could have had two or more SARS-CoV-2 infections during the study's period, each infection episode is considered an individual case. Henceforth, we use the terms “patients” and “cases” indifferently, although we are referring to a SARS-CoV-2 infection episode.

2.2 Data collection

Data was obtained from the routine surveillance system database of the Preventive Medicine and Epidemiology Department. This database received real-time information about positive SARS-CoV-2 test results from the Department of Microbiology and manual registration of SARS-CoV-2 infection by healthcare teams in case the patient reported a positive ART or an external PCR result. Complementary information about cases was completed by the surveillance team using data from hospital records. We included information about sociodemographic characteristics (age, sex, Basic Health Area (BHA) where the patient lives, country of birth, and nationality), relevant medical history and comorbidities, SARS-CoV-2 vaccination, symptomatology at the time of the visit, and health outcomes such as duration of hospitalisation, Intensive Care Unit (ICU) admission, mechanical ventilation and mortality.

2.3 Variable definitions

We considered five unfavourable health outcomes associated with SARS-CoV-2 infection among patients of the cohort. These included the presence of symptomatology at the time of hospital visit, hospitalisation, in-hospital 30-day mortality, ICU admission, and use of mechanical ventilation (MV) during SARS-CoV-2 infection. ICU admission and MV information could only be obtained for patients hospitalised at our centre. In-hospital 30-day all-cause mortality was

considered for patients with SARS-CoV-2 who had been hospitalised 30 days or less at the time of in-hospital death or if they had been diagnosed with a SARS-CoV-2 infection in the 30 days before their death, if it was a hospital-onset infection. Although a distinction is made between orotracheal intubation (OTI) and non-invasive mechanical ventilation (NIMV), for the multivariate logistic regression of MV use, we have dichotomised the variable and considered both OTI and NIMV as MV use.

Our exposure of interest was migration status as a dichotomous variable (migrant vs. native Spanish). We have defined migrant status as being born outside of Spain (being a non-native of Spain) irrespective of legal nationality. Native Spanish patients were considered as a reference category. We also analysed health outcomes disaggregating by region of birth. We decided to use the World Health Organization classification of the world's regions due to its widespread use and recognition: African Region, Region of the Americas, South-East Asian Region, European Region, Eastern Mediterranean Region, and Western Pacific Region.

We considered the following covariates: socio-demographic and geographical factors, SARS-CoV-2 vaccination, epidemiological period of contagion, presence of symptoms, a constructed comorbidity score and seven individual comorbidities. Age was described as a continuous variable, but we also categorised it into quartiles (18–40, 41–57, 58–73, and >73 years old). The Catalanian socioeconomic deprivation index (SDI) based on the BHA of residence used in this study was created by the Healthcare Quality and Evaluation Agency of Catalonia (AQuAS) (25). There was an important proportion of missing data which we could not entirely attribute to randomness, which is why we decided not to adjust the models for these variables.

We selected seven comorbidity groups that may be associated with the risk of SARS-CoV-2 infection and with higher mortality due to SARS-CoV-2: cardiovascular diseases (CVD), cardiovascular risk factors (CVRF) (obesity, dyslipidaemia, hypertension, alcohol consumption and/or smoking), diabetes mellitus (DM), chronic respiratory diseases (CRD), immunosuppression (solid organ transplant, hematopoietic transplant, cancer, and other types of immunosuppression), chronic kidney disease (CKD), and hepatic disease. As information on comorbidities was not sufficiently specific to use a known comorbidity index, we constructed our own comorbidity score by adding the number of each of these groups of comorbidities (26, 27). The categories of this score are 0, 1, 2 and ≥ 3 comorbidities. For the vaccination variable, we considered the person fully vaccinated if two or more doses were registered and the last dose was administered at least 14 days before the SARS-CoV-2 infection diagnosis.

We considered six pandemic epidemiological periods consistent with infection dynamics described in Spain: first period from 1st March 2020 to 31st May 2020; second period from 1st June 2020 to 30th November 2020; third period from 1st December 2020 to 28th February 2021; fourth period from 1st March 2021 to 30th June 2021; fifth period from 1st July 2021 to 15th October 2021; sixth period from 16th October 2021 to 31st March 2022.

2.4 Statistical analysis

We carried out a descriptive analysis of all relevant sociodemographic variables for the entire population and for the

native and migrant groups. Continuous variables were summarised as means with standard deviation or medians with interquartile ranges (IQR) if not normally distributed. Categorical variables were described as absolute frequency (n) and relative frequency (in percentages). For the comparative analysis between the native and the migrant group, we used either Chi² test or Fisher test for qualitative variables, and t-student or Mann–Whitney U test for quantitative variables.

Bivariate and multivariate analysis was performed with a subset of complete cases in the variables that we would theoretically fit into the model. We used logistic regression models only adjusted by sex and age quartile to obtain minimally adjusted Odds Ratios (mOR) and their 95% confidence intervals (95% CI) for each of the five outcomes. Multivariate logistic regression models were also performed to obtain a fully adjusted Odds Ratios (aOR) with 95% CI. Relevant covariates additionally to sex and age quartiles were first selected based on theoretical hypotheses; then the model was further refined using backward stepwise selection, considering the significance of each variable and the performance of Likelihood Ratio Tests (LRT) and Akaike's criterion (AICc) values. The R-project® statistical program in its 4.1 version was used for data processing and analysis. A value of $p < 0.05$ was statistically significant (28). Microsoft Excel® was also used for the odds ratio Forest plots.

3 Results

3.1 Descriptive analysis of the population

We included 11,589 SARS-CoV-2 infection cases between the 1st of March 2020 and the 31st of March 2022. A total of 3,914 (33.8%) SARS-CoV-2 infections were diagnosed in migrants, but 34% (Figure 1C) of them had Spanish nationality and 49.4% had a long-term or short-term visa, with the remaining 613 migrant patients being either tourists or undocumented migrants. Additionally, another 531 migrants (14%) had a European Union/European Economic Area (EU/EEA) nationality (Figure 1C). Most migrant patients were born in the Americas Region (60%), followed by the European Region (18%) (Figure 1B). A more detailed distribution of migrants by country of birth, WHO Region of birth, type of nationality, and place of residence can be found in Figures 1A–D.

The median of age was 57 years [IQR 40–73] and 46.8% were female. The descriptive analysis of all variables in native and migrant populations can be found in Table 1. A higher percentage of migrants compared to Spanish were female (49.6% vs. 44.9%). Migrants were significantly younger than Spanish with a median of 43 years [IQR 33–55] vs. 65 years [IQR 49–78]. Nearly 80% of migrants were younger than 58 years of age, compared to 36.7% of Spanish in those age categories.

The Catalanian SDI associated to the BHA of residence was significantly higher (more deprivation) for migrants (median of 25.6 [IQR 13.3–40.1] vs. 19.25 [IQR 10.7–35.4]), although there was an important proportion of missing information (18.4%). At the time of the hospital visit, 97.0% of native patients had been registered with a general practitioner (GP) in Catalunya and had a sanitary card that allowed them access to healthcare services without charge; however, only 89.4% of migrant population had been registered.

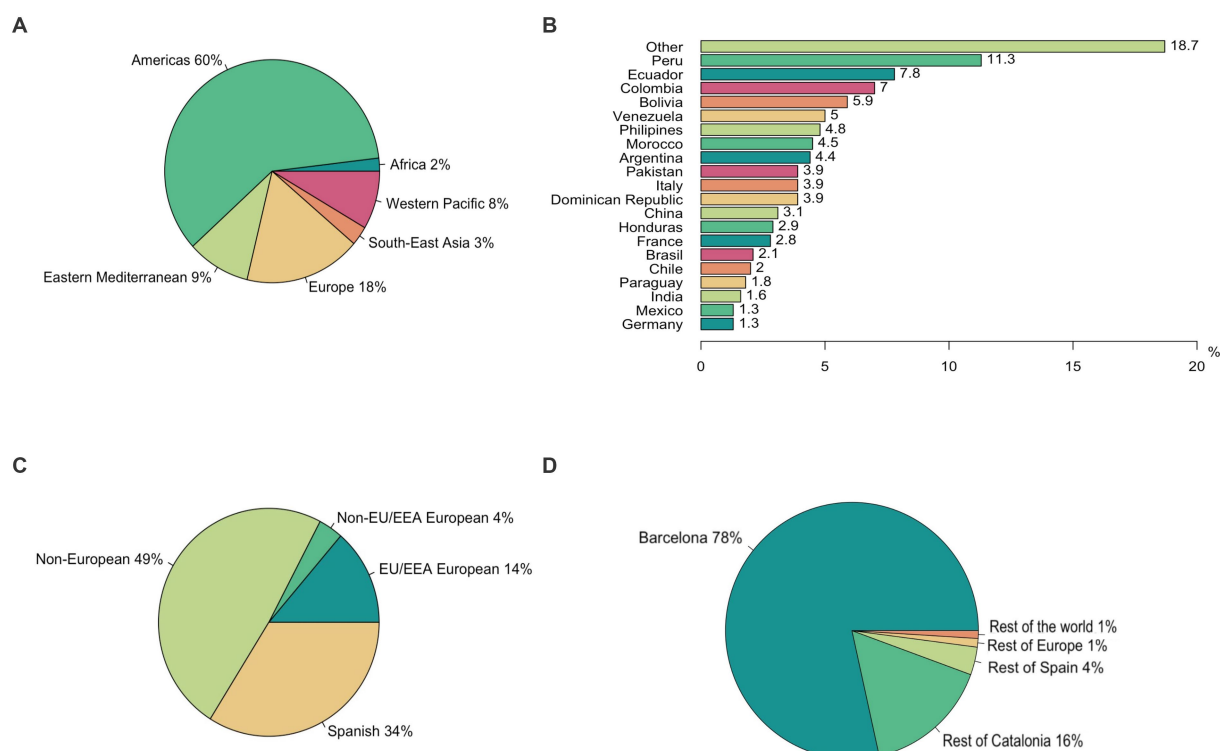


FIGURE 1

Geodemographic characteristics of non-native patients. (A) Distribution of migrant patients by country of birth (only the twenty most frequent countries are displayed). (B) Distribution of migrants by WHO region of birth. (C) Distribution of migrants by nationality type (distinction made between Spanish, EU/EEA Europeans, non-EU/EEA Europeans, and non-Europeans). (D) Distribution of migrants by place of residence at the time of the COVID-19 infection.

There were significant differences in the prevalence of each individual comorbidity between both groups (Table 1); the proportion of patients with each of the comorbidities was always larger for Spanish. The most prevalent type of comorbidity in both groups was cardiovascular risk factors (49.2% in natives and 27.1% in migrants). The number of comorbidities also varied widely, showing statistically significant differences ($p < 0.001$), with 54.3% of migrant patients vs. only 28.2% natives having no comorbidities. A considerable percentage of Spanish native patients had three or more comorbidities (21.1%), while only 5.7% of migrant patients were in this category.

Full vaccination at the time of infection was only present in 26.1% of Spanish patients and 15.4% of migrants ($p < 0.001$). There were also differences in the distribution of both groups through the six epidemiological periods. Although the number of native cases were always higher than the number of migrant cases throughout all periods, during the 2nd, 5th, and 6th periods there was a high concentration of migrant patients compared to other periods (Table 1). A weekly incidence graphic by region of birth can be found in Figure 2.

There was a higher percentage of migrant patients presenting symptoms at the time of the hospital's visit compared to Spanish patients (85.8% vs. 80.6%). However, there were no statistical differences in the duration of symptoms until diagnosis (median of 4 [IQR 2–8] days for Spanish and of 4 [IQR 2–7] days for migrants, $p = 0.654$). More native patients were clinically or radiologically diagnosed with pneumonia during the initial

healthcare contact (36.0% vs. 33.1%, $p < 0.001$). However, it is important to consider the large proportion of missing information for this variable (9.6%).

A total of 6,155 (53.1%) cases were hospitalised during a SARS-CoV-2 infection, although 213 ended up being admitted to another centre and were lost at follow-up. Out of the remaining 5,942 cases hospitalised at our centre, 13.7% had no symptoms compatible with SARS-CoV-2 infection at the time of admission. We admitted a lower proportion of migrant patients compared with native patients (41.6% vs. 56.2%) and the length of stay was also shorter for migrants (8 days [IQR 5–12] vs. 10 days [IQR 6–17], $p < 0.001$).

In-hospital 30-day all-cause mortality was registered in 3.4 and 13.6% of migrant and native hospitalised patients, respectively ($p < 0.001$); however, there were no statistical differences in the time between admission (or diagnosis if it was an in-hospital onset infection) and death for both groups (7 days [IQR 2.5–16] vs. 9 days [4–15], $p = 0.486$). We also compared immediate deaths, defined as death of a patient in the 24h of hospital arrival (death at the Emergency Department), and no statistical difference were found (12.7% in migrants vs. 7.4% in natives, $p = 0.183$).

3.2 Presence of symptoms at the time of hospital visit

Migrants had a minimally adjusted OR by sex and age quartile (mOR) of 1.53 (95%CI 1.36–1.73) and a fully adjusted OR (aOR) of

TABLE 1 Sociodemographic, clinical, and epidemiologic characteristic of the total population, comparing Spanish and migrant groups.

Variable	Total ¹	Spanish natives ¹	Migrants ¹	<i>p</i> -value ²
Total	11,589	7,675 (66.2%)	3,914 (33.8%)	
Female	549 (46.8)	3,446 (44.9)	1,973 (49.6)	<0.001
Age (years)	57 [40–73]	65 [49–78]	43 [33–55]	<0.001
Age categories				
18–40 years	2,922 (25.2)	1,211 (15.8)	1,711 (43.7)	<0.001
41–57 years	2,989 (25.8)	1,603 (20.9)	1,386 (35.4)	
58–73 years	2,919 (25.2)	2,279 (29.7)	640 (16.34)	
>73 years	2,759 (23.8)	2,582 (33.6)	177 (4.5)	
Registered with Catalanian GP	10,943 (94.4)	7,444 (97.0)	3,499 (89.4)	<0.001
Socioeconomic privation index	23.0 [10.7–35.4]	19.3 [10.7–35.4]	25.6 [13.3–40.1]	<0.001
Socioeconomic privation index by categories				
0–12.0%	2,422 (20.9)	1,766 (23.0)	656 (16.8)	<0.001
12.1–25.0%	2,669 (23.0)	1,831 (23.9)	838 (21.4)	
25.1–40.0%	2,359 (20.4)	1,508 (19.6)	851 (21.7)	
40.1–100%	2,006 (17.3)	1,202 (15.7)	804 (20.5)	
Missing	2,133 (18.4)	1,368 (17.8)	765 (19.5)	
Comorbidities				
CV risk factors	4,832 (41.7)	3,773 (49.2)	1,059 (27.1)	<0.001
Cardiovascular disease	1,822 (15.7)	1,614 (21.0)	208 (5.3)	<0.001
Diabetes mellitus	1,525 (13.2)	1,207 (15.7)	318 (8.1)	<0.001
Respiratory disease	1,507 (13.0)	1,216 (15.8)	291 (7.4)	<0.001
Immunosuppression	1,927 (16.6)	1,558 (20.3)	369 (9.4)	<0.001
Renal disease	1,012 (8.7)	871 (11.3)	141 (3.6)	<0.001
Hepatic disease	484 (4.2)	364 (4.7)	120 (3.1)	<0.001
Missing	748 (6.5)	529 (6.9)	219 (5.6)	
Comorbidity score				
0	4,288 (37.0)	2,163 (28.2)	2,125 (54.3)	<0.001
1	2,825 (24.4)	1,875 (24.4)	950 (24.3)	
2	1,884 (16.2)	1,486 (19.4)	398 (10.2)	
≥3	1,844 (15.9)	1,622 (21.1)	222 (5.7)	
Missing	748 (6.5)	529 (6.9)	219 (5.6)	
SARS-CoV-2 vaccination				
<2 doses	8,981 (77.5)	5,669 (73.9)	3,312 (84.6)	<0.001
≥2 doses	2,608 (22.5)	2,006 (26.1)	602 (15.4)	
SARS-CoV-2 epidemiological period ³				
1st period	2,123 (18.3)	1,576 (20.5)	547 (14.0)	<0.001
2nd period	1,621 (14.0)	881 (11.5)	740 (18.9)	
3rd period	1,891 (16.3)	1,434 (18.4)	457 (11.7)	
4th period	1,280 (11.0)	812 (10.6)	468 (12.0)	
5th period	1,623 (14.0)	845 (11.0)	778 (19.9)	
6th period	3,051 (26.3)	2,127 (27.7)	924 (23.6)	
Symptoms	9,544 (82.4)	6,186 (80.6)	3,358 (85.8)	<0.001
Days from symptoms onset to hospital visit	4 [2–8]	4 [2–8]	4 [2–7]	0.654
Pneumonia at the time of hospital visit				
Yes	4,060 (35.0)	2,764 (36.0)	1,296 (33.1)	<0.001

(Continued)

TABLE 1 (Continued)

Variable	Total ¹	Spanish natives ¹	Migrants ¹	p-value ²
Missing	1,111 (9.6)	816 (10.6)	295 (7.5)	
Hospitalisation				
Hospital Clínic de Barcelona (HCB)	5,942 (51.3)	4,314 (56.2)	1,628 (41.6)	<0.001
Other hospital	213 (1.8)	163 (2.1)	50 (1.3)	
No hospitalisation	5,434 (46.9)	3,198 (41.7)	2,236 (57.1)	
Duration of hospital stay (days) (n = 5942)	9 [6–16]	10 [6–17]	8 [5–12]	<0.001
ICU admission in HCB patients (n = 5942)	1,463 (24.6)	1,070 (24.8)	393 (24.1)	0.620
Duration of ICU stay (days) (n = 1463)	6 [3–12]	6 [3–12]	6 [3–11]	0.324
Mechanical ventilation in HCB hospitalised patients (n = 5942)				
Orotracheal intubation	465 (7.8)	342 (7.9)	123 (7.6)	0.863
Non-invasive ventilation	367 (6.2)	264 (6.1)	103 (6.3)	
No ventilation	5,110 (86.0)	3,708 (86.0)	1,402 (86.1)	
In-hospital 30-day mortality				
(% of all patients)	640 (5.5)	585 (7.6)	55 (1.4)	<0.001
(% of HCB hospitalised patients)	640 (10.8)	585 (13.6)	55 (3.4)	<0.001
Days from admission to death	8 [4–15]	9 [4–15]	7 [2.5–16]	0.486
Immediate death at hospital arrival ⁴	50 (7.8)	43 (7.4)	7 (12.7)	0.183

¹n (%); Median [IQR].²Pearson's Chi-squared test; Wilcoxon-Mann-Whitney test.³SARS-CoV-2 epidemiologic periods: from 25th February 2020 to 31st May 2020; from 1st June 2020 to 30th November 2020; from 1st December 2020 to 28th February 2021; from 1st March 2021 to 30th June 2021; from 1st July 2021 to 15th October 2021; and from 16th October 2021 to 31st March 2022.⁴Fisher test.

1.36 (95%CI 1.20–1.54). We adjusted the multivariate model for sex, age category, epidemiologic period of SARS-CoV-2 transmission, vaccination status, CVD, CRD, immunosuppressive condition, and hepatic disease (Figure 3).

We also performed a sub-analysis using region of birth as the explanatory variable instead of dichotomic migration status. The fully adjusted model for this outcome was adjusted by the covariates shown in Figure 4. It showed increased odds of presenting with symptoms at diagnosis only for patients born in the Americas Region (OR 1.54, 95%CI 1.33–1.80) and in the Western Pacific Region (OR 1.59, 95%CI 1.13–2.29) (Figure 4).

3.3 Hospitalisation

When using a minimally adjusted model, migrants had a mOR of 1.10 (95%CI 1.00–1.21, $p=0.04$). The change of the OR after fully adjusting the logistic regression model was minimal (aOR 1.11, 95%CI 1.00–1.23, $p=0.04$). More information about the minimally adjusted model and the fully adjusted model for this outcome and the covariates used can be found in Figure 5.

Subsequently, we performed a sub-analysis using the region of birth as the explanatory variable, which was adjusted by the same covariates. Only patients born in Southeast Asia and in the Western Pacific Region, had significantly increased odds of being hospitalised compared to patients born in Europe, with fully adjusted ORs (aOR) of 1.86 (95%CI 1.22–2.84, $p=0.004$) and 1.56 (95%CI 1.21–2.01, $p<0.001$), respectively (Figure 6).

3.4 Intensive care unit admission

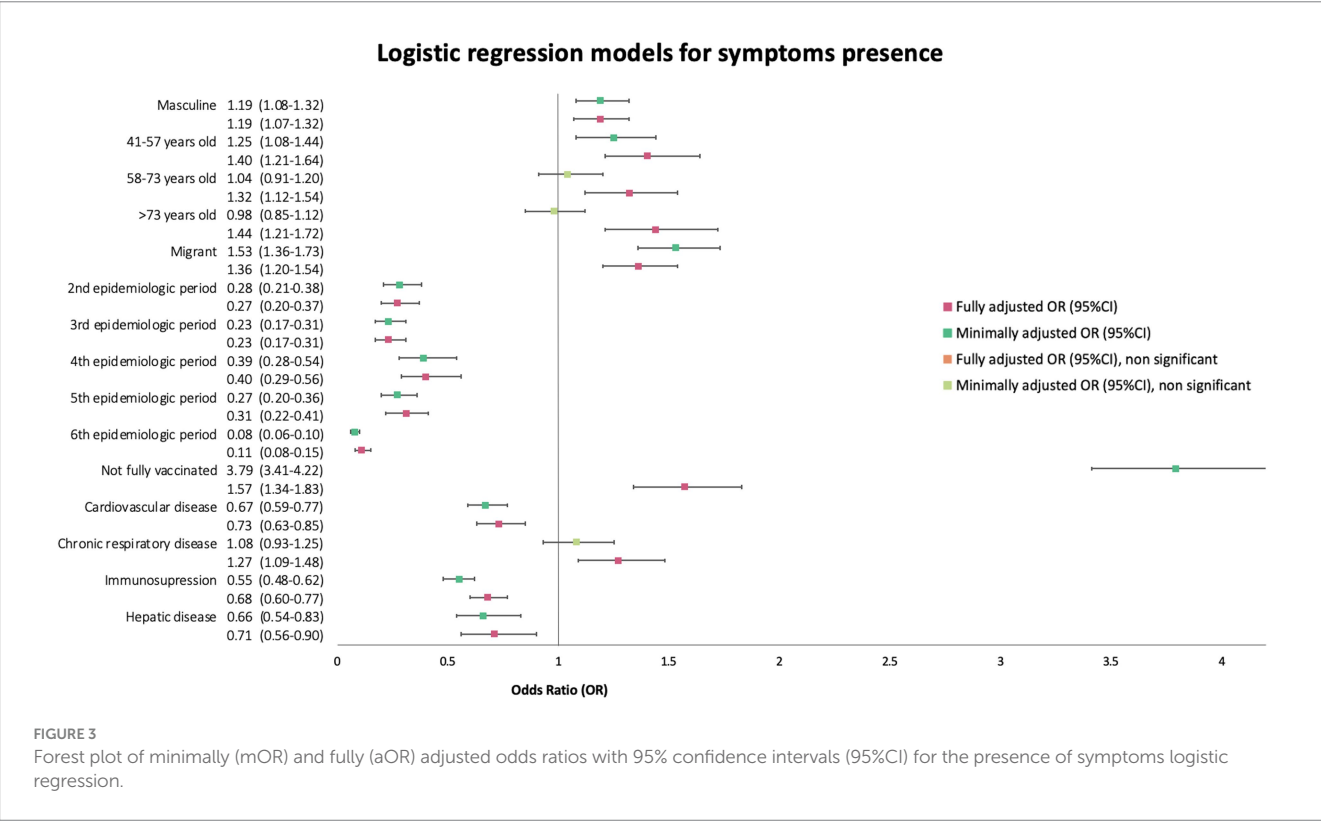
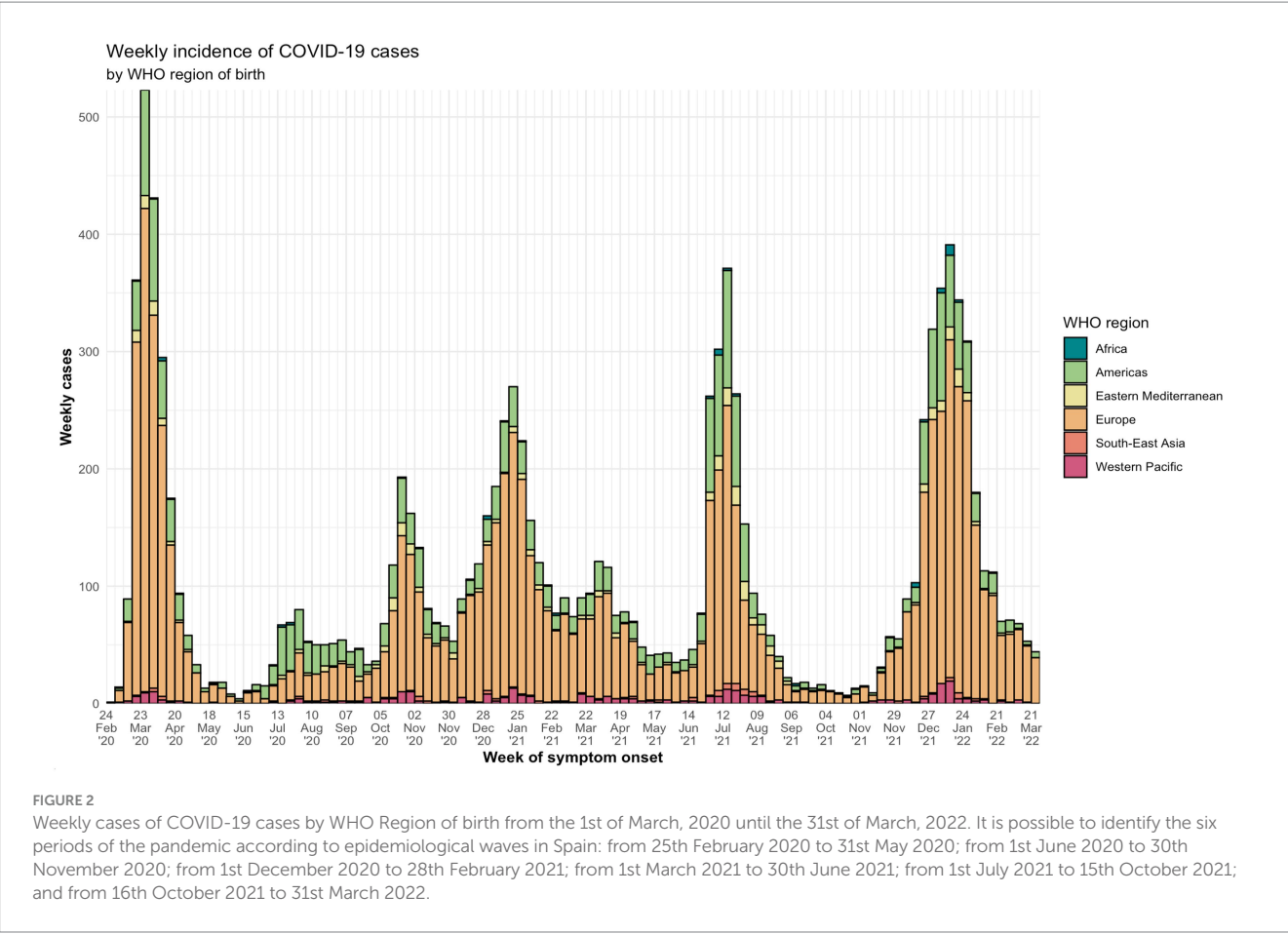
Minimally adjusted OR for migration status as an explanatory variable for ICU admission was 1.05 (95%CI, 0.90–1.22, $p=0.89$). After fully adjusting a model, the aOR for migrants remained non-significant (aOR 1.02, 95%CI 0.87–1.20, $p=0.8$). A detailed graphic account on odds ratio for all covariates considered can be found in Figure 7. Although a sub analysis by region of birth was performed and a multivariate regression model was also created, aOR of ICU admission was non-significant for all regions (data not shown).

3.5 Use of mechanical ventilation

Similarly, to the outcome of ICU admission, neither the minimally adjusted nor the fully adjusted logistic regression for the use of any type of mechanical ventilation rendered increased or reduced odds for migrant patients compared to Spanish natives with an mOR 1.20 (95%CI 0.99–1.45, $p=0.06$) of and an aOR of 1.08 (95%CI 0.88–1.31). A graphic representation of the bivariate and the multivariate model can be found in Figure 8. Sub-analysis by region of birth did not provide additional information, so data on this model is not shown.

3.6 In-hospital 30-day mortality

According to the minimally adjusted logistic regression, migrant patients were at reduced odds of in-hospital 30-day all-cause mortality



Logistic regression fully adjusted model for symptoms presence by region of birth

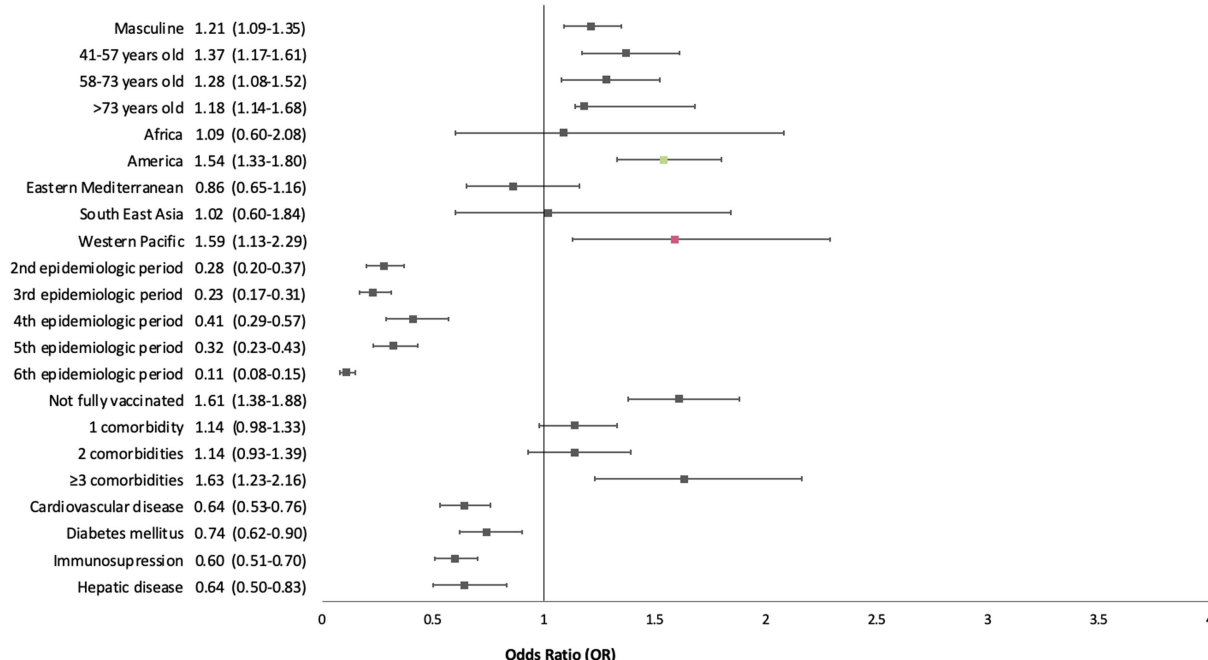


FIGURE 4

Forest plot of fully adjusted odds (aOR) ratios with 95% confidence intervals of the sub-analysis using region of birth as the explanatory variable for the presence of symptoms logistic regression.

Logistic regression models for hospitalisation

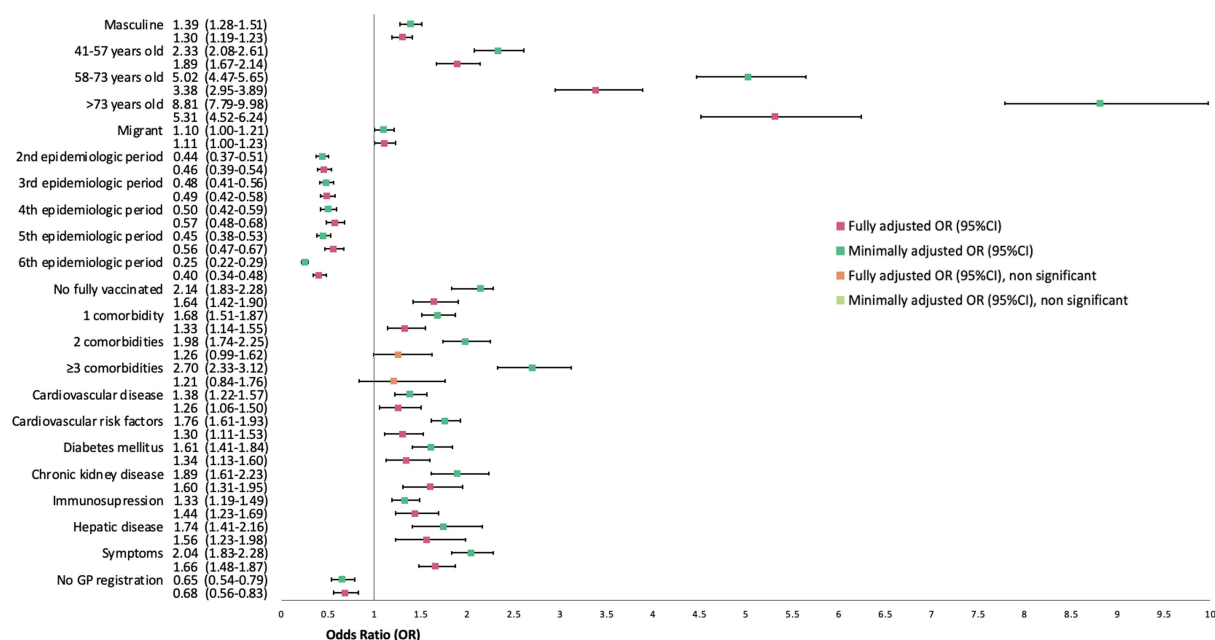


FIGURE 5

Forest plot of minimally (mOR) and fully (aOR) adjusted odds ratios with 95% confidence intervals (95%CI) for hospitalisation logistic regression.

with a mOR of 0.65 (95%CI 0.47–0.89, $p=0.01$). When fully adjusted it rendered a similar OR of 0.67 (95%CI 0.47–0.93, $p=0.02$) for migrant patients. A graphic summarising these models can be found

in Figure 9. We also performed a sub-analysis considering the region of birth as the explanatory variable but found no significant results for patients of any region in comparison to European patients. Patients

Logistic regression fully adjusted model for hospitalisation by region of birth

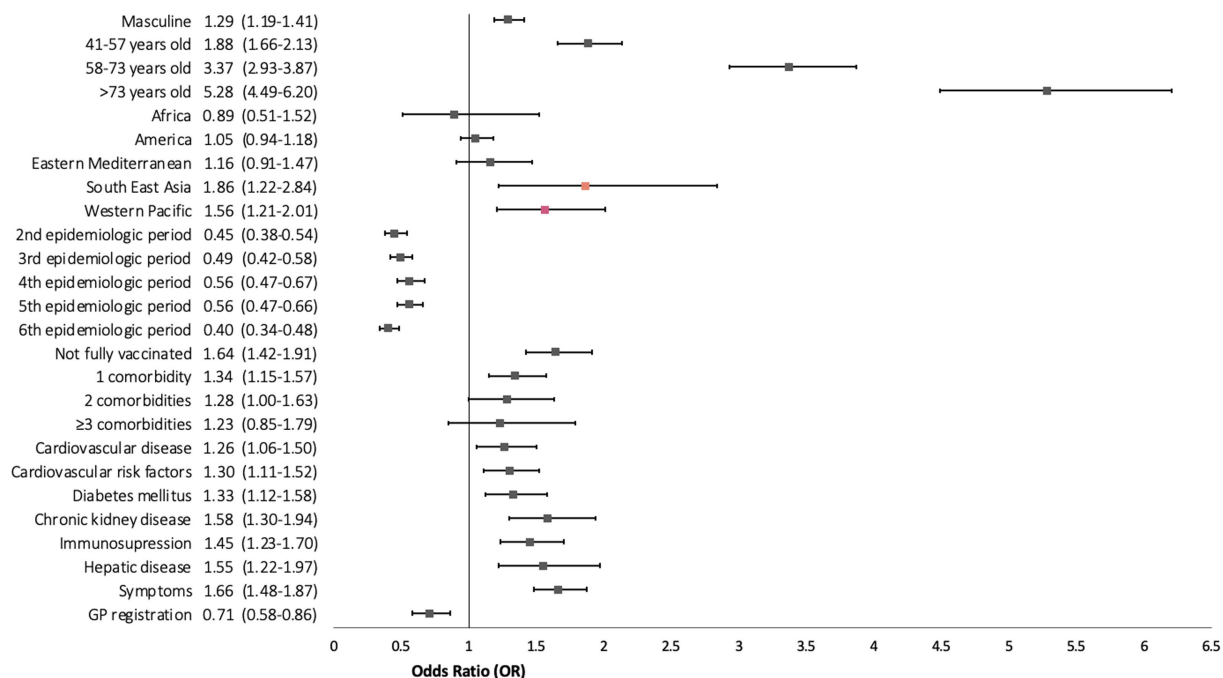


FIGURE 6

Forest plot of fully adjusted odds ratios with 95% confidence intervals of the sub-analysis using region of birth as the explanatory variable for hospitalisation logistic regression.

Logistic regression models for Intensive Care Unit admission

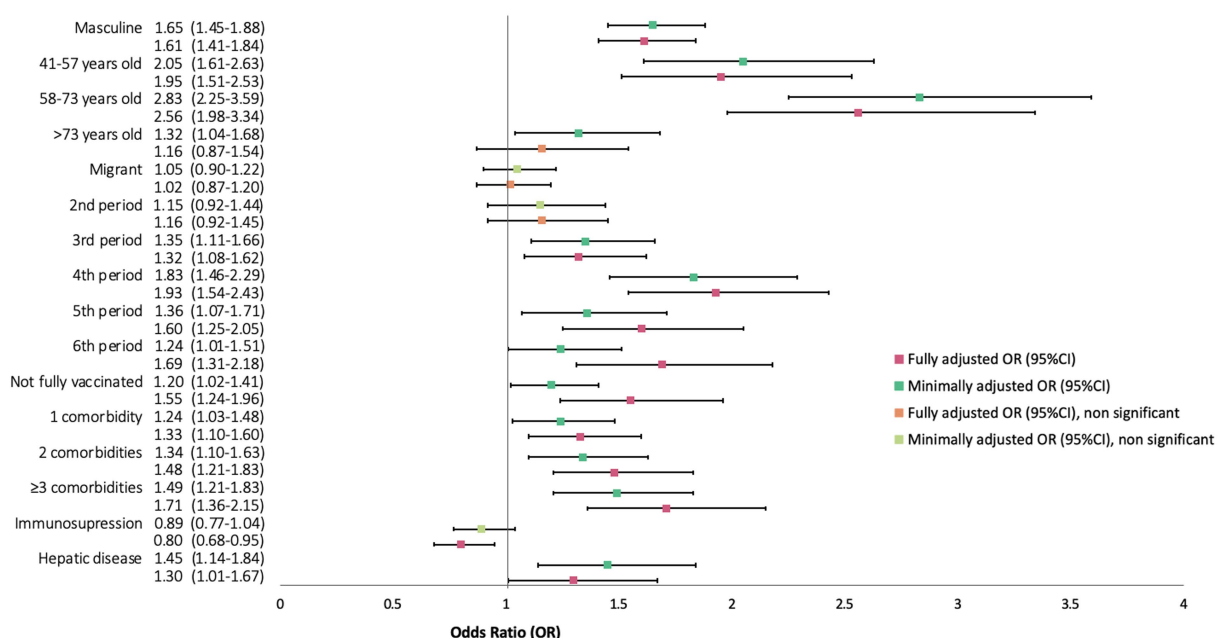


FIGURE 7

Forest plot of minimally (mOR) and fully (aOR) adjusted odds ratios with 95% confidence intervals (95%CI) for ICU admission logistic regression.

from the African region had an aOR of 0.84 (95%CI 0.13–3.31); patients from America an aOR of 0.71 (95%CI 0.45–1.08); patients from other European countries, an aOR of 0.67 (95%CI 0.22–1.61);

patients from the South East Asia region, an aOR of 0.6 (95%CI 0.03–3.86); and patients from the Western Pacific had an aOR of 0.4 (95%CI 0.11–1.06).

Logistic regression models for Mechanical Ventilation use

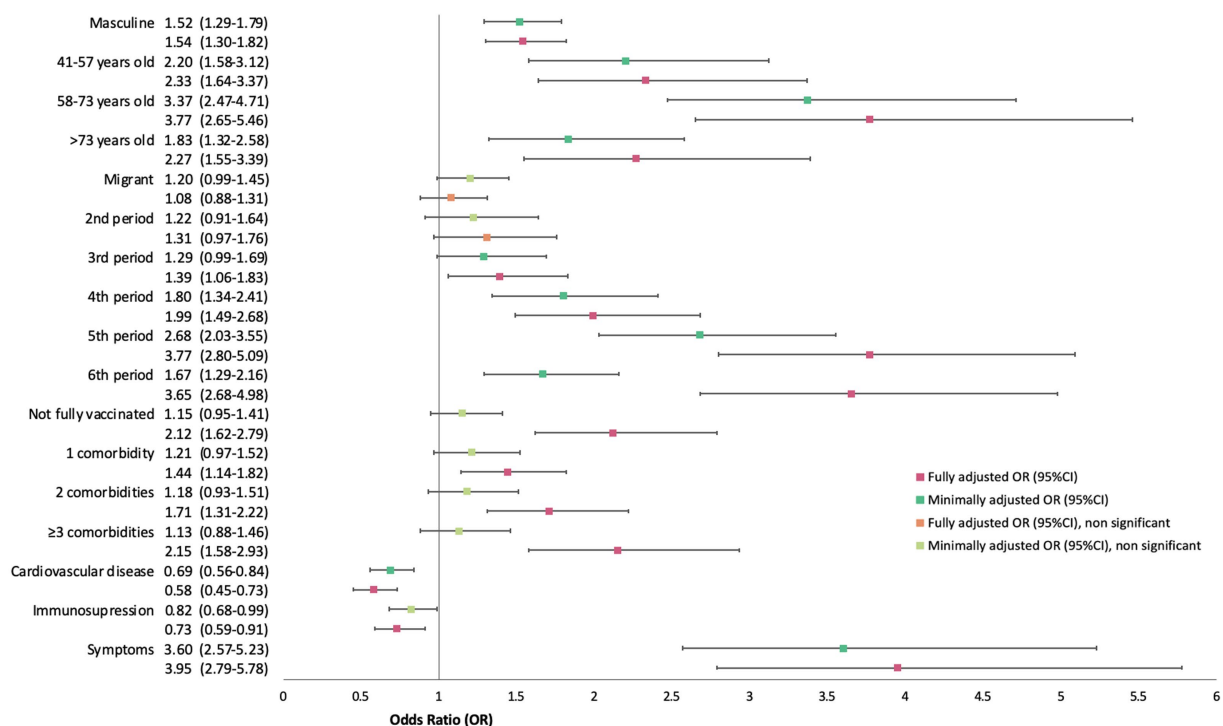


FIGURE 8

Forest plot of minimally (mOR) and fully (aOR) adjusted odds ratios with 95% confidence intervals (95%CI) for any type of mechanical ventilation use.

Logistic regression models for in-hospital 30-day mortality

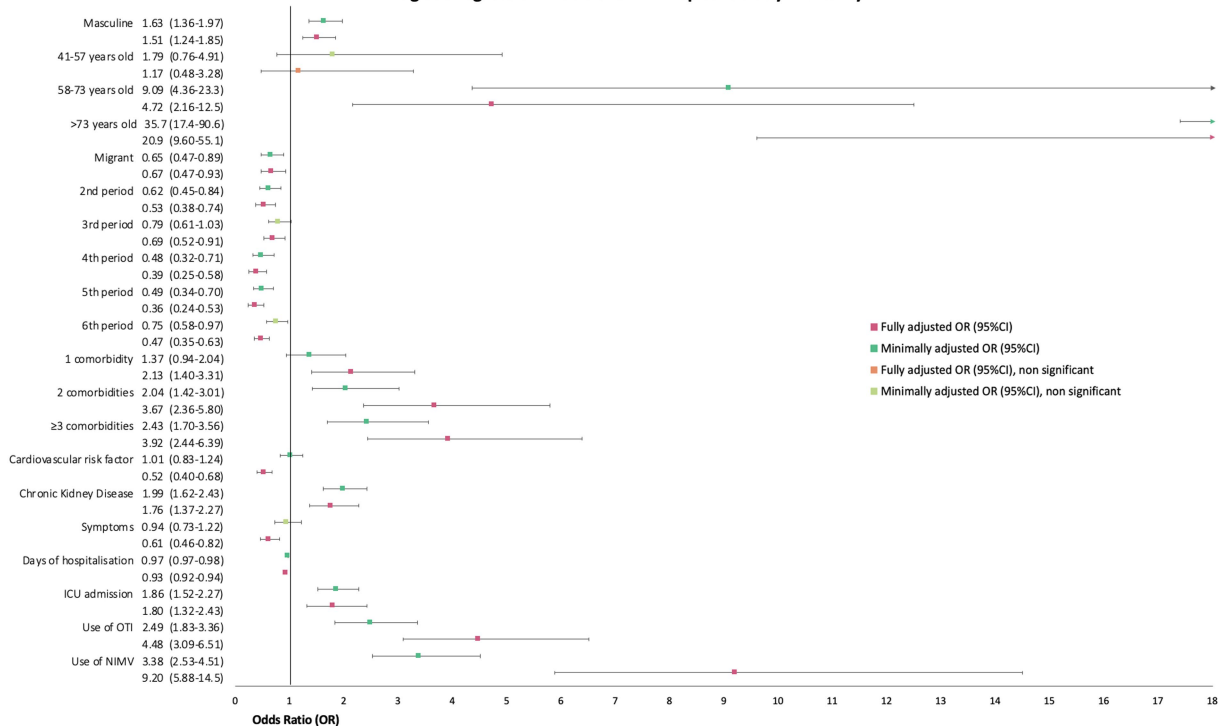


FIGURE 9

Forest plot of unadjusted and adjusted odds ratios (OR) with 95% confidence intervals (95%CI) for in-hospital 30-day mortality.

4 Discussion

Our study found a higher risk of hospitalisation and symptomatology at onset for migrants. The obtained ORs for each of the outcomes did not change significantly between models minimally adjusted by sex and age quartiles and models fully adjusted by covariates. As mentioned earlier, research on migrant health disparities during the pandemic has shown highly conflicting results. While some found no significant differences in health outcomes between the native and migrant population, others stated that some groups of migrants, such as those coming from Latin America, had a higher risk of ICU admission, use of MV, or to a lower extent, IHM (14–24, 29). Our results were consistent with some other studies' results on the subject, as we found that migrants had reduced odds of IHM and no significant differences in ICU admission or MV use. Although, the analysis did not show that migrants were at higher odds of these unfavourable health outcomes, it is important to acknowledge other important differences in our migrant population characteristics and in the odds of presenting with symptomatology and the need for hospitalisation.

Despite the widespread acknowledgement that migrants are at risk of worse health outcomes (16, 19–24), especially during times of crises, lower mortality and SARS-CoV-2 severity can be partially explained by the “healthy migrant effect,” a theory which has been thoroughly discussed in literature (4, 5, 13, 30–33). As in many other studies, we found that migrants were younger and had less comorbidities than natives, consistent with the abovementioned theory (13, 14, 19, 23, 24). It hypothesises that the majority of those who migrate, specially from low and middle-income countries to high-income countries, are the younger and healthier of the population. Most of these people are considered “economic migrants,” who usually migrate to work in developed countries, as a mean to improve their livelihoods (31). As many of the jobs they can get imply hard physical work, they are usually among the healthiest and youngest of their own societies (33). The low number of older migrants could partially explain some of the Odds Ratios' wide confidence intervals in the multivariate analysis.

Our hospital is in a rather affluent neighbourhood of Barcelona city and, therefore, its referral population usually comes from nearby BHA, meaning most of them have a lower deprivation index. Our results show that migrant patients in Barcelona city tend to live in more deprived neighbourhoods (BHA). It is highly probable that if we studied migrant population from other hospitals' referral areas, specially from smaller towns outside of Barcelona, the higher socioeconomic deprivation of migrants would be further evidenced, supporting the theory that an important proportion of them are “economic migrants” (31).

The results on the geographical distribution of migrant patients in this study are similar to those of other studies carried out in Spain that show that a higher proportion of migrants come from the Americas Region, specifically from Latin America (20–24, 30). This differs from the geographical origin of migrants living in other countries and could be explained by the shared language and historical colonial ties. The small number of patients born in certain regions also explain the OR's wide confidence intervals in the sub-analyses by region.

We found that a third of the patients with a SARS-CoV-2 infection who visited our hospital were migrants, which is a higher percentage than the ones reported in other studies (21, 24, 30). It also represents a higher percentage than the one of the migrant populations living in our hospital's referral area (34). A plausible explanation is that some patients living in

other hospitals' health areas with potentially higher migrant density could be referred to our centre because it is a high complexity hospital.

Important abovementioned differences in age and comorbidities could partially explain why fewer migrants were fully vaccinated at the time of the SARS-CoV-2 infection. Especially in the earlier vaccination campaigns, eligibility was based on advanced age and certain comorbidities, leading to the delay of young and healthy people's vaccination to later stages of the pandemic. However, it is also important to consider barriers to healthcare access, lower acceptability of vaccines, and conflicting relationships with state institutions as potential limitations to getting vaccinated (13, 16, 35).

Although most patients, regardless of migration status, presented to the hospital with symptoms associated with a SARS-CoV-2 infection, it is important to notice that, after adjusting by sex, age, vaccination status, and relevant comorbidities, migrants were 1.36 times more likely to seek healthcare attention with symptoms compared with native patients, with an even higher OR in the population born in Latin America. There are very few studies that considered this as an outcome, possibly because it does not necessarily translate into more disease severity or need for hospitalisation; nonetheless, it is relevant. This difference in the presence of symptomatology could be due to a few reasons, one of them being that asymptomatic Spanish natives were more frequently diagnosed incidentally with SARS-CoV-2 infection because they were already linked to healthcare and being followed up for comorbidities or future interventions or procedures. This makes us wonder if migrants enjoy the same level of healthcare linkage and if their lower prevalence of chronic diseases could in fact be explained not because of good health but because of a lack of diagnosis and follow-up.

The fact that more natives are already registered with a GP and are familiar with how the health system works in Spain, could have also determined a higher use of primary healthcare services when developing mild symptomatology instead of going directly to a tertiary hospital. It could reflect a more selective use of high-complexity healthcare facilities to treat chronic or high-complexity pathologies rather than acute respiratory infections.

Several factors come in to play to explain the phenomenon of misuse of health services by migrant population, but their relationship with the health system at their countries of origin is a very important one. Many developing countries have inefficient primary healthcare facilities with many material and human resources shortcomings which is why patients tend to seek attention at larger health centres. Furthermore, for many people, only by migrating to a high-income country they can finally access free and quality healthcare, which could also explain the overuse of these services. Lack of trust and long-lasting relationship with primary health physicians should also be considered. There could also be difficulties in identifying what constitutes as an emergency. This is partially due to lower health literacy and lack of patients' empowerment, concepts poorly explored and promoted in most developing countries (13, 16, 36).

We also reported slightly higher odds of being hospitalised for migrants. This is consistent with previously published research from other European countries (13, 15, 17, 18, 31, 36). These differences in hospitalisation for migrant patients could be attributed to a delay in seeking healthcare attention by migrants. This could be explained by a combination of factors, such as a lower level of health literacy, difficulties in recognising SARS-CoV-2 symptoms, problems to differentiate between health issues that require immediate attention

and those which can be managed at home or by the GP. It is also important to mention that an important percentage of these patients could be considered “economic migrants” and that they tend to prioritise work commitments over their own health, as they fear losing their jobs or not being able to send money back home.

On the other hand, migrants could have fewer resources, worse home conditions, and insufficient social or family support to receive care at home, and therefore would need to be admitted even though the severity of the illness by itself does not require hospitalisation. The same is true for patients who would not be able to follow adequate preventive measures and social distance recommendations at home, either because of specific living conditions, overcrowding, or lack of knowledge and support. This could partially explain why there is a lower IHM among migrants when they require hospital admission more often. We should also consider other genetic and biological factors that are beyond the scope of this study to explain these kind of health outcomes discrepancies in migrant population.

4.1 Limitations

One of the most important issues was the accuracy of migration specific information. For example, we were unable to identify those migrants with refugee status, who are possibly at the highest risk of unfavourable health outcomes. The type of document used for registration at the hospital was used as a proxy for nationality, but we did not have information about the exact type of visa migrants had.

There was an important mismatch between nationality and country of birth. This could be due to the fact that a considerable percentage of migrants hold dual citizenship and also that Spanish laws allow migrants of certain nationalities (mainly former Spanish colonies) to request permanent residence and subsequent naturalisation after 2 years of living in Spain. We could not confirm how long ago a migrant with Spanish nationality had been naturalised. Although we decided to define migration status by the country of birth, instead of the nationality because we believe inequalities and vulnerability do not completely disappear after naturalisation, we do believe that legal status is a factor that should also be explored in migrants (30).

It is very difficult to gather patients' data relevant to all intersectional factors that contribute towards health outcomes disparities. We recognise that there are several other characteristics that interact with migration status and that we could not get information on. Data on the country or region of birth does not adequately reflect the extent of structural inequalities or if a patient belongs to an underserved or marginalised community (30). It was not possible to assume other sociocultural factors related to migration status, such as language barriers, religion, education, or ethnicity. We believe this last factor to be a social determinant of health, based on genetic vulnerability and on discrimination (35). An important limitation to explore this subject is that, generally, in EU countries it is not possible to collect data on ethnic background. Although many studies use migrant status as a proxy for ethnicity, we believe they are different determinants (24–34, 36).

Although we collected data on the majority of patients' BHA of residence, we were not able to consider the socioeconomic deprivation index for the multivariate analysis because there was information missing for a large percentage. It is possible that patients living in other cities of Catalonia, from whom we could not get exact

information on BHA of residence, were more socioeconomically deprived. Characteristics of migrant patients described in this study largely differ from those of migrant communities living outside of Barcelona city, where there is a higher migrant density. Furthermore, as most patients in this study belong to our hospital's referral area, which is located between neighbourhoods with less socioeconomic deprivation, the results in this study should not be extrapolated to all migrant communities living in Catalonia.

5 Conclusion

Our study's results show that even though migrants have reduced odds of in-hospital mortality during SARS-CoV-2 infection, there are other important characteristics and outcomes that should be considered. The fact that migrants are more likely to present with symptomatology at onset and less likely to be diagnosed incidentally could translate into an inefficient use of health services, lower levels of healthcare linkage and follow-up, mistrust in health professionals, and lower health literacy. The higher odds of being hospitalised could also reflect the abovementioned issues, as well as a delay in seeking healthcare attention, lack of their own health's prioritisation, difficulty in recognising symptoms of disease, lack of resources and support to receive care at home, and other genetic and biological factors that are beyond the scope of this study.

The results of this study further evidence the fact that even in states with inclusive, universal, and free healthcare systems, such as Spain, diverse inequalities in health outcomes persist for minorities. These disparities cannot be explained simply by socioeconomic status or limitations in access healthcare services because of migratory status or the elevated price of healthcare. Although we believe the findings of this study will be useful to evidence existing inequalities between native and migrant communities living in high-income countries, we also think that more research is needed to identify more clearly the underlying structural factors that determine these disparities. Furthermore, we strongly believe that there is enough evidence to direct more efforts into interventions and health policies that could help reduce these gaps in access to healthcare and health literacy in minorities and other vulnerable groups, such as migrants.

Data availability statement

The data analysed in this study is subject to the following licenses/restrictions: This is a database created and maintained until this day for purposes of epidemiologic surveillance. Although the original database is anonymized it does contain information about infection dates, age, and specific comorbidities that would be better protected from the public. However, if it is necessary to make them publicly available we could ask for permission from the Ethics Committee. Requests to access these datasets should be directed to vperez@clinic.cat.

Ethics statement

The studies involving humans were approved by Research Ethics Committee of Hospital Clinic Barcelona. 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 Dissociated data was used so that no information included allows the identification of participants. Written informed consent for participation was waived for this study in accordance with the national legislation (Spain's Organic Law 3/2018 of December 5 on the Protection of Personal Data and Guarantee of digital rights) and the institutional requirements.

Author contributions

VP-M: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing – review & editing. MB: Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing. LB-M: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – review & editing. IT-R: Formal analysis, Methodology, Software, Supervision, Validation, Writing – review & editing. JG-G: Data curation, Investigation, Software, Writing – review & editing. AV: Conceptualization, Methodology, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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

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Ready for new waves: optimizing SARS-CoV-2 variants monitoring in pooled samples with droplet digital PCR

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Introduction: The declaration of the end of the Public Health Emergency for COVID-19 on May 11th, 2023, has shifted the global focus led by WHO and CDC towards monitoring the evolution of SARS-CoV-2. Augmenting these international endeavors with local initiatives becomes crucial to not only track the emergence of new variants but also to understand their spread. We present a cost-effective digital PCR-based pooled sample testing methodology tailored for early variant surveillance.

Methods: Using 1200 retrospective SARS-CoV-2 positive samples, either negative or positive for Delta or Omicron, we assessed the sensitivity and specificity of our detection strategy employing commercial TaqMan variant probes in a 1:9 ratio of variant-positive to variant-negative samples.

Results: The study achieved 100% sensitivity and 99% specificity in 10-sample pools, with an Area Under the Curve (AUC) exceeding 0.998 in ROC curves, using distinct commercial TaqMan variant probes.

Discussion: The employment of two separate TaqMan probes for both Delta and Omicron establishes dual validation routes, emphasizing the method's robustness. Although we used known samples to model realistic emergence scenarios of the Delta and Omicron variants, our main objective is to demonstrate the versatility of this strategy to identify future variant appearances. The utilization of two divergent variants and distinct probes for each confirms the method's independence from specific variants and probes. This flexibility ensures it can be tailored to recognize any subsequent variant emergence, given the availability of its sequence and a specific probe. Consequently, our approach stands as a robust tool for tracking and managing any new variant outbreak, reinforcing our global readiness against possible future SARS-CoV-2 waves.

KEYWORDS

Pool testing, droplet digital PCR, COVID-19 pandemic, new variant outbreak, SARS-CoV-2 wave surveillance, Delta/Omicron tracking

Introduction

The dynamic nature of COVID-19 pandemic is characterized by the emergence and succession of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) variants, categorized as variants of concern (VOCs), variants of interest (VOIs), and, more recently, variants being monitored (VBM) (1–5).

As of this writing, the World Health Organization (WHO) recognizes Omicron (B.1.1.529 and descendant lineages) as the only VOC, while the Alpha (B.1.1.7), Beta (B.1.351), Gamma (P1), and Delta (B.1.617.2) are considered VBM (1). The declaration of the end of the Public Health Emergency for COVID-19 on May 11th, 2023, has shifted the global focus led by WHO and CDC towards monitoring the evolution of SARS-CoV-2.

Traditional methods for tracking variants of SARS-CoV-2, such as Next-Generation Sequencing (NGS), provide definitive identification of mutations. NGS would be the method of choice when one seeks to monitor the emergence of new variants with unknown sequences. However, in the event of a wave of a new VOC, the timeliness of NGS falls short of the immediate demands for treatment decisions and for the surveillance required in the face of a quickly spreading virus (6, 7). Furthermore, the cost and infrastructure required for NGS are substantial, limiting its reach to countries with the necessary resources. In this context, more accessible and rapid methods would be needed. One such alternative is RT-PCR genotyping panels, which have enabled the quick identification of circulating SARS-CoV-2 variants (8, 9). However, these strategies primarily rely on individual testing, thereby escalating the time, labor, and costs involved.

Droplet digital PCR (ddPCR), an increasingly popular method for nucleic acid detection and quantification, offers an alternative approach (10–12). This technique compartmentalizes DNA molecules into thousands of droplets, amplifying them simultaneously, leading to several advantages, including decreased inhibitor concentration, increased relative sample concentration, and improved accuracy. The benefits of ddPCR have led to its widespread adoption in clinical research for the diagnosis of various diseases (13–16). Within our ddPCR process, two TaqMan probes with different fluorophores are utilized for amplification. These probes only differ in the sequence corresponding to the mutation of the variant under study. The probe with the HEX-fluorophore typically hybridizes specifically to the wild-type (WT) sequence, while the probe with the FAM fluorophore is specific for the sequence carrying the point mutation of interest. Our current digital PCR technology, featuring two channels of detection, supports the use of multiple probes, as evidenced in several studies (17, 18). Effective multiplexing is achieved by employing the same fluorophore for various probes, with distinct signal differentiation accomplished by adjusting the concentration of each probe. This allows for the separation of signal clusters in a two-dimensional plot, enhancing the versatility of digital PCR. Our system is

designed with primers and probes that amplify a specific fragment size, typically between 60 to 100 base pairs. Thus, the size of the target marker does not affect the intensity of fluorescence in our assays. Rather, the fluorescence intensity within a positive droplet is predominantly influenced by the concentration of primers and probes used in the reaction. A higher concentration of these components generally results in stronger fluorescence signals, which is a crucial aspect for multiplexing applications. Therefore, by varying the concentrations of different probes, we can effectively multiplex without compromising the accuracy and efficiency of the assay.

In keeping with advancements in the field, newer versions of ddPCR technology now allow up to six channels to be read, significantly enhancing the analytical capabilities of ddPCR. This advancement enables the quantification of up to 12 targets in a single well, representing a substantial increase in throughput and efficiency for complex analyses, further broadening its application in research and diagnostics.

Following amplification, the fluorescence of each droplet is read, and the concentrations of mutant and WT molecules are determined using a Poisson distribution (19).

The utilization of pooling assays in diagnostic PCR offers significant advantages, including reduced costs, increased testing capacity, and shorter turnaround times, as multiple samples can be simultaneously analyzed in a single assay, providing an efficient and cost-effective approach to detect and monitor pathogens in diagnostics assays (20–22).

In this paper, we present a systematic variant detection approach that we evaluated on a total of 1,200 retrospective samples collected between August 2021 and May 2022 using commercially TaqMan variant probes. We analyzed the Delta and Omicron variants in pools of 10 SARS-CoV-2 positive samples, achieving a sensitivity of 100% and a specificity of 99% compared to individual samples.

During the initial phase of the Delta variant's emergence, we evaluated our platform using a double-entry chart combinatorial pool-testing strategy on 41 SARS-CoV-2 positive samples. This approach efficiently reduced the number of samples needing ddPCR testing from 41 to 13. Upon identifying positive intersections of rows and columns, we performed individual RT-PCR tests on the intersecting samples and were able to detect all three that carried the Delta variant. This led to notable savings in both reagents and time.

While our study focused on known, retrospective samples and targeted SARS-CoV-2 variants that may no longer be relevant for current testing, this was a necessary step to fine-tune the method. The true intent behind this work is to ensure that, in the future, this methodology can be applied to monitor the circulation of any newly emerging variant. The ddPCR pooling strategy is especially effective for detecting these SARS-CoV-2 variants when they first begin to circulate and their incidence is very low, allowing for pooled testing. In this landscape, both NGS and

ddPCR techniques can complement each other. While NGS stands out in the identification of new mutations, ddPCR excels in tracking their distribution, making it an invaluable tool for rapid response to any rapidly evolving pathogens with pandemic potential.

Materials and methods

Sample collection

Nasopharyngeal samples were collected between August 2021 and May 2022, mixed with 2 mL saline solution and stored at 4°C until extraction. In Biosafety Level 2 containment, the virus was inactivated and then extracted automatically using the Applied Biosystems™ MagMAX™ kit and the Thermo Scientific™ KingFisher™ purification system.

Individual RT-qPCR test

RT-qPCR was performed in the clinical laboratory to detect the presence of SARS-CoV-2 RNA with the PerkinElmer SARS-CoV-2 RT-qPCR Reagent kit®, following manufacturer instructions. We received de-identified positive samples with a code. SARS-CoV-2 positive samples were genotyped by RT-PCR to detect the variants of concern (Delta and Omicron). For that, probes P681H.CCT.CAT, L452R.CTG.CGG, Q954H.CAA.CAT and P681R.CCT.CGT (ThermoFisher Scientific®) and CoV_B1429_L452R (Biorad®) were used. At the time of assay, the spike protein mutation P681R was used to identify and distinguish the Delta variant and L452R was specific for Delta and Epsilon. On the other hand, Q954H was specific for Omicron while P681H was specific for Omicron and Alpha variants.

Pooling preparation

To confirm that the samples employed for pool creation were devoid of the variants under study, we utilized samples collected prior to the emergence of these variants. We designated these samples, which tested positive for SARS-CoV-2 but negative for the specific variant in study, as “WT-SARS-CoV-2 samples.” The SARS-CoV-2 sample carrying the mutation in study was designated VARIANT-SARS-CoV-2 (Va-SARS-CoV-2) sample. For the construction of sample pools, we combined one Va-SARS-CoV-2 sample with nine WT-SARS-CoV-2 samples.

Primer/probe thermal gradient optimization

Optimus pool size and dilution were used as a template to assess the optimal annealing temperature of the individual primer/probes. The standard ddPCR cycling program was modified by replacing the annealing temperature step with a thermal gradient from 55°C to 60°C for 1 min extension time. These experiments were performed with P681H and Q954H from Thermo Fisher Scientific® and L452R from Thermo Fisher Scientific® and Biorad®.

Pooled ddPCR test

One-Step RT-ddPCR Advanced Kit for Probes (BioRad) was used for RT-ddPCR according to the manufacturer's recommendations. 5.5 µL of pooled samples were added to 16.5 µL of master mix with variants/WT primers and probes according to the manufacturer's recommendations. VOCs probes (P681H, L452R, Q954H, and P681R) were labeled with carboxyfluorescein (FAM), WT probes were labeled with HEX fluorophore. RT-ddPCR reactions were set up in 96-well ddPCR plates. Plates were covered with a pierceable foil heat seal, sealed using a PX1 PCR Plate Sealer (BioRad), mixed by vortexing, and centrifuged before droplet generation. Droplet generation was done in the QX200 AUTO DG (BioRad), which dispensed droplets into a new 96-well plate. Then, plates were heat-sealed with a pierceable foil and thermocycled on a C1000 touch thermal cycler (BioRad) as follows: 50°C for 50 min; 95°C for 10 min; 5 cycles of 94°C for 15 s and 55°C for 50 s, 35 cycles of 94°C for 10 s and 55°C for 45 s, 98°C for 10 min, followed by a 25 min hold at 4°C for droplet stabilization. Droplets were then read on a QX200 Droplet Reader (BioRad) set up to read FAM and HEX channels and analyzed using the QuantaSoft™ Analysis Pro 1.0.596 software. The software performed the automated droplet count for each case, which was then manually reviewed to determine whether positive droplets fell outside gating parameters. Besides the cut-off determined and according to manufacturer instructions, a minimum number of accepted droplets is required to ensure optimal detection in the analysis: positive assays must have at least 6,000 accepted droplets, negative ones must have 10,000 or more (23).

Statistical analysis

The overall performance of the new technique was evaluated by calculating the area under the Receiver Operator Characteristics (ROC) curve and its 95% confidence interval using the method described by Hanley and McNeil (24). The best cut-off point was selected using the Youden Index (25), with a priority for the test to have a 100% sensitivity. The estimates of sensitivity and specificity were determined using a 95% confidence interval for previously established cut-off values.

Data were processed using R. Among other packages, pROC and OptimalCutpoints were utilized.

Analysis of the ddPCR data was performed with QuantaSoft analysis software v.1.7.4.0917 (Bio-Rad) to calculate the concentration of the targets.

Ethical approval

The study protocol was approved on December 10th, 2020 by the Bioethics Committee of Universidad Nacional de Rosario, Facultad de Ciencias Médicas (resolution: 3733/2022). Signed informed consent was not necessary as de-identified samples of SARS-CoV-2 tests by HOSPITAL PROVINCIAL DE ROSARIO were used. This study was conducted in accordance with the principles of the 1964 Declaration of Helsinki.

Results

Assay optimization: pool size and thermal gradient

While our previous work demonstrated the ability to detect a SARS-CoV-2 positive sample within a 34-sample pool (26), identifying specific Va-SARS-CoV-2 sequences among SARS-CoV-2 positive samples proves to be more complex. This complexity arises because, in the former case, we were seeking a specific sequence within a vast array of DNA without similar sequences. In contrast, now we face the added challenge of differentiating between the target sequence and the wild-type sequence, which differ by only a single nucleotide.

In our initial tests, we purposefully mixed one Delta sample (with a CT value of 27) with nine WT-SARS-CoV-2 samples. We intentionally chose a sample with a high CT value, which holds clinical relevance, to challenge the system's capability in detecting variants at a low concentration (27). Assay conditions were then modified for optimization. As depicted in Figures 1A–D the dilution of the pool with water (1/2, 1/4, and 1/30) and the reduction of the annealing temperature expanded the separation between the WT and mutant droplet clouds (Figures 2A,B). This increase in the separation of the clouds in a graph was already described by the manufacturer (28). The optimal condition was found to be a 1/30 pool dilution in water combined with an annealing temperature of 55°C.

Specificity and sensitivity assessment

Recognizing the competitive amplification environment where the mutant sequence is outnumbered by the predominance of WT sequence, it was crucial to establish conditions enhancing the method's specificity. We assessed the specificity of our method in accordance with the guidelines of the European Pharmacopoeia (29). We obtained

1,000 WT SARS-CoV-2 samples that predate the Delta variants' emergence. These samples were divided into 100 pools for analysis, and each pool was examined by ddPCR with the 4 probes named in Table 1. Each probe had to be run independently to ascertain their distinct cutoff points. The cut-off points were the maximum number of positive drops tolerated in a negative sample.

Evaluating the sensitivity of our platform presented a unique challenge due to the need to detect a specific variant sequence amidst a majority of WT-SARS-CoV-2 samples. To accurately measure this sensitivity, we designed our tests according to the guidelines provided by the European Pharmacopoeia (27). Our sensitivity analysis was initiated with a Delta SARS-CoV-2 sample (CT = 27) combined with nine WT SARS-CoV-2 samples (with CT values ranging from 14 to 20). This high-CT Delta sample was purposefully chosen to test the system's capacity to detect a low-concentration variant amid a higher-concentration of WT SARS-CoV-2 samples. To ensure the robustness of our findings, each measurement was repeated six times across four independent experiments, resulting in a total of 24 repetitions for each probe. This process was conducted separately for each of the two Delta variant-specific probes. A similar study design was applied for the two probes specific to the Omicron variant. Table 1 presents the results. The sensitivity at the best cut-off value was 100% for all probes, while the specificity was 100% for the Q954H and L452R probes, and 99% for the P681R and P681H probes.

The Receiver Operating Characteristic (ROC) curves were used to analyze the specificity and sensitivity of the four different probes (Figures 3A–D). Table 1 shows that the areas under the curve (AUC) were 1 for the Q954H and L452R probes, and above 0.998 for the P681R and P681H probes.

In regards to the P681R and P681H probes, we prioritized having 100% sensitivity over specificity as we did not want to miss any positive sample. Conversely, if a pool tested false positive, this error could be eliminated by de-pooling and individually testing each sample via RT-PCR.

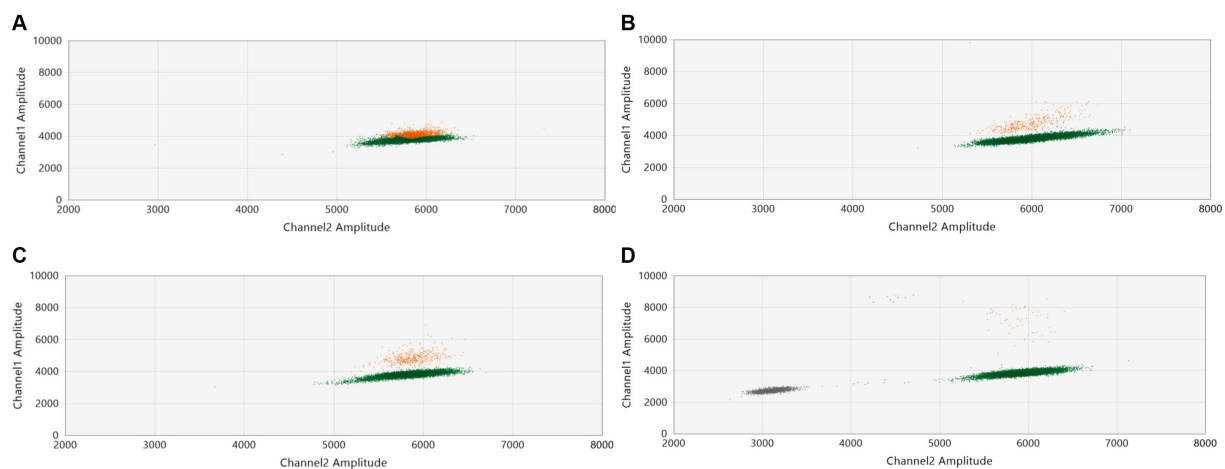


FIGURE 1

Assay optimization: influence of sample dilution on assay performance. A Delta sample (CT = 27) was combined with nine WT-SARS-CoV-2 samples (CT values ranging from 14 to 20). The combined pool was then analyzed by ddPCR with the L452R probe (Catalogue # CVA5AAD). We examined the distribution of droplet populations across various dilution conditions with water: (A) undiluted, (B) 1/2 dilution, (C) 1/4 dilution, and (D) 1/30 dilution. The diagrams illustrate the variation in droplet distribution under each dilution condition.

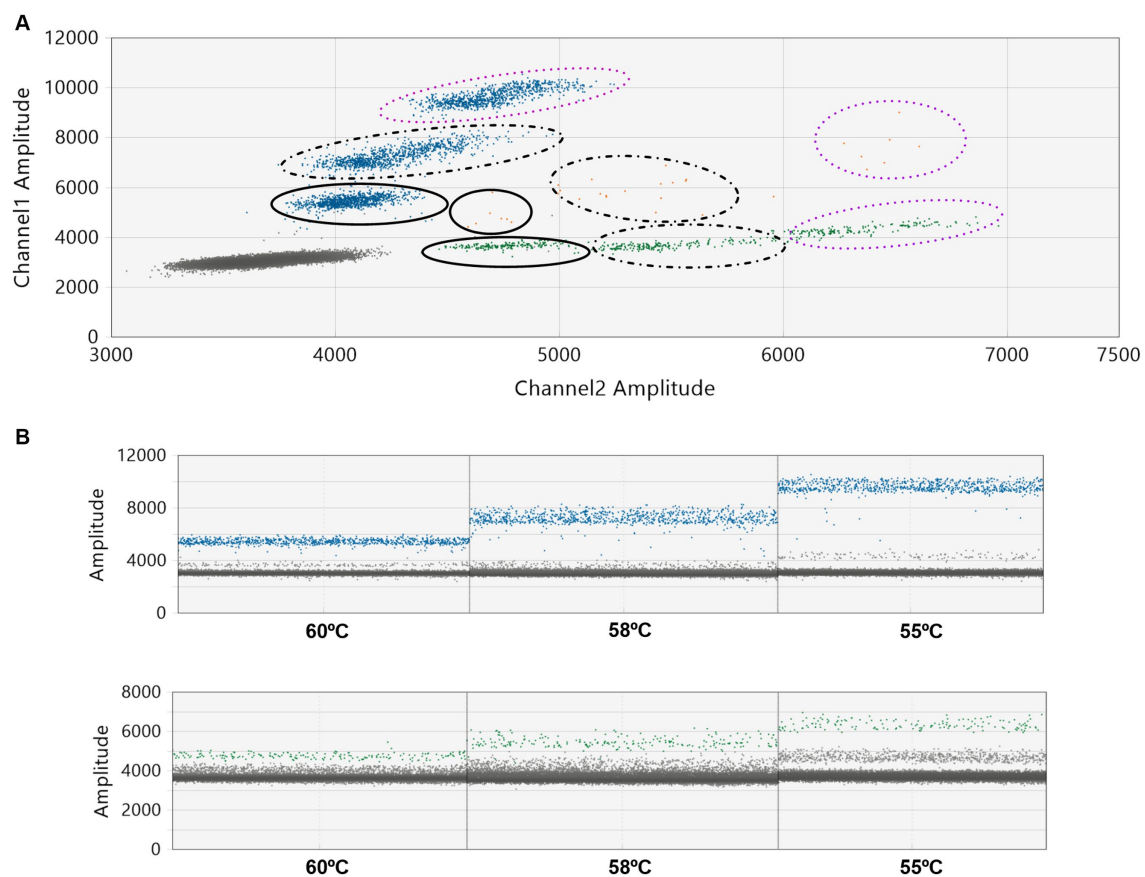


FIGURE 2

Assay optimization: impact of thermal gradient on annealing temperature. A Delta sample (CT = 24) was combined with nine WT-SARS-CoV-2 samples (CT values ranging from 14 to 20). This pooled sample was then analyzed by ddPCR with the L452R probe (CoV_B1429_L452R Biorad®). Assays were conducted at three different annealing temperatures: 55°C, 58°C, and 60°C. (A) A 2-D plot displaying the distribution of the droplet populations at each of the three annealing temperatures. The smooth line represents 60°C, the dashed line represents 58°C, and the purple dotted line represents 55°C. (B) 1-D plots of the ddPCR reactions at different temperatures, with blue representing positive droplets for the variant, green for the WT, and gray for negative droplets.

TABLE 1 Details of sensitivity, specificity, and area under the curve (AUC) for all four probes used for SARS-CoV-2 variant detection.

Variant	Amino acid change	Cut off number	AUC	Specificity $n = 100$	Sensitivity $n = 24$
Omicron	P681H	4,6	0.999	99%	100%
Delta	P681R	2,8	0.998	99%	100%
Delta	L452R	2,75	1	100%	100%
Omicron	Q954H	7,45	1	100%	100%

Clinical case study

We had the opportunity to evaluate our platform during the emergence of the Delta variant wave from September 17th to 22nd, 2021. At that time, the incidence of Delta cases in our country was still quite low, representing about less than 2% of total cases (30). We selected 41 leftover SARS-CoV-2 positive samples from that week and applied them to a double-entry chart combinatorial pool-testing strategy, also known as matrix or grid testing (20). This approach entails testing each sample twice in a grid format, which facilitates the cross-matching of results to accurately identify positive samples. The methodology is visually represented in Table 2. As the incidence was 2%, pools were assembled with either six or seven samples each.

Table 2 shows that pools B, E, 5, 6, and 7 returned positive results for the Delta variant (light gray). The cells located at the intersection of these columns and rows, indicated in dark gray, were designated as “putative positive samples.” We proceeded to “deconstruct” these five pools and individually re-tested the six dark gray samples using RT-PCR. This process resulted in the confirmation of samples 19–509, 19–510, and 22–626 as Delta variant positives.

It is important to highlight that this approach effectively reduced the number of samples requiring ddPCR testing from 41 down to 13 (plus an additional 6 samples individually tested by RT-PCR). Furthermore, this method ensured each sample was tested twice: once in column pools and once in row pools.

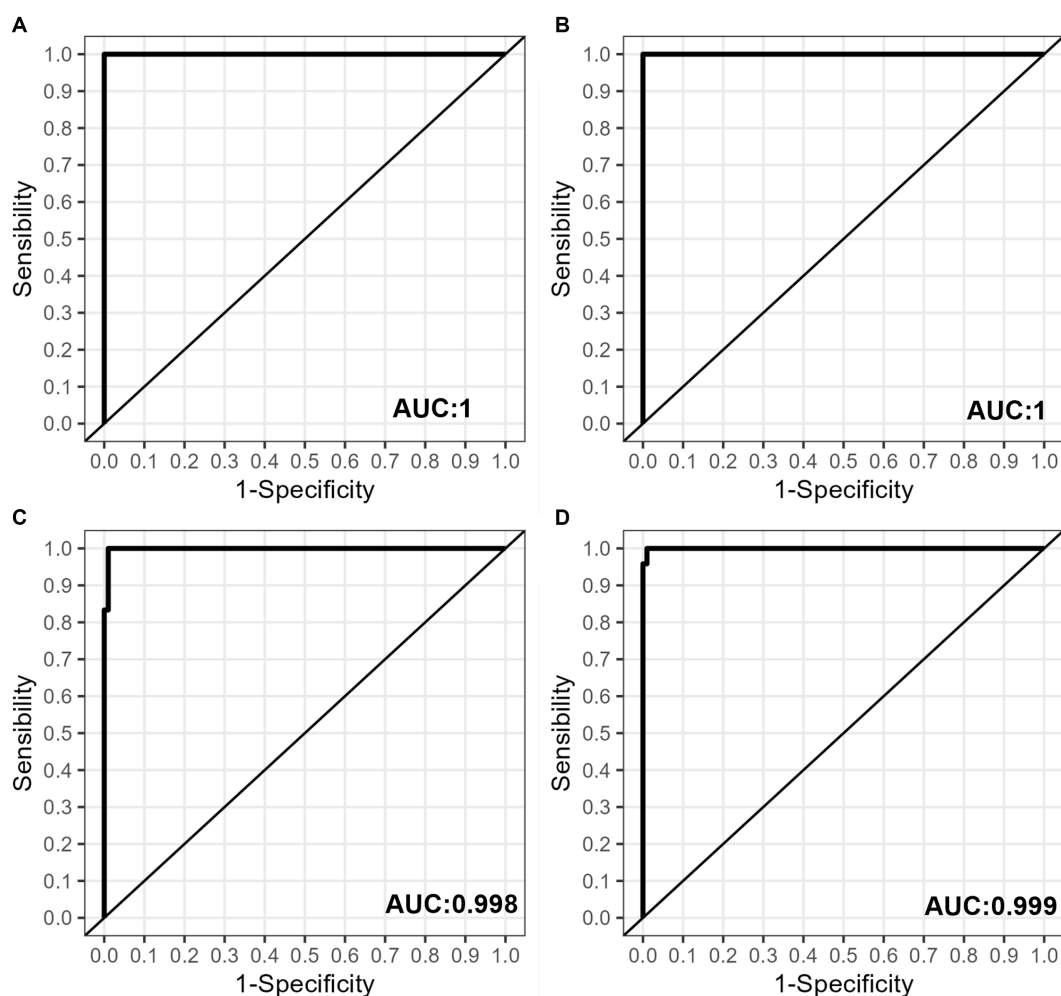


FIGURE 3

Sensitivity and specificity assessment. Receiver operating characteristic (ROC) curves are illustrated for the following probes: (A) L452R, (B) Q954H, (C) P681R, and (D) P681H. These curves were derived from the analysis of pooled samples consisting of one Va-SARS-CoV-2 sample (CT = 27) and nine WT-SARS-CoV-2 samples (CT values ranging from 14 to 25).

We employed this technology briefly. Within just one week, the prevalence of the delta variant in our country jumped from 2% to over 10%. Given this rapid increase and the subsequent high prevalence, pooling samples became impractical, as the majority were expected to test positive for the variant (30).

Conclusion

Despite the declaration of the end of the Public Health Emergency for COVID-19 on May 11th, 2023, national efforts to monitor the emergence and spread of new variants continue. While these strategies may vary from country to country, they generally involve genomic surveillance of a percentage of patients, travelers, and wastewater samples (1).

Although next-generation sequencing remains the primary method for detecting and tracing new SARS-CoV-2 variants, there are scenarios where its inherent challenges – such as cost, extended turnaround times, and the need for specialized personnel – could benefit from complementary methodologies (6, 7). Techniques like Sanger sequencing and RT-PCR genotyping using single-nucleotide

polymorphism (SNP) can serve as supplementary tools, providing faster and more cost-effective solutions in certain contexts (8, 31, 32). Digital PCR is a sophistication of the traditional RT-PCR that has higher sensitivity to detect a low copy number mutation (33). Additionally, pooling samples with ddPCR offers the significant benefit of reducing reagent costs. The extent of these savings is influenced by several factors, including the positivity rate, which in turn guides the optimal group size, as well as the inherent cost of ddPCR testing. When comparing the costs, if ddPCR is approximately twice as expensive as RT-PCR, pooling 1,000 samples in groups of 10 with a positivity rate of 2% can result in reagent cost reductions of at least 50%.

Our study presents a robust and flexible platform for the detection of specific SARS-CoV-2 variants amidst a backdrop of 10 WT-SARS-CoV-2 samples. We have successfully adapted and optimized our earlier SARS-CoV-2 detection methodology (26) leveraging a ddPCR pooling strategy, to identify two different variants, Delta and Omicron.

A pivotal strength of our platform lies in its inherent adaptability. It is designed to be modifiable with minimal alterations needed to detect future emerging variants. Through the strategic selection of two distinct TaqMan probes for each variant, we established two

TABLE 2 Double-entry chart with analyzed samples for the real case.

	POOL A	POOL B	POOL C	POOL D	POOL E	POOL F
POOL 1	17,664	17,673	18,587	18,595	21,675	22,627
POOL 2	17,667	17,674	18,588	20,597	21,525	22,628
POOL 3	17,668	18,592	18,589	20,598	21,671	22,629
POOL 4	17,669	20,567	18,590	20,599	21,672	22,630
POOL 5	17,670	19,509	18,591	20,600	21,673	22,631
POOL 6	17,671	19,510	18,593	20,601	22,657	22,616
POOL 7	17,672	19,511	18,594	21,674	22,626	

Samples that were used for the preparation of 13 pools are shown in this table (numbers in the cells are internal codes of identification of patients used in the hospital). Pools 1–7 were prepared with samples described under each line, and pools A–F were prepared with samples described following each column. Considering this distribution and preparation of pools, each sample was tested twice, once in pool 1–7 and once in pool A–F. In dark gray we show the “putative positive samples” to be retested by RT-qPCR after finding that pools 5, 6, 7, B and E were positives by ddPCR.

independent methods for each variant detection, enhancing the reliability and versatility of our platform.

Our platform's sensitivity and specificity were carefully evaluated following the European Pharmacopoeia. We demonstrated 100% sensitivity at the best cut-off value for all probes, with specificity ranging from 99% to 100% across the four probes used. The real-world efficacy of our platform was validated during the emergence of the Delta variant, accurately identifying Delta-positive samples within the tested pools.

In conclusion, we have developed a robust, sensitive, and flexible platform capable of accurately identifying specific SARS-CoV-2 variants within predominantly WT-SARS-CoV-2 sample pools. Moreover, the underlying principles and techniques of this platform hold significant potential for broader applications. It is not only adaptable for detecting variants from other viruses beyond SARS-CoV-2 but also versatile enough to work effectively across various sample matrices. Digital PCR's ability to detect different types of viruses, including respiratory pathogens and others like Ebola and Dengue, in blood samples, has been validated (34, 35). For instance, our work on the detection of SARS-CoV-2 in saliva (26), and the effectiveness of digital PCR in other matrices such as urine and feces for SARS-CoV-2 (36) and bacteria in blood (34), underlines the flexibility of this technology in a variety of diagnostic scenarios.

The adaptability and precision of this platform may thus contribute profoundly not only to the current fight against COVID-19, but also to future endeavors in viral epidemiology and public health. By simply modifying the targeted genetic sequences, our methodology could serve as a versatile foundation for rapid response to new viral threats, enhancing our capabilities in global health surveillance and response.

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 following link: <https://doi.org/10.6084/m9.figshare.24915639>.

Ethics statement

The studies involving humans were approved by COMITÉ DE BIOSEGURIDAD, Facultad de Ciencias Medicas, Universidad

Nacional de Rosario. The studies were conducted in accordance with the local legislation and institutional requirements. The human samples used in this study were acquired from remnants from SARS-CoV-2 tests conducted at the Hospital Provincial de Rosario. Signed informed consent was waived as patient information was anonymized and de-identified prior to analysis. This study was carried out in accordance with the principles of the 1964 Declaration of Helsinki. Written informed consent for participation was not required from the participants or the participants' legal guardians/next of kin in accordance with the national legislation and institutional requirements.

Author contributions

AP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. FP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. SH: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. GI: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. MVP: Conceptualization, Formal analysis, Investigation, Methodology, Writing – original draft. MaP: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft. YA: Investigation, Methodology, Writing – review & editing. LP: Formal analysis, Writing – review & editing. MA: Formal analysis, Writing – review & editing. LC: Investigation, Writing – original draft. AC: Conceptualization, Funding acquisition, Investigation, Resources, Writing – review & editing. JS: Conceptualization, Formal analysis, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing – review & editing.

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Epidemiology of human papillomavirus infection in women from Xiamen, China, 2013 to 2023

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Background: Cervical cancer is primarily caused by HPV infection. The epidemiology of HPV infection in specific areas is of great meaning of guide cervical cancer screening and formulating HPV vaccination strategies. Here, we evaluated the epidemiological characteristics of HPV infection in Xiamen population.

Methods: In total, 159,049 cervical exfoliated cell samples collected from female outpatients in Women and Children's Hospital, School of Medicine, Xiamen between January 2013 and July 2023 were analyzed. HPV DNA detection was performed using HPV genotyping kits (HybriBio Limited Corp, China). An analysis was conducted on the prevalence of HPV infection, taking into account factors such as age, year, and multiple patterns of HPV infection. The differences in prevalence among age groups and years were compared using χ^2 test.

Results: The overall prevalence of any 21 HPV genotypes was 18.4%, of which the high-risk HPV (HR-HPV) positive rate was 14.6%. The age-specific prevalence of HPV infection showed a bimodal distribution, with two distinct peaks, one at <25 years (31.2%) and the other at 60–64 years (32.9%). There was a downward trend in the prevalence of HPV infection over time, decreasing from 26.2% in 2013 to 14.5% in 2021, and then increasing to 19.0% in 2023. The five most prevalent HR-HPV genotypes were HPV52 (4.0%), 58 (2.6%), 16 (2.5%), 51 (1.8%), and 39 (1.7%). Among the positive cases, 76.7% were detected with only one genotype and 23.3% with multiple genotypes. The most common co-infection was HPV52 + HPV58 (0.24%), followed by HPV16 + HPV52 (0.24%), HPV52 + HPV53 (0.21%), HPV52 + HPV81 (0.21%), HPV51 + HPV52 (0.19%), HPV16 + HPV58 (0.18%), and HPV39 + HPV52 (0.17%).

Conclusion: The study provided the largest scale information on the recent epidemiological characteristics of HPV infection in Xiamen, and even in Fujian Province, China, which would support making the prevention and control strategies for cervical cancer in the region.

KEYWORDS

human papillomavirus (HPV), infection, genotype, prevalence, cervical cancer

Introduction

Cervical cancer is the fourth most prevalent cancer among women worldwide (1). More than 85% of cervical cancer cases and deaths occur in developing countries, such as China (2). In recent years, the incidence and mortality of cervical cancer in China have been increasing. An estimated number of 109,741 new cases and 59,060 deaths from cervical cancer were recorded annually in China (3), accounting for 20% of the annual global incidence (604,127 new cases) and 17% of the annual global mortality (341,831 deaths) (1). Therefore, it is urgent to take effective prevention and control measures to reduce the burden of cervical cancer in China.

It is widely acknowledged that Human papillomavirus (HPV) infection is an etiological factor for cervical cancer (4, 5). HPV is a common sexually transmitted pathogenic microorganism. More than 200 HPV genotypes have been identified to date (6). Of which, approximately 40 genotypes can infect genital tract and cause related lesions, and they are categorized as high-risk (HR-HPV) and low-risk (LR-HPV) according to their potential carcinogenicity in humans (7, 8). The HR-HPV include HPV16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 68, and it has been suggested that there was high variation in potential carcinogenicity among these genotypes (9). HPV16 and 18, the most carcinogenic genotypes, cause about 70% of cervical cancers around the world (1). Whereas, LR-HPV, such as HPV6 and HPV11, may cause genital warts and some other hyperplastic lesions.

The prophylactic HPV vaccine is the most effective primary prevention and control measure for cervical cancer or other HPV-related diseases. Currently, there are 6 licensed prophylactic HPV vaccines, including three bivalent vaccines (HPV16/18, Cervarix®, Cecolin®, and Walrinvax®), two quadrivalent vaccines (HPV6/11/16/18, Gardasil®9 and Cervavax®), and one nonavalent vaccine (HPV6/11/16/18/31/33/45/52/58, Gardasil®9) (10). The HPV vaccine has been widely used worldwide and has shown a significant effect. A significant decrease of vaccine-type related high-grade cervical lesions and cervical cancer has been observed in countries with high HPV vaccine coverage. However, HPV vaccines showed prominently genotype restricted efficacy, which can only prevent vaccine-type infection and vaccine-type related lesions. It has been reported that the prevalence and distribution of HPV genotypes varied quite a lot across geographic regions (1), and even varied among regions within the same country (11). Therefore, investigating the epidemiological characteristics of HPV infection in a certain population is the foundation of making HPV vaccination strategies in this area.

In addition, the prophylactic HPV vaccine cannot clear the acquired infection, and the current HPV vaccines do not cover all high-risk genotypes, cervical cancer screening should still be performed even after HPV vaccination. Due to the important role of HPV infection in the development of cervical cancer and high-sensitive, objective, and repeatability of HPV DNA detection, the role of HPV DNA detection has been constantly improved in cervical cancer screening. It is important to note that HPV DNA detection was recommended as the preferred screening method for cervical cancer screening by the WHO guideline (12). The implementation of HPV screening, particularly for HR-HPV, has the potential to decrease the risk of cervical cancer. Hence, it is essential to update information on the prevalence of type-specific HPV and its distribution in different geographic regions for regional HPV screening strategies.

Xiamen, an economically developed city in Fujian province, is situated in southeast China. To be our knowledge, a population-based study on the epidemiology of HPV infection is limited and outdated. Our colleagues Shen et al. (13) have previously investigated the prevalence and distribution of HPV using the data collected from November 2019 to June 2021, however, the study period coincided with the COVID-19 epidemic period, which may have impact on the characteristics of HPV. Thus, to confirm the results and to obtain additional information, in the study, we conducted a larger sample size study over a long period of time to investigate the epidemiological characteristics of HPV infections, which would support making regional prevention and control strategies for cervical cancer in Xiamen, China. In addition, from September 2020, adolescent girls aged 13.5 to 14.5 years in Xiamen were vaccinated free with HPV bivalent vaccine (Cecolin®), and the free cervical cancer screening program for women aged 35–64 years was implemented in Xiamen from 2022, our results would provide baseline information for estimating effect of HPV vaccination and HPV screening in this region.

Methods

Study population

The study population consisted of women who underwent HPV testing in gynecological outpatient and health examination center of Women and Children's Hospital, School of Medicine, Xiamen University, China from January 2013 to July 2023. All women met following conditions before sampling: (1) no sexual intercourse in 48 h; (2) no intravaginal medication in 48 h; (3) during a non-menstrual period. Women with available HPV genotyping results were included in the study, the exclusion criteria were pregnancy, incomplete identity information and baseline information (e.g., age, report date). This study was approved by the Ethics Committee of Women and Children's Hospital, School of Medicine, Xiamen University (approval number KY-2023-073-H01).

Sample collection and HPV DNA detection

Gynecologists collected cervical exfoliated cell samples from outpatients according to the established operating procedure, and stored the samples in preservation solution for HPV DNA testing. HPV DNA typing was performed using one of the two commercial HPV GenoArray Diagnostic kits, HBGA-21PKG and HBGA-37PKG (HybriBio Limited Corp, Chaozhou, Guangdong, China), which were based on DNA amplification with HPV L1 consensus polymerase chain reaction primers, can detect 21 and 37 HPV genotypes, respectively. The 21 genotypes kit was used from 2013 to 2017, which detects 13 HR-HPV genotypes (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68) and 8 LR-HPV genotypes (6, 11, 42, 43, 44, 53, 66, and 81). Since 2018, the upgraded version, the 37 genotypes kit was used, which can detect other 16 genotypes, including HPV26, 34, 40, 54, 55, 57, 61, 67, 69, 70, 71, 72, 73, 82, 83, 84. These two kits have been approved by the National Medical Products Administration (NMPA). The tests were conducted strictly in accordance with the manufacturer's instruction. In brief, the main points of the experimental protocol are as follows: (1) DNA extraction: DNA was extracted based on magnetic

beads method. The principle of the method is that the powerful protein denaturant in magnetic bead binding solution dissolve the protein and dissociate the DNA, the released DNA is bound to the magnetic bead, and then the impurities are removed by the magnetic bead washing solution, and the pure DNA is eluted down by the eluent. DNA extraction was performed by an automated nucleic acid extraction instrument using a pre-packed nucleic acid extraction kit (DaAn Gene Co, Ltd., China). (2) PCR amplification: the extracted DNA was subject to PCR amplification using HPV L1 consensus primers, the amplification reagent was configured according to the PCR mixture of 23.25 μ L, DNA polymerase 0.75 μ L and DNA 1 μ L per sample. Amplification was started with initial denaturation at 20°C for 10 min and then 95°C for 9 min, followed by 40 cycles of 95°C for 20s, 55°C for 30s, 72°C for 30s and a final extension at 72°C for 5 min. (3) hybridization: the 25 μ L of PCR products was further examined by flow through hybridization using HPV GenoArray Diagnostic Kit, the specific detection steps were as follows: 25 μ L of PCR products were denatured by heating at 95°C for 5 min and then bathed in ice for 2 min, mixed with 0.5 mL pre-warmed hybridization solution and incubated for 10 min, after which a blocking solution was added. This flow-through hybridization procedure was performed in a sample well atop a Hybrimem HPV-21/ HPV-37 membrane containing immobilized probes against which target molecules were directed. Streptavidin-horseradish peroxidase conjugate was added to bind to the biotinylated PCR products. The direct visualization of the breakdown product (purple dot) of the substrate nitroblue tetrazolium-5-bromo-4-chloro-3-indolylphosphate was interpreted as positive for the corresponding HPV DNA type as indicated on the schematic diagram of the membrane provided with the test kit. HPV negative and positive controls provided in the kit were simultaneously detected in every test for quality control (13–15).

Statistical analysis

The first sample for HPV genotype evaluation from each woman collected between January 2013 and July 2023 was considered, and therefore ensured that women were only included once in the analysis. In the study, the common 21 genotypes detected by both HBGA-21PKG and HBGA-37PKG were analyzed. We calculated the overall, age-specific, and year-specific prevalence of HPV infection, respectively. The difference in the prevalence of HPV infection among age groups and year were analyzed using χ^2 test. The age-specific infection pattern (single, double and multiple infection) of HPV genotypes was also evaluated. Subsequently, we calculated the prevalence of single and multiple infection, respectively. Moreover, to quantify the co-infection preference of 21 HPV genotypes, we also created a heatmap of prevalence of any two HPV genotypes. $p < 0.05$ was considered statistically significant. Statistical analyses were performed using SAS version 9.4 software (SAS Institute, Cary, North Carolina).

Results

HPV prevalence

From January 2013 to July 2023, a total of 159,049 female outpatients aged 15 to 92 (mean age: 35.9 ± 9.2) years old were involved

in the study. Of the 159,049 specimens, 29,198 women were positive for HPV, with an overall HPV infection rate of 18.4% (29,198/159,049). The prevalence of HR-HPV and LR-HPV were 14.6% (23,252/159,049) and 5.9% (9,399/159,049), respectively. The prevalence of overall HPV infection showed a downward trend over time (Cochran-Armitage $\chi^2 = 21.03$, $p < 0.0001$), the overall prevalence of HPV infection decreased from 26.2% (1,471/5,608) in 2013 to 14.5% (3,063/21,076) in 2021, and then increased to 19.0% (1,351/7,094) in 2023. Similar trends were observed for the HR-HPV (decreased from 20.7% in 2013 to 11.5% in 2021, and then increased to 14.4% in 2023) and LR-HPV (decreased from 9.0% in 2013 to 4.4% in 2021, and then increased to 6.8% in 2023; [Figure 1](#); [Supplementary Table S1](#)).

When women were stratified into 10 age groups of <25 years, 25–29 years, 30–34 years, 35–39 years, 40–44 years, 45–49 years, 50–54 years, 55–59 years, 60–64 years, and ≥ 65 years, we found that the prevalence was significantly different ($\chi^2 = 1697.20$, $p < 0.0001$), and the age-specific prevalence of HPV infection was “U-shaped” distribution, with the highest overall prevalence of HPV was found in women aged 60–64 years (32.9%, 520/1,579), while the lowest HPV prevalence was found in women aged 30–34 years (15.8%, 6,910/43,835). Similarly, the prevalence of HR-HPV and LR-HPV also differed among these age groups ($p < 0.0001$). The highest prevalences of HR-HPV and LR-HPV were found in women aged 60–64 years (28.0%, 442/1,579) and women <25 years (13.2%, 1,105/8,347), respectively. While the lowest rates were both found in women aged 30–34 years (HR-HPV: 12.5%, 5,466/43,835; LR-HPV: 4.8%, 2,090/43,835; [Figure 2](#); [Supplementary Table S2](#)).

Single and multiple HPV infections

Single HPV infection was observed to be the most common pattern, accounting for 76.7% (22,407/29,198) of all HPV positive cases, with an overall prevalence of 14.1% (22,407/159,049). The prevalence of multiple infections was relatively low (4.3%, 6,791/159,049), accounting for 23.3% (6,791/29,198) of HPV positive cases. And of which, most women were infected with 2 genotypes, with a prevalence of 3.2% (5,006/159,049), accounting for 17.1% (5,006/29,198) of HPV positive cases. Three or more genotypes were found in 1,785 women with a prevalence of 1.1% (1,785/159,049), accounting for 6.1% (1,785/29,198) of HPV positive cases. We further analyzed multiple infection cases with HR-HPV types only, LR-HPV types only, and combination of HR-HPV and LR-HPV types. HR-HPV only infection accounted for 44.6% (3,030/6,791) of multiple infections, with a prevalence of 1.9% (3,030/159,049). 50.8% (3,453/6,791) of multiple infections showed both HR-HPV and LR-HPV infections for a prevalence of 2.2% (3,453/159,049) for all tested women. While only 308 women had 2 or more LR-HPV types, accounting for 4.5% (308/6,791) of multiple infections. The prevalences of multiple infections were also different among age groups, however, the pattern was similar, co-infection with both HR-HPV and LR-HPV was most common in all age groups ([Table 1](#)).

HPV genotype distribution

As shown in [Figure 3](#), HPV52 (4.0%, 6,358/159,049) was the most commonly detected genotypes, followed by HPV58 (2.6%,

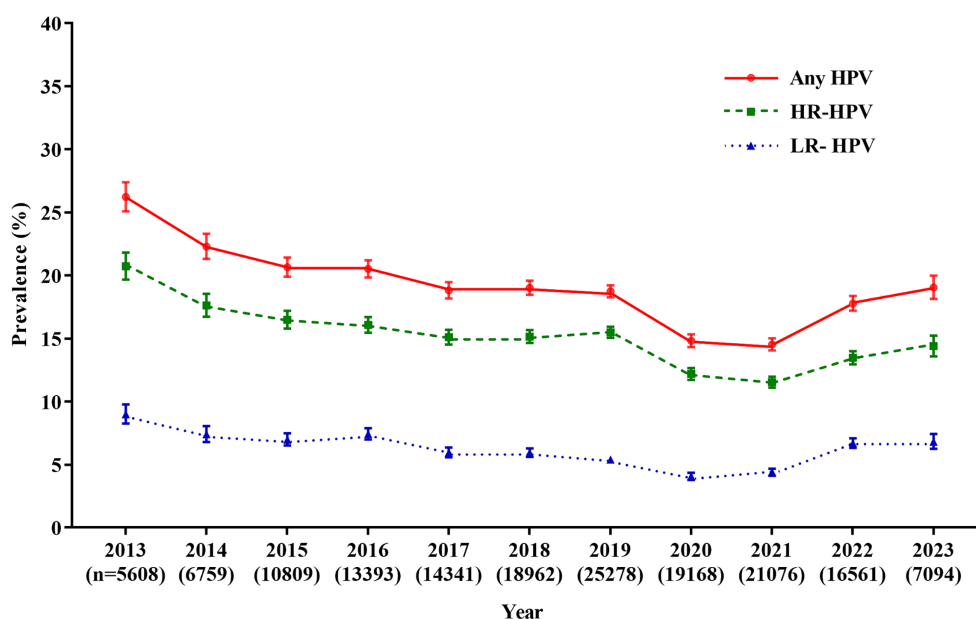


FIGURE 1

Prevalence of HPV infection over the past 10.5 years. Any HPV: any of 21 detected HPV types; HR-HPV, high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68); LR-HPV, low-risk HPV types (6, 11, 42, 43, 44, 53, 66, and 81); HPV, human papillomavirus.

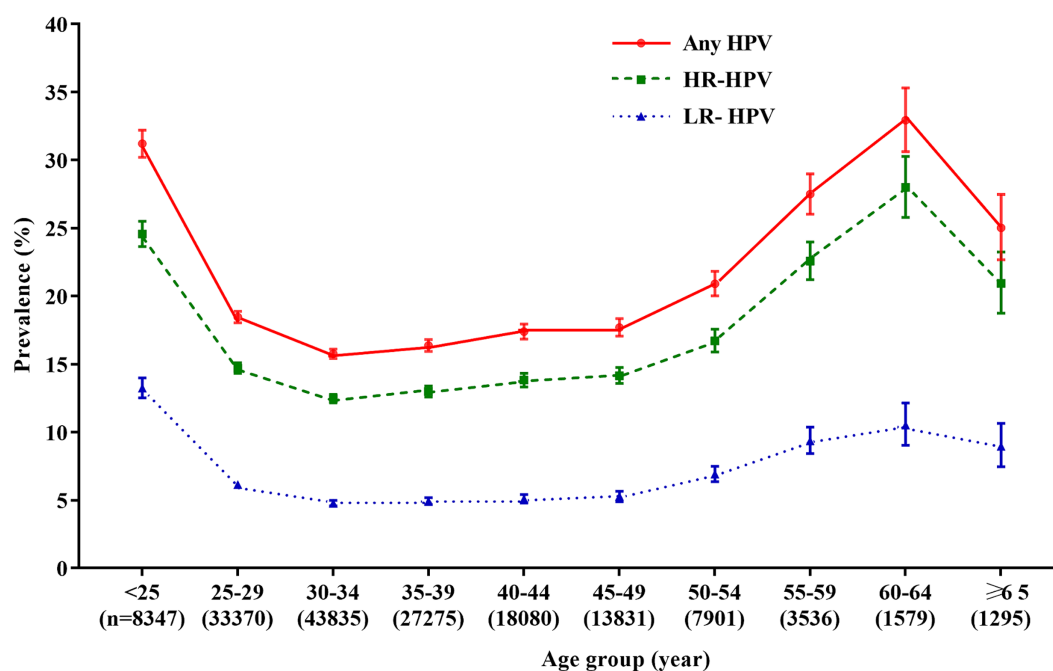


FIGURE 2

Age-specific prevalence of HPV infection. Any HPV: any of 21 detected HPV types; HR-HPV, high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68); LR-HPV, low-risk HPV types (6, 11, 42, 43, 44, 53, 66, and 81); HPV, human papillomavirus.

4,097/159,049), 16 (2.5%, 3,958/159,049), 51 (1.8%, 2,905/159,049), and 53 (1.7%, 2,772/159,049). And other genotypes with a prevalence of more than 1.0% included HPV39 (1.7%), 81 (1.6%), 18 (1.1%), and 68 (1.0%). Additionally, the five most prevalent HPV genotypes also accounted for the highest rates in the top five of both single and multiple infections. In single infection, the prevalences of the top five

prevalent genotypes were HPV52 (2.6%, 4,099/159,049), HPV58 (1.6%, 2,520/159,049), HPV16 (1.5%, 2,438/159,049), HPV51 (1.1%, 1,678/159,049), and HPV53 (1.0%, 1,601/159,049), and that were HPV52 (1.4%, 2,259/159,049), HPV58 (1.0%, 1,577/159,049), HPV16 (1.0%, 1,520/159,049), HPV51 (0.8%, 1,227/159,049), and HPV53 (0.7%, 1,171/159,049) in multiple infections.

TABLE 1 Prevalence of HPV single/multiple infection in different age groups.

Age group	<25 (N = 8,347)	25–29 (N = 33,370)	30–34 (N = 43,835)	35–39 (N = 27,275)	40–44 (N = 18,080)	45–49 (N = 13,831)	50–54 (N = 7,901)	55–59 (N = 3,536)	60–64 (N = 1,579)	≥65 (n = 1,295)	Total (N = 159,049)
Age, years, mean (SD)	22.6 (1.5)	27.3 (1.4)	31.8 (1.4)	36.8 (1.4)	41.9 (1.4)	46.8 (1.4)	51.7 (1.4)	56.7 (1.4)	61.7 (1.4)	69.8 (5.1)	35.9 (9.2)
Positive for HPV Infection, n (%)											
Single infection	1,651 (19.8)	4,608 (13.8)	5,520 (12.6)	3,582 (13.1)	2,570 (14.2)	1,964 (14.2)	1,270 (16.1)	689 (19.5)	349 (22.1)	204 (15.8)	22,407 (14.1)
multiple infections	954 (11.4)	1,551 (4.6)	1,390 (3.2)	883 (3.2)	574 (3.2)	483 (3.5)	382 (4.8)	283 (8.0)	171 (10.8)	120 (9.3)	6,791 (4.3)
2 infections	606 (7.3)	1,128 (3.4)	1,078 (2.5)	701 (2.6)	457 (2.5)	388 (2.8)	283 (3.6)	185 (5.2)	117 (7.4)	63 (4.9)	5,006 (3.2)
≥3 infections	348 (4.2)	423 (1.3)	312 (0.7)	182 (0.7)	117 (0.7)	95 (0.7)	99 (1.3)	98 (2.8)	54 (3.4)	57 (4.4)	1,785 (1.1)
Pattern of multiple Infections, n (%)											
HR-HPV + HR-HPV	355 (4.3)	683 (2.0)	672 (1.5)	420 (1.5)	277 (1.5)	224 (1.6)	157 (2.0)	118 (3.3)	73 (4.6)	51 (3.9)	3,030 (1.9)
HR-HPV + LR-HPV	550 (6.6)	801 (2.4)	646 (1.5)	420 (1.5)	275 (1.5)	239 (1.7)	214 (2.7)	157 (4.4)	88 (5.6)	63 (4.9)	3,453 (2.2)
LR-HPV + LR-HPV	49 (0.6)	67 (0.2)	72 (0.2)	43 (0.2)	22 (0.1)	20 (0.1)	11 (0.1)	8 (0.2)	10 (0.6)	6 (0.5)	308 (0.2)

HR-HPV, high-risk HPV types (16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68); LR-HPV, low-risk HPV types (6, 11, 42, 43, 44, 53, 66, and 81); HPV, human papillomavirus.

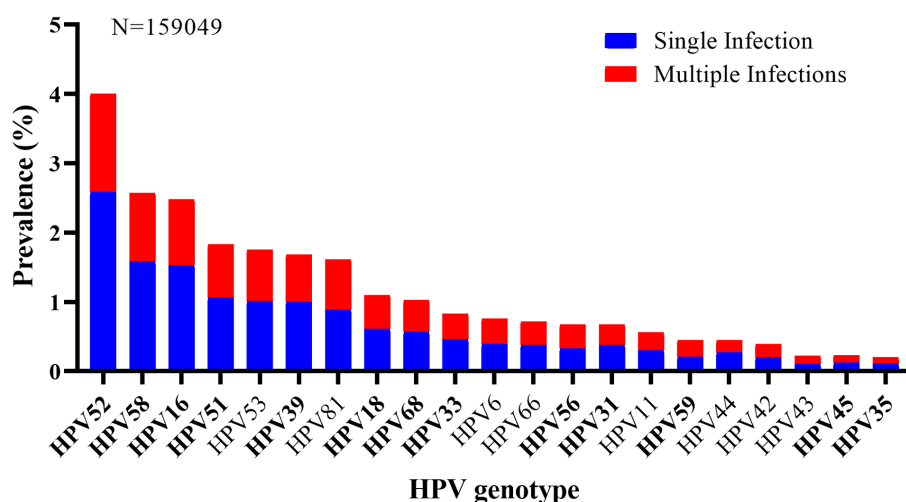


FIGURE 3
HPV genotypes distribution of single (in blue) and multiple infections (in red).

We further calculated the prevalence of co-infection with any 2 genotypes out of the 21 HPV genotypes. The most common co-infection was HPV52 + HPV58 (0.24%), followed by HPV16 + HPV52 (0.24%), HPV52 + HPV53 (0.21%), HPV52 + HPV81 (0.21%), HPV51 + HPV52 (0.19%), HPV16 + HPV58 (0.18%), HPV39 + HPV52 (0.17%). Interestingly, we found that HPV35, HPV45, HPV43, HPV44 were rarely co-infected with other genotypes (Figure 4; Supplementary Table S3).

Discussion

Epidemiological characteristics of HPV infection in a region are one of the important steps toward cervical cancer prevention. Here, we conducted a large clinic-base study to investigate the characteristics of HPV infection in Xiamen, southeast China. The study population was composed of gynecological outpatients and asymptomatic women, which would be more representative of the overall HPV infection in women from Xiamen.

The prevalence of HPV infection varied considerably across geographic regions. Globally, the highest prevalence of HPV infection was in some countries in Africa, South America and Europe ($\geq 33.87\%$), followed by some European countries and Australia (16.93–33.87%), and some countries in North America and South America, and China ($< 16.93\%$) (1). Even in one country, especially in China, the prevalence of HPV infection varies greatly due to its large population and territory. In the current study, the prevalence of HPV was 18.4%, which was higher than that reported in a pooled analysis in China (15.5%) (16), but lower than that in Guangzhou (21.7%) (17), Zhejiang (22.3%) (18), Jiangxi (22.5%) (19), and other northern regions, such as Heilongjiang (27.1%) (20) and Henan (19.7%) (21). Significantly, the prevalence of HPV was also much lower than that in a previous study in Fuzhou (38.3%) (22). The reported prevalence varies among studies because of the difference in population composition, testing methods, and region. In addition, the sampling periods may be part of the reason. We found the prevalence of overall HPV, HR-HPV and LR-HPV showed a

downward trend from 2013 to 2021 (overall HPV: from 26.2 to 14.5%, HR-HPV: from 20.7 to 11.5%, LR-HPV: from 9.0 to 4.4%). Similarly, the overall HPV prevalence was found to decline from 28.7% in 2011 to 17.8% in 2014 in Zhejiang (23). The reason for the decline in HPV prevalence may be due to the improvement of health awareness and the popularization of HPV. Considering an increasing number of asymptomatic women attended HPV screening, the prevalence of HPV decreased, and tended to be natural infection rate. Interestingly, the prevalence of HPV increased in 2022 and 2023, which may be attributed to the free cervical cancer screening program implemented in Xiamen from 2022. The data of free cervical cancer screening program was not included in this study, participation of asymptomatic women in cervical cancer screening programs has led to an increase in the proportion of gynecological outpatients in our study population, which in turn contributed to the increase in HPV infection rates. However, the reasons for the increase of HPV infection in these 2 years remain unknown and should be investigated. The changes of HPV infection should be monitored continuously in Xiamen.

The prevalence of HPV infection was also different among age groups. Globally, the prevalence of HPV infection peaks ($\sim 30\%$) shortly after the debut of sexual activity (< 25 years), and then quickly decreases and keeps at low rate ($< 10\%$) in women aged 35–44 years, and then increases slightly in women aged ≥ 65 years (1). However, numerous studies have demonstrated that the age-specific prevalence of HPV showed a bimodal distribution in Chinese women (23–28). In line with previous studies, our study found that the age-specific prevalences of overall HPV, HR-HPV, and LR-HPV all showed a bimodal distribution, with a peak at the women < 25 years and another at the women aged 60–64 years. Although the age of infection peak varied in different studies, the first peak was generally found in young women with early twenties, and the second peak was usually found in menopausal women. The first peak observed in young women may be related to sexual activity and immature immunity to HPV. It is reported that the risk of acquiring HPV infection was 46% at 3 years after first intercourse, and the median time from first intercourse to first detection of HPV was only 2.6 months (29). Thus, considering the

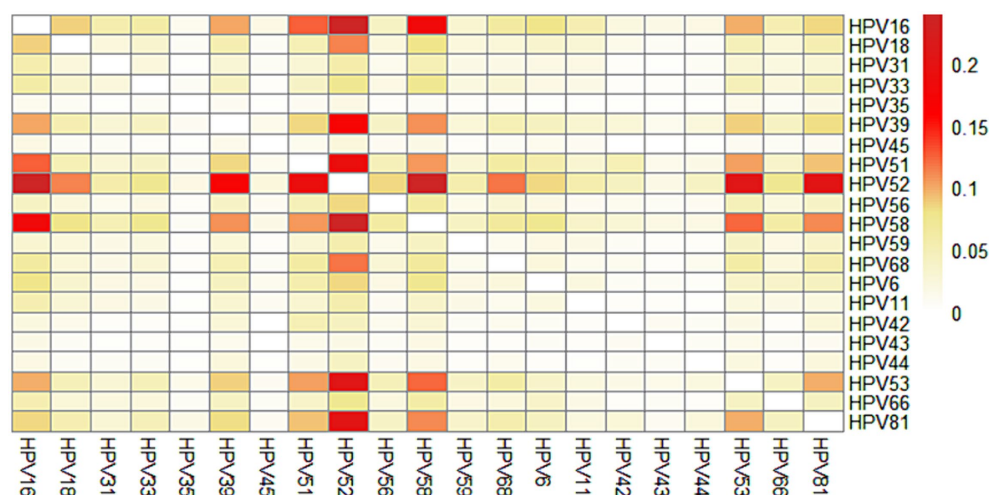


FIGURE 4
Prevalence of HPV involved in women with HPV co-infection by genotypes.

nature of prophylactic HPV vaccines, it is the optimum time for adolescent females to receive HPV vaccines before the onset of sexual activity (i.e., before exposure to HPV). The understanding of the second peak in older women was limited, HPV persistence or the reactivation of potential HPV caused by physiological and immune disorders during the period of menopause, and changes in the sexual behavior during middle age may be the explanations (30). Therefore, it is necessary to conduct HPV screening in perimenopausal women in China.

According to HPV prevalence surveys, HPV16 and 18 are the most prevalent genotypes worldwide, which cause approximately 70% of cervical cancer (1). However, it was reported that HPV52 and HPV58 are more prevalent in Asian, especially in China (31–33). In our study, HPV52, 58, 16, 51 and 53 were found to be the five most prevalent HPV genotypes in Xiamen, which was similar to previous surveys in Sichuan and Yunnan province. However, in a previous study conducted in Fujian, HPV16 was identified to be the most frequent (8.6%) (22). This might be due to the difference in population composition and survey period. It has been reported that HPV52 and 58 account for 33.3% of high-grade lesions and 14.7% of cervical cancer, and HPV51 accounts for 3.9% of high-grade lesions in China (1). Hao et al. (34) suggested that HPV51 and HPV53 were found in 6.3% of HPV infection and 8.9% of cervical cancer in Jilin province, China. Considering the high prevalence and carcinogenicity of HPV52, 58, 51 and 53, except for HPV16 and 18, more attention also should be paid to preventing and control these genotypes infection in Xiamen, including the development of HPV prophylactic vaccines based on HPV16 and HPV18, covering HPV52, 58, 51 and 53, and implementing the HPV screening programs which focus on HPV52, 58, 51 and 53, as with HPV16 and 18.

Co-infection with multiple genotypes is common in HPV-positive individuals. In our study, multiple infections accounted for 23.3% of all infections, which is lower than that in Guangzhou (26.5%) (17), Shanghai (36.6%) and Beijing (27.7%), and similar to that in Zhejiang (22.5%) and Shanxi (24.3%) (35). There is ongoing debate regarding the impact of multiple HPV infections on the development of cervical cancer. Several studies have suggested an extended duration of

multiple HPV infections and a stronger association between multiple HPV infections and precancerous lesions/cervical cancer (36–39). However, some other studies have reported that multiple HPV infections were no additive or synergistic effect on the development of cervical precancerous lesions and cervical cancer (40, 41). The carcinogenicity of multiple infections may be related to the different HPV genotypes combinations. However, few studies focus on whether different HPV genotypes combinations could interact on promoting or decreasing the oncogenicity. Carrillo-Garcia et al. (38) found that the co-infection of 68 with 16 increases the risk of ICC/HISL. Wang et al. (39) suggested that multiple HR-HPV infection with HPV16/18 had a higher risk of CIN2+, while multiple HR-HPV infection without HPV16/18 did not increase the risk significantly. Brun et al. (42) also found it is probable that only specific combinations of HPV can be associated with a clinically significant impact. In the study, we found that HPV52 + HPV58 and HPV16 + HPV52 were the most prevalent combinations of genotypes, which is consistent with the findings of Wang et al. (43), while Chen et al. (23) reported that HPV16 + HPV58, HPV16 + HPV18 and HPV16 + HPV52 were the most frequent in Zhejiang. Further study is necessary to explore the potential role of multiple HPV infections, especially the interactions among these common specific HPV genotypes in the development of cervical cancer.

To our knowledge, this study provides the largest scale information on the recent HPV prevalence and genotype distribution in Xiamen, and even in Fujian Province, China. However, several limitations existed. First, our study only included those women who underwent HPV testing in the gynecological outpatient and health examination center of Women and Children's Hospital, School of Medicine, Xiamen University, and most of them were outpatients with gynecological disorders. In this respect, the findings may not represent all women in Xiamen, even in Fujian. Second, we did not collect detailed behavioral information such as the number of sexual partners, smoking habits, to assess the impact of these factors on the prevalence of HPV infection. Third, the results of cervical cytology or histology abnormalities were not collected, thus, we cannot further analysis the characteristics of HPV infection based on cervical lesion classification.

In conclusion, the age-specific prevalences of any HPV showed a bimodal distribution, with peaks at the women aged <25 years and 60–64 years, which provide evidence for the younger females for HPV vaccination and provide clinical guideline for perimenopausal women in cervical cancer screening in Xiamen. The prevalent genotypes were HPV52, 58, 16, 51, and 53, which suggests that more attention should be paid to these genotypes in vaccine development and cervical cancer screening process in Xiamen. In addition, we found the prevalence of HPV increased in 2022 and 2023, although it may be related to the free cervical cancer screening program implemented, it still deserves to pay closer attention and monitor the changes of HPV infection in the region in the future.

Data availability statement

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

Ethics statement

The studies involving humans were approved by Women and Children's Hospital, School of Medicine, Xiamen Ethical Review Board. The studies were conducted in accordance with the local legislation and institutional requirements. Written informed consent from the patients was waived due to the retrospective nature of the study.

Author contributions

XY: Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Visualization, Writing – original draft, Writing – review & editing. QL: Conceptualization, Data curation, Investigation, Resources, Writing – review & editing. YC: Formal analysis, Validation, Visualization, Writing – original draft. ZD: Data curation, Formal analysis, Writing – review & editing. YH: Data curation, Writing – review & editing. YiZ: Data curation, Validation, Writing – review & editing. JZ: Data curation, Validation, Writing – review & editing. WW: Data curation, Validation, Writing – review & editing. LZ: Data curation, Validation, Writing – review & editing. JX: Data curation, Validation, Writing – review & editing. CX: Data curation, Validation, Writing – review & editing. YG: Conceptualization, Data

curation, Resources, Validation, Writing – review & editing. YuZ: Conceptualization, Funding acquisition, Resources, Supervision, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2024.1332696/full#supplementary-material>

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Comparison of multiplex PCR capillary electrophoresis assay and PCR-reverse dot blot assay for human papillomavirus DNA genotyping detection in cervical cancer tissue specimens

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Background: The study aimed to evaluate the positivity rates and genotype distribution of the multiplex PCR capillary electrophoresis (MPCE) and PCR-Reverse Dot Blot (PCR-RDB) assays for human papillomavirus (HPV) detection in cervical cancer tissue specimens, and to explore their detection principles and applications in large-scale population screening.

Methods: The MPCE and PCR-RDB assays were performed separately on 425 diagnosed cervical cancer tissue specimens. Subsequently, the results of both assays were compared based on the HPV infection positivity rates and genotype distribution.

Results: The overall positive rates of HPV genotypes for the MPCE and PCR-RDB assays were 97.9% and 92.9%, respectively. A p -value < 0.001 indicated a statistically significance difference in consistency between the two assays. The kappa value was 0.390, indicating that the consistency between both assays was fair. HPV16 was the most common single-genotype infection type, with infection rates detected via MPCE and PCR-RDB assays being 75.7% and 68.3%, respectively. In the age group >50 years, the HPV multiple-type infection rate detected via MPCE assay was significantly higher than that detected by the PCR-RDB assay, with a statistically significant difference ($p = 0.002$).

Conclusion: To reduce the false-negative rate and improve screening efficiency, the MPCE assay, which targets the oncogenic gene E6/E7 segments, can be extended to the general female population for the early detection, diagnosis, and treatment of cervical cancer.

KEYWORDS

HPV DNA detection, polymerase chain reaction, cervical cancer tissue specimen, multiplex PCR capillary electrophoresis assay, PCR-reverse dot blot assay

1 Introduction

Cervical cancer ranks as the fourth most common malignant tumor threatening the life and health of women worldwide (1). The primary cause of cervical cancer and its pre-cancerous lesions is the persistent infection of oncogenic human papillomavirus (HPV) (2). An epidemiological study indicates that almost all cases of cervical cancer are attributable to HPV (3). However, approximately 5% of tumors are not associated with persistent HPV infection (4). Cervical cancer stands out as the only gynaecological malignant tumor with well-defined etiology globally. It is also unique among malignant tumors in that it can be eradicated through comprehensive tertiary preventive measures.

In 2021, the “WHO guideline for screening and treatment of cervical pre-cancer lesions for cervical cancer prevention” recommends using HPV DNA testing as part of a comprehensive approach to screening, triage, and treatment. This guideline advises regular screening at the age of 30 years, with intervals of 5 to 10 years between screenings (5). In 2022, the latest Cervical Cancer Screening Programme in China included HPV nucleic acid testing as one of the primary screening methods (6). HPV DNA detection can be performed on cervical specimens using signal amplification techniques or nucleic acid amplification through polymerase chain reaction (7). These assays have proven to be more sensitive and efficient than cytology methods. Additionally, they are more objective, as they do not rely on visual inspection expertise (8).

HPV is a closed circular double-stranded DNA virus with a genome length of approximately 8 kilobase pairs (kbp). Its genome encodes early regulatory proteins (E1, E2, E4, E5, E6, and E7), late structural proteins (L1 and L2), and the long control region. The early proteins primarily participate in virus replication and transcriptional regulation, while the late proteins are responsible for forming the virus capsid. The long control region is a regulatory area of the virus closely associated with virus replication and transcriptional processes. Genotypes with genome sequence differences >10% are categorized as different genotypes (9–11). Over 200 HPV genotypes have been identified and classified into High-Risk (HR-HPV) and Low-Risk (LR-HPV) types based on their potential to cause cancer (12). HPV16 and 18 are widely recognized as the predominant HR-HPV types worldwide, while HPV52 and HPV58 are commonly found in Asia (13–15).

The significant geographical variation in cervical cancer rates is largely due to non-existent or inadequate screening in public health care settings, coupled with limited access to standard treatment options. Detecting HPV infection in cervical cancer specimens is considered the gold standard for confirming persistent high-risk HPV infection and establishing the pathogenetic association between HPV infection and cervical cancer. However, the challenge lies in obtaining pathologic tissue specimens, which poses a challenge for scientific research. Formalin-fixed, paraffin-embedded (FFPE) tissue specimens stored in pathology departments worldwide are crucial resources for diagnostic purposes when fresh clinical material is unavailable. They also serve as invaluable resources for retrospective molecular and epidemiological studies, especially when investigating rare clinical conditions where prospective data collection is impractical. The quality of extracted DNA is usually assessed via PCR amplification of housekeeping genes. DNA fragmentation can significantly impair PCR efficiency. Hence, primers that amplify a relatively small fragment

of the human genome (<270 bp) when working with FFPE material should be used preferably. An amplifiable internal control ensures successful DNA extraction, the absence of PCR inhibitors, and reliable HPV detection, enabling the performance of HPV genotyping on small molecular fragments in cervical cancer tissues (16).

Screening measures are essential for patients with detected early cervical lesions (17). In China, the predominant techniques for HPV nucleic acid detection rely on nucleic acid amplification and its derivatives, which are effective in detecting the entire HPV genome or a specific segment of it (18, 19). In recent years, nearly 100 different HPV assays have become commercially available, varying in the principles, types, and number of HPV genotypes they detect, as well as their clinical utility. Target region selection has a significant impact on an HPV DNA test's ability to detect cancers. The majority of assays target the L1 region of the virus, and the gene is prone to deletion during virus integration as the disease progresses (20). Therefore, selecting an appropriate and validated test for clinical accuracy, reproducibility, and cost-effectiveness is crucial before implementing screening programs (21–23). This study aimed to assess the diagnostic value of two commonly used HPV DNA genotyping assays: the Multiplex PCR Capillary Electrophoresis (MPCE; targeting E6/E7 segments) and PCR-Reverse Dot Blot (PCR-RDB; targeting L1 segment) assays. McNemar's test was performed to compare the consistency of genotyping results of both assays in cervical cancer tissue specimens (CCTS) from 425 patients with cervical cancer who underwent surgery. The findings of this study could help to facilitate the selection of appropriate HPV nucleic acid detection reagents and systems based on national cervical cancer prevention and control strategies, laboratory requirements, consideration methodology, different detection targets and types, automation levels, and accessibility. This selection could provide a theoretical basis for the strategy of ‘HPV vaccination with HPV nucleic acid detection-based cervical cancer screening’ in the Chinese female population.

2 Materials and methods

2.1 Samples

Overall, 425 samples of FFPE tissue specimens were selected from patients diagnosed with cervical cancer by the pathology department at four hospitals in eastern China, spanning from August 2021 to the end of July 2023 (Supplementary Table S1). Two experienced pathologists reviewed the samples according to the classification standards for gynecological tumors (24). They examined the FFPE cervical cancer tissue sections and the clinical pathology data of the patients to ensure the quality of the cancer tissue samples. The age of patients ranged from 21 to 88 years old, with an average age of 54.69 ± 11.92 years. Simultaneously, patient demographic data were collected. This study was approved by the ethics committee of the hospitals, and all participants provided informed consent.

2.2 DNA extraction from FFPE cervical cancer tissue specimens

The QIAamp DNA FFPE Tissue Kit (Catalog Number: 56404, QIAGEN GmbH, Germany) was used to purify genomic DNA from

FFPE tissue sections. Firstly, the excess paraffin around each paraffin-embedded tissue was removed and each paraffin-embedded tissue was cut into 5 µm thick slices, yielding for 5–8 slices. The tissue pellet was resuspended in 180 µL Buffer ATL buffer and 20 µL proteinase K, then incubated at 56°C for 1 h, to partially reverse the formaldehyde modification of DNA, the samples were then incubated at 90°C for 1 h. After brief centrifugation, we added 2 µL RNase A (100 mg/mL) and incubated samples at room temperature for 2 min to avoid RNA contamination. In the following two steps, AL buffer and ethanol were consecutively added to samples and vortexed thoroughly. Next, we transferred the entire lysate to the QIAamp MinElute column and placed it in a 2 mL collection tube. After centrifugation, we placed the QIAamp MinElute column in a clean 2 mL collection tube. The nucleic acid was adsorbed to the membrane of the QIAamp MinElute column and then washed by AW1 and AW2 buffer. Finally, 50 µL ATE buffer was added to the center of the membrane. After incubating at room temperature for 5 min, the samples were centrifuged at 14,000 rpm for 2 min, and the DNA was collected into new sterile 1.5 mL micro-centrifuge tubes. The Experiment was performed according to the QIAamp DNA FFPE Tissue Kit manufacturer's handbook.

2.3 HPV-DNA genotyping test

Both MPCE and PCR-RDB assays were employed separately to detect and obtain HPV genotyping results from the 425 DNA samples. Table 1 shows the characteristics of the two PCR HPV-DNA genotyping assays: MPCE and PCR-RDB assays. The MPCE and PCR-RDB assays targeted E6/E7 and L1, respectively. Table 1 shows that the MPCE assay can detect one additional HR-HPV26 type and one additional LR-HPV44 type compared to the PCR-RDB assay.

2.3.1 MPCE assay

HPV genotyping was conducted on 425 samples using the MPCE assay (Ningbo HEALTH Gene Technologies Co., Ltd. China). The SureX® HPV 25X Genotyping Kit (Catalog Number: 06937044500040), Real-time PCR System SLAN-96P (Fosun Diagnostics (Shanghai) Co., Ltd. China), and CE2400 Automated Capillary Electrophoresis

Analyzer (Ningbo HEALTH Gene Technologies Co., Ltd. China) were employed to detect HPV targeting the carcinogenic E6/E7 gene segments, allowing for specific amplification and capillary electrophoresis separation of genes from different HPV genotypes. The PCR program included an initial denaturation step at 42°C for 5 min and 95°C for 8 min, followed by 35 cycles of 94°C for 30 s, 60°C for 30 s, 70°C for 60 s, with a final extension at 70°C for 1 min. Internal reference controls, pcDNA, and human genomic reference β-globin were used to monitor the PCR reaction process and sample handling, respectively. The detection procedure strictly adhered to the instructions provided in the reagent kit and detection system manual. The testing process was fully automated and integrated.

2.3.2 PCR-RDB assay

HPV genotyping was performed on 425 samples using PCR-RDB assay (Yaneng Bioscience, Shenzhen, China). The HPV Genotyping Test Kit (Catalog Number: 210608), Real-time PCR System SLAN-96P (Fosun Diagnostics (Shanghai) Co., Ltd. China), and HPV Genotyping Gene Chip Detection System (Yaneng BIOScience Shenzhen Co., Ltd.) were used to detect 23 HPV genotypes, including 17 HR HPV and 6 LR HPV genes, targeting L1 segment (Table 1). The detection process was conducted following the manufacturer's instructions on the test kit and detection system, including DNA extraction, amplification, hybridization, membrane washing, and data interpretation. The PCR program included an initial denaturation step at 50°C for 15 min 95°C for 10 min, followed by 10 cycles of 94°C for 10 s, 42°C for 90 s, 72°C for 30 s. This was followed by 30 cycles of 94°C for 10 s, 46°C for 60 s, and 72°C for 20 s, with a final extension at 72°C for 5 min. Interpretation was conducted based on the specific HPV genotype sites on the chip. Negative and positive controls were included throughout the experiment. Simultaneously, internal and external quality evaluations were conducted to ensure that the results met the requirements of the laboratory.

2.4 Statistical analysis

A paired design χ^2 test (McNemar's) was performed to compare the positivity rates of the MPCE and PCR-RDB assays with the gold standard (pathological histological diagnosis results). The kappa value was used to assess the consistency between both assays. The positivity rates of two different HPV genotyping assays were analyzed in CCTS, exploring the differences in positivity rates between both assays and their corresponding pathological basis. The chi-square and Fisher's exact tests were used to assess association and compare genotypes by age. Statistical significance was set at $p < 0.05$. All statistical analyses were performed using SPSS 19.0 (IBM Corp., Armonk, NY, United States).

3 Results

3.1 Analysis of the results obtained from the two assays

Table 2 shows that among the total 425 CCTS, the MPCE assay detected nine negative results, accounting for 2.1% (9/425), while the PCR-RDB assay detected 30 negative results, accounting for 7.1% (30/425). There were eight cases in which neither assay detected HPV infection. The overall positive detection rates of HPV genotypes for the

TABLE 1 Characteristics of the two PCR HPV-DNA genotyping assays.

Assay	Type of technology	Gene targeted	HPV DNA genotyping	Internal quality control
MPCE	Multiplex PCR capillary electrophoresis	E6/E7	HR-HPV: 16, 18, 26, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82	Human b-globin gene
			LR-HPV: 6, 11, 42, 43, 44, 81, 83	And PcDNA
PCR-RDB	PCR-Reverse Dot Blot	L1	HR-HPV: 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82	β-actin gene segment
			LR-HPV: 6, 11, 42, 43, 81, 83	

TABLE 2 Overall positive rates of HPV DNA between the two assays.

		MPCE		Total	McNemar test	Kappa value
		Positive	Negative			
		N (%)	N (%)			
PCR-RDB	Positive	394(94.7)	1(11.1)	395(92.9)	$p < 0.001^*$	0.390
	Negative	22(5.3)	8(88.9)	30(7.1)		
	Total	416(97.9)	9(2.1)	425(100.0)		

*A P -value < 0.001 indicated a statistical difference in consistency between the two assays. The kappa value is 0.390 indicated that the consistency between the two assays was fair.

MPCE and PCR-RDB assays were 97.9% and 92.9%, respectively, both $>90\%$. This suggests that both assays demonstrated relatively high overall positive rates for HPV detection in CCTS. A p -value < 0.001 indicated a statistical difference in consistency between both assays. Agreement between the tests was assessed based on the kappa value. Kappa values range from 0.81–1.00, indicating almost perfect agreement, 0.61–0.80 substantial agreement, 0.41–0.60 moderate agreement, 0.21–0.40 fair agreement, and 0.00–0.20 slight agreement. The kappa value was 0.390, indicating that the consistency between both assays was fair.

3.2 HPV genotyping distribution in HPV-positive CCTS

Table 3 presents the distribution of the top nine high-frequency single HPV genotype infections and mixed infections detected via both assays in CCTS. The results showed that the positive rates of single-type HPV-16 infection detected via the MPCE and PCR-RDB assays were 75.7% and 68.3%, respectively, in CCTS. Furthermore, the positive rates of single-type HPV-18 infection detected through the MPCE and PCR-RDB assays were 9.9% and 11.8% in CCTS, respectively.

In the MPCE assay, among the total 416 HPV-positive detections, single and multiple infections accounted for 73.1% (304/416) and 26.9% (112/416), respectively. Among the 112 cases with multiple infections, HPV16 + 18 (25.9%, 29/112) was the most common genotype combination, followed by HPV16 + 58 (11.6%, 13/112) and HPV16 + 33 (9.8%, 11/112; Figures 1, 2).

In the PCR-RDB assay, among the 395 HPV-positive detections, single and multiple infections accounted for 83.8% (331/395) and 16.2% (64/395), respectively. Among the 64 cases with multiple infections, HPV16 + 18 (14.1%, 9/64) and HPV16 + 58 (14.1%, 9/64) were the most common genotype combinations, followed by HPV16 + 6 (9.4%, 6/64; Figures 1, 2).

Despite the differences between both assays, HPV16 was the most frequent type in single-type and multiple-type HPV infections within the CCTS. Furthermore, no statistical difference was observed in the HPV Genotyping distribution rate between both assays.

3.3 Comparison of positive rates of single-type and multiple-type HPV DNA genotyping infections across different age groups between the two detection assays

Among all the detected positive cases in both assays, in the population over 50 years old, the HPV multiple-type infection rate detected via the MPCE assay was significantly higher than that

detected by the PCR-RDB assay, with statistical significance ($p = 0.002$). This indicates that in the age group over 50 years old within the CCTS, the MPCE detection assay demonstrates a higher proportion of detecting multiple HPV infections (Table 4).

4 Discussion

HPV was identified in 99.7% of patients with cervical cancer (25). Persistent infection with HR HPV is the primary cause of pre-cancerous lesions leading to cervical cancer. HPV genes encode three early proteins: E5, E6, and E7. The E5 protein has a relatively weak transforming effect but activates membrane-associated protein kinases. E6 and E7 are viral oncogenes that regulate the expression and replication of viral genes in host cells. The E6 protein binds to p53, while the E7 protein binds to the Rb protein, thereby inactivating these tumor suppressor genes and enabling unlimited cell growth (11, 26, 27). The oncoproteins encoded by the E6 and E7 genes are crucial in cervical epithelial carcinogenesis. In the early stages up to CIN I, HPV primarily exists freely within host cells. As the lesion advances, the HPV genome integrates into the host cell genome. As the lesion severity increases, HPV eventually exists in an integrated state. During integration, the E6/E7 genes persist while the L1 gene may be lost, posing a risk of false-negative detection results. Most PCR-based tests only amplify the L1 region of the virus. Therefore, PCR false negatives may occur due to the loss of this region during viral integration. In contrast, evaluating E6/E7 mRNA expression can indicate high-grade lesions or cervical cancer, as E6/E7 mRNA levels increase after the viral genome integrates into host cells (28).

In this study, CCTS from patients diagnosed through pathological histology was assessed, providing a more direct validation of the association between cervical cancer and HPV infection than traditional cytological testing. However, both assays were effective for HPV DNA genotyping. The overall positivity rate of HPV detection with the MPCE assay was higher than that with the PCR-RDB assay, with $p < 0.05$ indicating a statistically significant difference between the two assays. Using the oncogenic genes E6/E7 DNA as detection targets reduced the missed detection rate in CCTS by approximately 5.0% compared to that targeting the L1 gene. Another study produced similar results. A comparison of detection using universal primers for the L1 region and typing primers for E6/E7 in 56 biopsy samples of invasive cervical cancer tissue revealed that 23 samples exhibited L1 region deletions (29). For cervical cancer, a malignancy with a well-defined pathogenic mechanism, higher detection rates of oncogenic HPV facilitate earlier identification of potential patients.

TABLE 3 HPV genotyping distribution in HPV-positive cervical cancer tissue specimens.

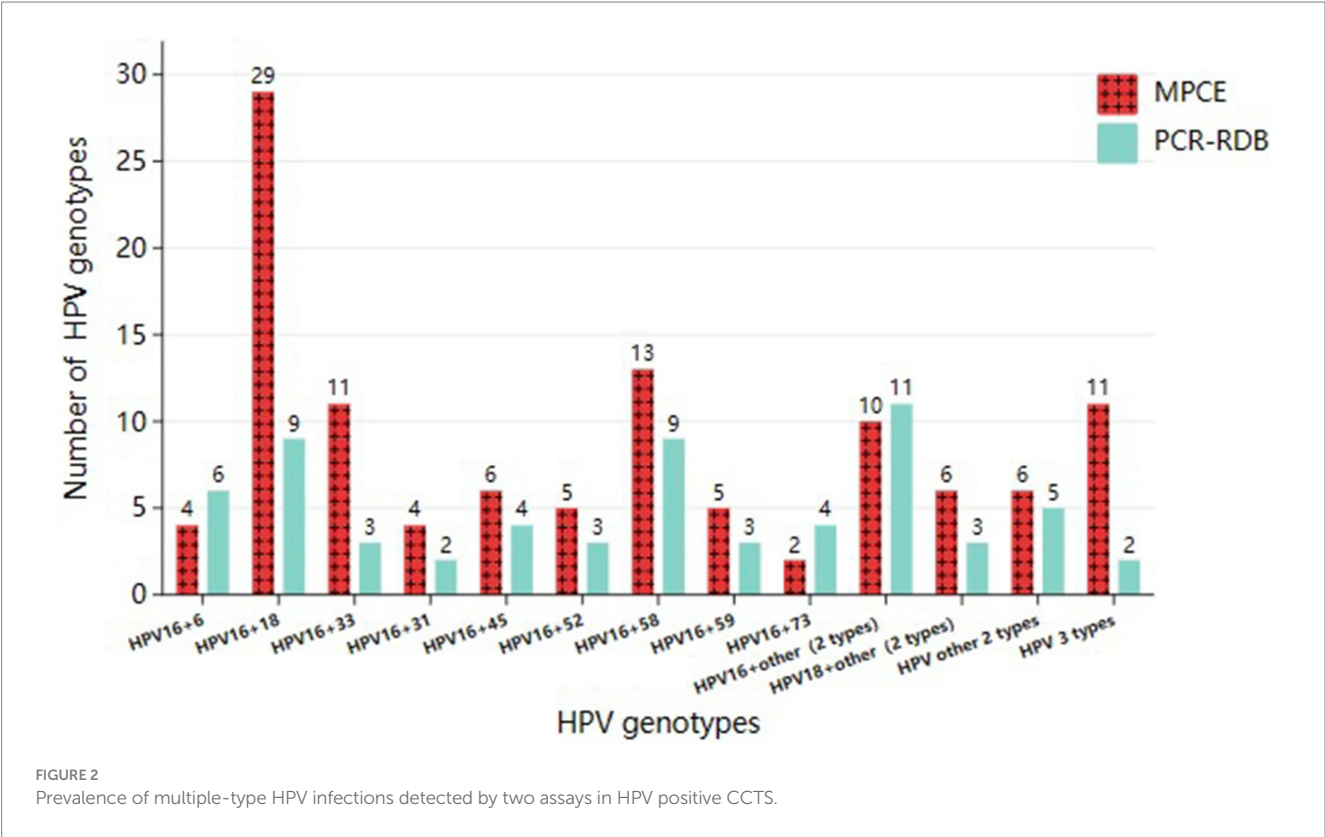
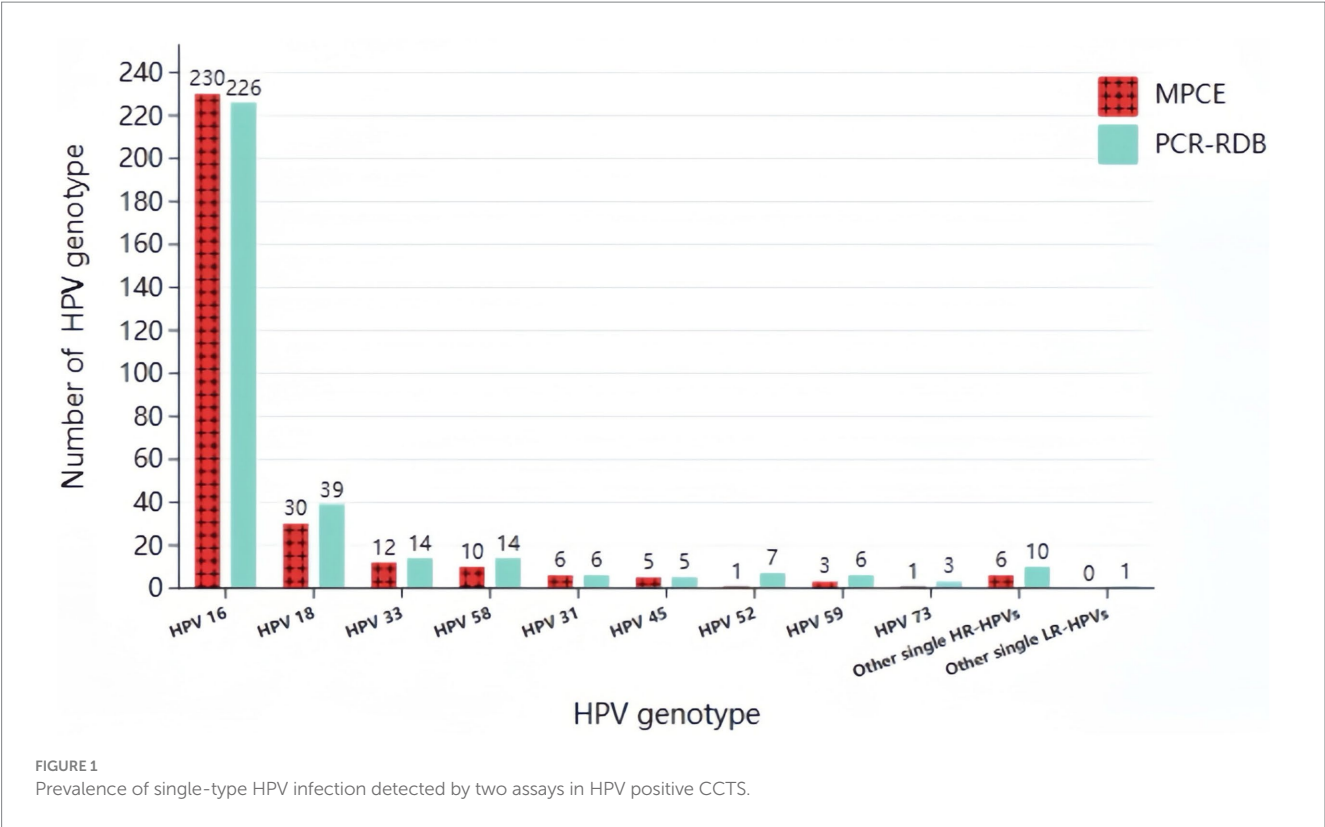
	Type	MPCE	Frequency	PCR-RDB	Frequency	χ^2 Test*	P
		N	%	N	%		
Single	HPV 16	230	75.7	226	68.3	9.213	0.511
	HPV 18	30	9.9	39	11.8		
	HPV 33	12	3.9	14	4.2		
	HPV 58	10	3.3	14	4.2		
	HPV 31	6	2.0	6	1.8		
	HPV 45	5	1.6	5	1.5		
	HPV 52	1	0.3	7	2.1		
	HPV 59	3	1.0	6	1.8		
	HPV 73	1	0.3	3	0.9		
	Other single HR-HPV	6	2.0	10	3.0		
	Other single LR-HPV	0	0.0	1	0.3		
	Total	304	100	331	100		
Multiple	HPV16 + 6	4	3.6	6	9.4	14.378	0.265
	HPV16 + 18	29	25.9	9	14.1		
	HPV16 + 33	11	9.8	3	4.7		
	HPV16 + 31	4	3.6	2	3.1		
	HPV16 + 45	6	5.4	4	6.2		
	HPV16 + 52	5	4.5	3	4.7		
	HPV16 + 58	13	11.6	9	14.1		
	HPV16 + 59	5	4.5	3	4.7		
	HPV 16 + 73	2	1.8	4	6.2		
	HPV16 + others (2types)	10	8.9	11	17.2		
	HPV18 + others (2types)	6	5.4	3	4.7		
	HPV other 2 types	6	5.4	5	7.8		
	HPV 3 types	11	9.8	2	3.1		
	Total	112	100.0	64	100.0		

*Fisher's Exact Test; HPV16 was the most frequent type in both single-type and multiple-type HPV infections within the CCTS. There was no statistical difference in HPV Genotyping distribution rate between the two assays.

The genotyping results of positive HPV DNA detected through the two testing assays were more significant in terms of understanding the etiology. Our study revealed that the MPCE assay targeting the oncogenic genes E6/E7 DNA detected a higher rate of multiple-type infections in CCTS of patients over 50 years old. A study indicated that E6/E7 target regions are more sensitive than L1 (30). While the time of HPV infection in patients was unknown based on the process of HPV integration into host cells, we hypothesize that without intervention, as time progresses from HPV infection to cervical cell infiltration and eventual carcinogenesis, the increasing severity of persistent HPV infection makes E6/E7 fragments easier to detect in host cells than that of L1 fragments. This suggests that detection based on L1 fragments poses a risk of false-negative results. Therefore, PCR tests may yield false negatives owing to the loss of L1 fragments during viral integration. This may suggest that in older individuals with long-term HR-HPV infections, targeting HPV oncogenic genes E6/E7 are more effective for detection. One study showed that multiple HPV

infections exerted a synergistic effect on cervical cancer development (31). One study found that multiple HPV genotype infections correlate with poorer cervical cancer survival than that of single genotype infection (32). The presence of multiple HPV genotypes may extend the duration of persistent HPV infection and increase the risk of carcinogenesis.

Table 3 shows a high degree of overlap in the top high-frequency HR genotype distribution detected through the two assays, corresponding to the seven HR-HPV through the 9-valent HPV vaccine (HPV 16/18/31/33/45/52/58). This suggests that vaccinating large populations of women in the appropriate age group with the 9-valent HPV vaccine can significantly reduce the risk of cervical cancer, ultimately aiming to reduce or even eliminate the disease. The HPV vaccination rate in China remains relatively low (33, 34). While the HPV vaccine is not yet incorporated into the national immunization program, qualified regions should be encouraged to conduct trials.



Pathological diagnosis of cervical cancer tissue is considered the gold standard for diagnosing cervical cancer, although there is not a 100% definitive causal relationship between HPV infection and cervical cancer. The epidemiological study revealed that almost all cases of cervical cancer are caused by HPV. However, approximately 5% of tumors are not associated with

TABLE 4 Comparison between single and multiple HPV infections in different age groups.

Age	MPCE		PCR-RDB		χ^2 test	P-value
	Single-type	Multiple-type	Single-type	Multiple-type		
	N (%)	N (%)	N (%)	N (%)		
≤50	111(74.5)	38(25.5)	119(83.8)	23(16.2)	3.801	0.051
>50	193(72.3)	74(27.7)	212(83.8)	41(16.2)	9.991	0.002*
Total	304	112	331	64		

*In the age group over 50 years old within the CCTS, the MPCE assay demonstrated a higher proportion of detecting multiple HPV infections.

HPV-persistent infection. HPV-negative status can arise from various scenarios: (1) HPV-independent cancers (true negative), such as certain adenocarcinoma subtypes and a few cases of squamous carcinoma (4); (2) loss of the HPV genome during integration; (35); (3) presence of viral genotypes not included in the molecular tests; (4) failure of the detection of the diagnostic method employed; or (5) misclassification of cancers as primary cervical, including metastases or primary uterine corpus neoplasms.

In this study, the 22 samples that tested positive with the MPCE assay but negative with the PCR-RDB assay require further investigation in future research. This discrepancy is most likely attributable to the absence of the L gene, which led to the failure of the PCR-RDB assay to detect HPV. In future studies, we plan to expand the sample size to explore the rate of L gene loss during cervical cancer progression, its integration with host genes, and the timeframe of this occurrence. This could potentially provide significant insights for detecting and treating cervical cancer. In contrast, there were eight cases where neither assay detected HPV infection. One possibility is that the testing kits did not include specific HPV DNA types, leading to undetected results. Another possibility is true HPV negativity, indicating no HPV infection was present. Due to less focus on HPV-negative cervical cancer, the clinical significance of HPV-negative cervical cancer may have been underestimated. Studies suggest that HPV-negative cancer may exhibit worse clinical features and distinct biological characteristics than that of HPV-positive cervical cancer (36, 37). Some scholars advocate combining HPV genotyping with cytological evaluation in co-testing to mitigate the risk of false-negative results caused by associated with a single HPV genotyping (38). In 2020, the WHO updated the Female Genital Tumors classification (5th edition) and recognized that a proportion of cervical cancers, particularly adenocarcinomas, may not be linked to HPV infection (39). This area also warrants further focused research and attention.

Quality control of the kit significantly influences the reliability of the test. Both MPCE and PCR-RDB incorporate internal control to ensure successful DNA extraction and amplification steps. Nevertheless, the implementation of the dUTP-UNG system to prevent carry-over contamination, along with the inclusion of dual internal controls and the capacity to detect and genotype 25 HPV types simultaneously in a single reaction, as well as highly automated sample analysis process of the MPCE assay, have enabled its widespread application in clinical settings.

Our study had some limitations. This study focused solely on evaluating and comparing the performance of the newly developed

MPCE assay with the commonly used PCR-RDB. We did not analyze common screening indicators such as sensitivity and specificity. As the progression from HPV infection to cervical cancer is a relatively lengthy process, these indicators will be further analyzed and validated in subsequent large-scale population screenings in common people. During that period, we will focus on screening precancerous lesions, conducting staged studies on lesions, and providing reliable theoretical support for reducing cervical cancer incidence through effective detection methods and clear intervention measures. While cervical cancer is primarily associated with high-risk HPV infections, approximately 5% of cervical cancers are classified as true HPV-negative cervical cancers. This highlights the inherent complexity of cervical cancer. Here, we focused on samples diagnosed with cervical cancer based on histology, which does not entirely rule out cancer risk from other causes. The setting of the gold standard has certain limitations. However, it is undeniable that when appropriate experiments are selected for large-scale population screening, the benefits of early detection, diagnosis, and treatment of cervical cancer precursor lesions far outweigh the risks of missed diagnoses. Non-invasive examination methods and the detection of fluid biomarkers, applied in large-scale population screening, can significantly benefit the early detection, diagnosis, and treatment of cervical cancer precursor lesions.

However, further research necessitates increasing the sample size and conducting comparative testing on a larger sample of pathological tissues. Targeting E6/E7 genes instead of the L1 gene can mitigate false-negative results from HPV genome integration into the human genome. Therefore, the oncogenic genes E6/E7 are more suitable targets for HPV DNA detection.

Given the large population in China, uneven distribution of healthcare resources, and relatively low rates of cervical cancer screening and vaccination, adopting an HPV screening plan that aligns better with the national conditions in China to achieve early detection, diagnosis, and treatment of cervical cancer is crucial. Considering the screening value, economic cost of the detection assay, and healthcare resource allocation in China, adopting MPCE assay as one of the primary methods for population-based cervical cancer screening is recommended. A screening interval of 5 years should be established, along with the maintenance of corresponding records. Special attention should be given to the distribution of HPV genotypes among populations with precancerous lesions. The MPCE assay proposed and validated in this study can use cervical exfoliated cell samples as research specimens in practical screening efforts to identify more patients early, resulting in superior cost-effectiveness.

Data availability statement

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

Ethics statement

This study was approved by the Ethics Committee of Nanjing Traditional Chinese Medicine Hospital (approval no. 2012NJL008). 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. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

LQ: Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation, Conceptualization. DL: Writing – review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Data curation. ZW: Writing – review & editing, Resources, Methodology, Data curation. JL: Writing – review & editing, Resources, Methodology, Data curation. CH: Writing – review & editing, Resources, Methodology, Data curation. JM: Writing – review & editing, Resources, Methodology, Data curation. JG: 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.

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2024.1421774/full#supplementary-material>

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Pandemic paradox: the impact of the COVID-19 on the global and Brazilian tuberculosis epidemics

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KEYWORDS

SARS-CoV-2, tuberculosis, society impact, sanitary measures, pandemic, epidemic, public health

Throughout the nearly 3-year period of the SARS-CoV-2 pandemic, various strategies were implemented to contain the virus, including the use of masks, frequent hand washing, social distancing, and quarantine. This period represented an extraordinary threat to public health, impacting populations due to the severity of virus infection, often requiring hospitalization and intubation, with patients staying in intensive care units (ICUs) (1). Alongside these events, there were implications for mental health, characterized by a significant increase in reports of anxiety, depression, distress, sleep disorders, and post-traumatic stress during periods of confinement and social isolation. Furthermore, the repercussions of the pandemic extended beyond the health sector, manifesting in notable effects on economic, food, educational, and tourism domains (2).

The trajectory of the COVID-19 pandemic exerted substantial influence on the Tuberculosis (TB) epidemic, manifesting in a notable decrease in notifications at the peak of the pandemic in 2020. The number of new TB cases plummeted from 7.1 million in 2019 to 5.8 million in 2020, marking an approximate 18% decline in official TB notifications. As 2021 unfolded, numerous countries achieved significant strides in vaccination efforts, contributing to a lessening of the severity of COVID-19 cases and a reduction in the mortality rate associated with SARS-CoV-2. This positive trend facilitated a gradual normalization in the functioning of healthcare sectors, even amid substantial global spikes in COVID-19 cases. The severity of illness among SARS-CoV-2 carriers became less pronounced compared to the early stages of the pandemic, alleviating the strain on healthcare systems, and enabling a return to normalcy in healthcare services. This improvement was evident in the resumption of notifications for other diseases, including tuberculosis, which saw ~6.4 million new cases in 2021 (Figure 1A).

Despite the widespread use of masks and the implementation of various sanitary strategies, aimed not only at combating the COVID-19 virus but also other airborne pathogens, the incidence of tuberculosis cases increased substantially in 2022. Official notifications reported a staggering 7.5 million newly diagnosed individuals with TB, surpassing the 2019 figure of 7.1 million by 4.5% (Figure 1A). This paradoxical rise occurred despite 2 years of stringent sanitary measures, with people remaining apprehensive about venturing outside and predominantly confined to their homes.

In Brazil, from 2015 to 2022, the tuberculosis incidence rate also showed a similar trend with a 12.1% reduction in tuberculosis new cases from 2019 to 2020. On the other hand, in 2021, the incidence rate resumed its upward trajectory, reaching 34.9 cases per 100,000 inhabitants (74,385 new cases). This ascending trend persisted in 2022, with an increase to 36.3 cases per 100,000 inhabitants (78,057 cases; Figure 1B). As the global

profile, the influence of the COVID-19 pandemic had a controversially shown negative impact on efforts to combat tuberculosis, deviating from the global expectation of reducing the number of cases. Based on the presented numbers, the COVID-19 pandemic resulted in setbacks for Brazil's national plans, which aimed to achieve <10 cases per 100,000 inhabitants and restrict the annual number of deaths to <230 by 2035.

The global and Brazilian decline in new cases during 2020 and 2021 may be attributed to the reduced number of diagnoses, a consequence of the substantial increase in SARS-CoV-2 infection cases and their severity. This surge placed an overwhelming burden on all health-related departments, diverting attention and resources away from tuberculosis diagnosis, thereby negatively impacting tuberculosis control (5). Contrastingly, despite its low recombination and mutation rate, it is noteworthy to emphasize the significant increase in new tuberculosis cases in 2022. This uptick is particularly surprising considering the period followed the peak of the pandemic, during which the adoption of sanitary measures was expected to potentially contribute to the reduction in the transmission of various airborne pathogens (6, 7).

The rising trajectory of epidemic tuberculosis cases prompts three crucial questions: (1) Why have the implemented sanitary measures not effectively curtailed tuberculosis transmission, particularly given that both pathogens are airborne? (2) Could the surge in new cases in 2022 be attributed to a heightened activation of latent tuberculosis? (3) Have there been any advancements in TB diagnoses that might explain the increase in numbers?

Concerning Question 1, we can postulate that the elevated TB numbers might be attributable to delays in notifications from 2020 and 2021, coupled with reductions in access to diagnosis and treatment. These factors could potentially influence the latest health reports of 2023, indicating a significant increase beyond the observed average rates when compared to trends before the COVID-19 pandemic (8). It is also a hypothesis that individuals could also have contracted the TB in their homes. Despite the adoption of masks, their use was predominantly in public spaces, and the primary transmission chain and the higher risk of TB involve familial and frequent contact with active tuberculosis cases, with an infectivity rate of 22% (9, 10). Indeed, it is estimated that an individual with active TB can infect an average of 10–15 people during the course of a year. In this regard, the use of masks may not have significantly influenced TB transmission, and new strategies should be studied and implemented to minimize damage to various healthcare sectors in future pandemics.

Discussion of Question 2 is paramount. The activation of latent tuberculosis is a dynamic process that can occur at any point, with the condition of the immune system playing a pivotal role in its reactivation. Notably, during the pandemic, a substantial number of individuals contracted COVID-19, and even in milder cases, this could potentially influence the activation of tuberculosis. It is essential to emphasize the complex interplay between the two pathogens, intensifying the severity of cases as a result of the immunologically sensitized state in the presence of both the virus and the bacteria. Due to their airborne transmission and the potential for significant damage to lung structures and cells, a cycle of negative feedback developed between these two pathogenic agents (11, 12). Moreover, the extended duration of the pandemic

led to heightened psychological stresses, which may contribute to immunosuppression, further enhancing the likelihood of latent TB reactivation (13). In addition to psychosomatic factors, the SARS-CoV-2 infection itself is responsible for the excessive activation of the immune system, leading to an abundance of pro-inflammatory cytokine release, resulting in a “cytokine storm.” The presence of high levels and diversity of cytokines in the body triggers the hyperactivity of the immune system and the production of autoantibodies that can result in autoimmune diseases such as autoimmune hemolytic anemia, autoimmune thrombocytopenia, Guillain-Barré syndrome, vasculitis, multiple sclerosis, pro-thrombotic state, diffuse coagulopathy, and other autoinflammatory conditions, weakening the effectiveness of the immune system in maintaining chronic conditions such as latent tuberculosis, transitioning latent cases to active tuberculosis (14).

Regarding question 3, the increase in cases above 2019 observed in 2022 has been evidenced because of failures in containing new tuberculosis cases, lack of care, diagnoses, and notifications due to health system overload during the pandemic period, difficulties in the supply and transportation of medications experiencing disruptions due to flight cancellations and circulation restrictions. Additionally, quarantines and stay-at-home measures have increased the risk of TB transmission, especially among family members. Many of the strategies adopted during the COVID-19 pandemic may have been beneficial for containing SARS-CoV-2, but they have led to consequences that will still be listed in the coming years (15). There is another side of the coin that must be taken into consideration: despite the significant losses caused by the virus, numerous technological advancements have been developed with the intention of better facing the new challenges that would be encountered during the pandemic. Technological advancements were notable with various digital innovations, enabling remote learning, and the shift of major companies to the new “home office” format. In the realm of research and health, advancements can be highlighted in the search for faster and more effective diagnostic methods necessary for confirming COVID-19 cases and implementing necessary measures more rapidly. There have been developments in new platforms of machine learning for epidemiological studies and infectivity dynamics, as well as progress in vaccine development. In Brazil, innovations in the diagnosis of active tuberculosis cases began in 2020 with the implementation in the unified health system, through ordinance SCTIE/MS No. 34, of August 24, 2020, using a new methodology called “Automated Liquid Culture,” an automated technology for detecting mycobacteria and testing sensitivity to antimicrobials used in tuberculosis treatment simultaneously (16).

In Brazil, in 2022, Information Note No. 2/2022-CGDR/DCCI/SVS/MS detailed the adoption of new diagnostic tests known as Interferon-Gamma Release Assays (IGRA) for a more precise diagnosis of latent tuberculosis. The implementation process for latent tuberculosis diagnosis took place throughout 2022 and 2023, culminating in its permanent integration into the unified health system by 2024. This integration aims to facilitate frequent testing, particularly for children who have been in contact with active tuberculosis, HIV carriers, and patients undergoing immunosuppression, who are susceptible to developing active tuberculosis.

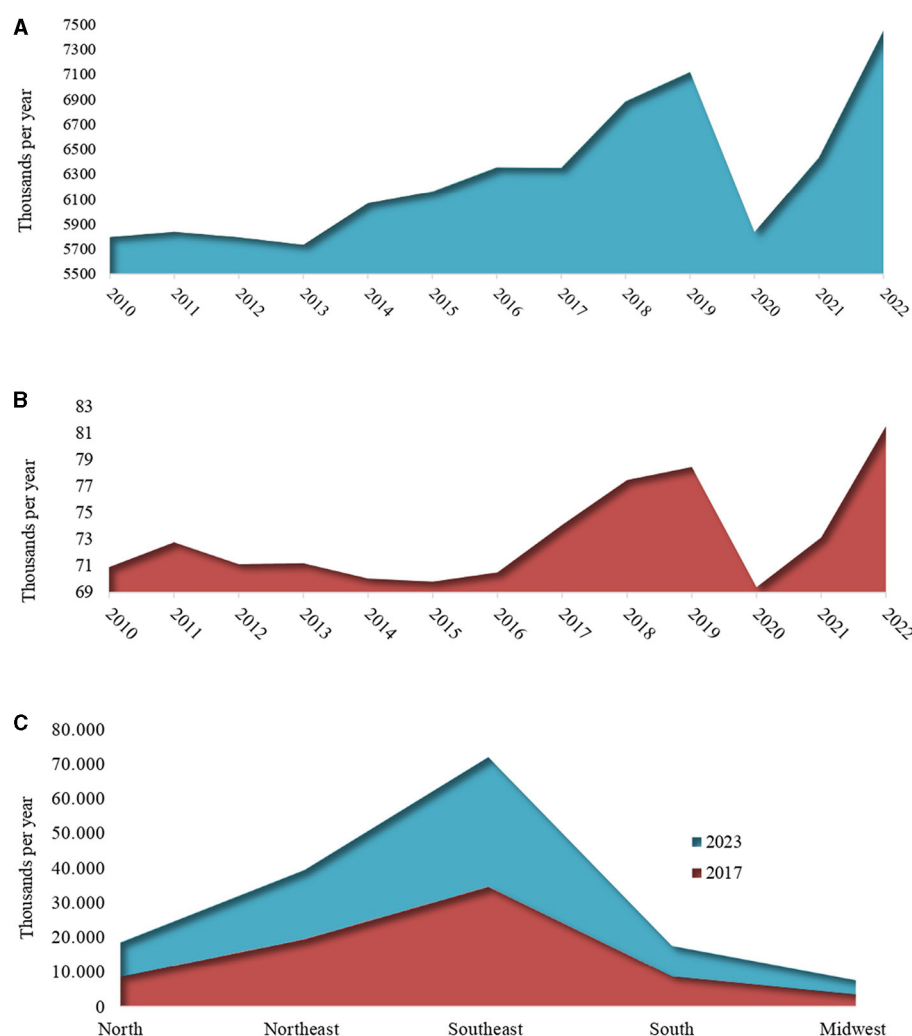


FIGURE 1

Trends in the estimated number of incident new tuberculosis cases globally (A), in Brazil (B), and epidemiological indicators of new tuberculosis cases by Federative Units of Brazil (C). Data source: WHO Global Tuberculosis Report, 2023 and the Epidemiological Bulletin (Brazil), 2017 and 2023 (3, 4).

The full impact of the pandemic is yet to be comprehensively studied in the coming years. The return to desired case control parameters may occur gradually, offering a more coherent understanding of the global tuberculosis landscape. This understanding will inform the implementation of strategies aimed at reducing tuberculosis cases on a global scale. Detecting latent tuberculosis cases is crucial for both investigation and treatment of the infection before it becomes active. Alongside the establishment of a new network for latent testing, it's vital to highlight the significance of past health awareness campaigns in Brazil. These initiatives have played a pivotal role in disseminating knowledge about tuberculosis vaccination and promoting testing for both latent and active cases. Therefore, implementing public campaigns emphasizing the importance of tuberculosis vaccination and testing for suspected cases can significantly contribute to disease control.

The national scenario mirrors the global trend of recent years, indicating a notable rise in cases between 2017 and 2024,

encompassing both pre- and post-pandemic periods (Figure 1C). To address the large number of tuberculosis cases in Brazil, it is essential to adopt a multifaceted approach that includes prevention, early diagnosis, effective treatment, and public awareness. In addition to the necessity of all these factors, another important point for recovery from the increase in cases is to improve treatment and adherence to treatment by ensuring the continuous and free supply of medications through the Unified Health System (SUS). Awareness and health education campaigns should be conducted across various media platforms and integrated into schools and communities to demystify the disease and reduce stigma, along with preventive measures such as promoting vaccination. The inclusion of epidemiological studies is necessary to better understand the patterns of transmission and risk factors in different regions of the country, where we can observe the heterogeneity in the distribution of cases (Figure 1C). Finally, it is crucial to ensure adequate and sustainable funding for tuberculosis control programs and to develop health

policies, implementing these strategies in a coordinated and continuous manner.

The intricate interplay highlighted above emphasizes the necessity for a thorough understanding of the complex factors influencing the dynamics of tuberculosis within the context of the ongoing global health crisis. This prompts a critical analysis of the situation. The epidemiological data presented not only indicate the potential impacts of the COVID-19 pandemic on public health extending beyond the direct effects on its victims but also reveal the persistent challenge posed by tuberculosis. Indeed, despite being described by Robert Koch in 1882, this ancient disease remains uncontrolled and far from eradication.

The call for an efficient flow of communication and collaboration among governmental and international organizations, economic sectors, and the scientific community is not just a recommendation but a crucial imperative. These entities play a fundamental role in establishing a global system resilient to emerging pandemics, thereby mitigating potential damages that could significantly impact society during future outbreaks. The lessons drawn from the intersection of COVID-19 and tuberculosis underscore the importance of proactive and coordinated efforts to address global health crises.

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EF: Writing – original draft, Writing – review & editing, Conceptualization, Investigation, Methodology, Resources. MP: Writing – original draft, Writing – review & editing, Conceptualization, Investigation, Supervision. JA: Writing –

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Hospitalised patients as stewards of their own antibiotic therapy: a qualitative analysis informing the strategic design of interventions to encourage shared decision-making in tertiary hospital settings in Singapore

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Background: Shared decision-making (SDM) on antibiotic therapy may improve antibiotic use in tertiary hospitals, but hospitalised patients are apprehensive about being involved in it. Understanding the facilitators and barriers to SDM can inform the design and implementation of interventions to empower these patients to engage in SDM on their antibiotic therapies.

Methods: We conducted qualitative interviews with 23 adult patients purposively sampled with maximum variation from the three largest tertiary-care hospitals in Singapore (April 2019–October 2020). Thematic analysis was conducted using the Theoretical Domains Framework and Capability, Opportunity, Motivation, Behaviour (COM-B) model to identify areas for intervention.

Results: Hospitalised patients lacked comprehensive knowledge of their antibiotic therapies and the majority did not have the skills to actively query their doctors about them. There was a lack of opportunities to meet and interact with doctors, and patients were less motivated to engage in SDM if they had a self-perceived paternalistic relationship with doctors, trusted their doctors to provide the best treatment, and had self-perceived poor knowledge to engage in SDM. To empower these patients, they should first be educated with antibiotic knowledge. Highlighting potential side effects of antibiotics could motivate them to ask questions about their antibiotic therapies. Environment restructuring, as facilitated by nurses and visual cues to nudge conversations, could create opportunities for interactions and motivating patients into SDM on their antibiotic therapies.

Conclusion: Education and environmental restructuring should be explored to empower hospitalised patients to engage in SDM on their antibiotic therapies.

KEYWORDS

antimicrobial resistance, antibiotic prescribing, shared decision-making, hospitalised patients, tertiary hospital

1 Introduction

Antimicrobial resistance (AMR) is expected to result in an annual incidence of 10 million global deaths by 2050, with nearly half of the deaths occurring in the Asian population (1). With AMR-attributable deaths already reaching 1.27 million in 2019 alone (2), there is an urgent need to ensure that antibiotics are appropriately used to mitigate this global public health threat (3).

Internationally, the use of antimicrobials in tertiary hospitals is not trivial. In a global point prevalence study conducted in 2015, 53 countries reported that 34% of patients warded in 303 study sites were receiving at least one antimicrobial (4). In Southeast Asia, in particular, more than half of the patients were receiving antibiotics during their hospital stay (5–7), with a prevalence of 51% reported in Singapore (5) – a developed country in the region.

Antibiotic stewardship programmes (ASPs) are the cornerstones of ensuring appropriate antibiotic use and reducing AMR (8–11) in tertiary hospitals through active monitoring and evaluation (12–15). However, the ASP team of a hospital often comprises a limited number of microbiologists, infectious disease doctors and pharmacists (16), who become easily overwhelmed by a large-scale epidemic or pandemic (17). New alternative strategies need to be explored to expand ASP teams beyond healthcare professionals, for example to involve hospitalised patients, to augment ongoing ASPs.

In primary care, patients play an important part in antibiotic stewardship by engaging in shared decision-making (SDM) with their doctors on antibiotic prescribing, which have been found to be effective in improving the appropriateness of antibiotics prescribed (18). In the handful of studies conducted, it was suggested that SDM on antibiotic therapy in tertiary hospitals may also yield favourable outcomes on optimising appropriate antibiotic decisions (19). However, the lack of opportunities provided by healthcare professionals to interact, as cited by hospitalised patients, have limited SDM (20, 21). In addition, the higher acuity of illness of these patients could have led to poorer confidence in them to engage in SDM with their clinical team. Furthermore, hospitalised patients have also shared doubts about their capabilities in comprehending medical information and therefore were more likely to trust their doctors to make the final antibiotic decision (21).

In order to explore the possibilities of involving hospitalised patients in future antibiotic stewardship efforts, it is imperative that we first understand the factors influencing these patients to be actively engaged in SDM for their antibiotic therapy. We aimed to use the Theoretical Domains Framework (TDF) (22) to identify the influencing factors. After which, the domains will be mapped onto the Capability, Opportunity, Motivation, Behaviour (COM-B) model (23), to identify potential areas for intervention to facilitate

hospitalised patients' engagement in SDM with healthcare professionals on their antibiotic therapies.

2 Materials and methods

2.1 Study design and study population

Semi-structured interviews were conducted with adult patients, aged 21 years and above, admitted in the general wards of three largest tertiary hospitals in Singapore, between April 2019 and October 2020. Patients receiving antibiotics and were aware that they were given antibiotics during their hospital stay were included in this study. To ensure maximum variation, patients were purposively recruited from the medical and surgical disciplines, with a good representation from different hospitals. For planning purposes, a sample size of 12–15 interviews per stratum (i.e., medical versus surgical disciplines) was estimated in order to achieve data saturation (24). The conduct and reporting of this study followed the Consolidated Criteria for Reporting Qualitative Research (COREQ) guidelines (25) and this study was approved by the National Healthcare Group Domain Specific Review Board.

2.2 Semi-structured interviews

A semi-structured interview guide (Supplementary material 1) was developed by HG (Female, PhD, Epidemiologist) and the questions were designed to explore hospitalised patients' knowledge, attitudes, perceptions and experiences related to antibiotics and AMR, and their interactions with their clinical team during the hospital stay. Pilot interviews were conducted by a study team member (Female, BSc, Research Assistant) with two staff members from the hospital who had at least one prior hospitalisation experience in a local tertiary hospital to ensure content validity and proper phrasing of the questions. These staff were from the same institution but they were not affiliated with the study team (they were neither from the same department as the study team members nor had working relationships with them). Interviews were predominantly conducted in English but options were available for the conduct of interviews in Mandarin or Malay languages. Three study team members (Females, 2 BSc and 1 MPH, Research Assistants respectively), who were bilingual and trained in qualitative research, conducted the interviews.

Patients were invited in-person by the study team in each hospital after checking with their respective clinical teams on their capacity to be interviewed. Only those who were deemed fit and without cognitive impairment were recruited into the study. Informed consent was taken

from interested patients on the same day of the interview and the duration of each interview was around 60 min. The interview was conducted physically at the bedside or virtually via Zoom during the coronavirus disease 2019 (COVID-19) pandemic, with curtains drawn to maintain the confidentiality of the patients. For virtual interviews, the study team would provide the patients with a digital tablet and set up the Zoom session before leaving the bedside to commence the interview. This was to minimise physical contact time with the patients to reduce COVID-19 transmission risks. No personal identifier was collected during the interview and each patient was provided with a unique participant number to be used through the study. Prior to the start of the interview, the interviewer would introduce herself as a public health researcher who did not work under the same department as the clinical team providing hospital care to the patient. This was to assure the patients that the care they received would not be compromised by taking part in the interview, and also to minimise social desirability bias to enhance the truthfulness of the responses received. All interviews were audio-recorded and transcribed verbatim.

2.3 Data analysis

Applied thematic analysis was performed (26). HG read and re-read the transcripts to familiarise with the data before developing the codebook according to the TDF (22). The preliminary codebook was reviewed by AC (Female, PhD, Associate Professor) and the final codebook was applied to all transcripts. The domains of TDF were later mapped onto and grouped under the elements of the COM-B model (23) as proposed by Fahim et al. (27). The six broad thematic codes were capability (psychological), capability (physical), opportunity (social), opportunity (physical), motivation (automatic)

and motivation (reflective). Any TDF domain not mapped onto the COM-B model was reported as well. Any discrepancies that arose during data analysis were resolved between AC and HG. Data saturation was achieved, in agreement with AC, at around two-thirds way through the interviews. The emergent themes (***bold italics***) and subthemes (*italics*) were organised using Atlas.ti, whilst basic descriptive data were tabulated using Microsoft Excel.

3 Results

3.1 Study participants

A total of 23 in-depth interviews were conducted (Table 1), with an almost equal representation from medical (43%) and surgical (57%) disciplines. The median age of the participants was 42 (range: 22–69) years, with a slight preponderance of males (61%) and Chinese (57%). Slightly more than half of the participants were lower educated (52%).

3.2 Qualitative findings

Capable of actively querying the clinical team on their antibiotic therapies if motivated by perceived negative consequences of antibiotics.

A small proportion of participants revealed that they would actively query the clinical team on any antibiotic therapy prescribed to them. It was observed that these hospitalised patients were *motivated to raise questions due to side effects of antibiotics experienced in the past, or due to perceived negative outcomes from the use of antibiotics*, such as fear of driving antibiotic resistance.

TABLE 1 Basic characteristics of the study participants.

Characteristics	Hospital A (N = 9)	Hospital B (N = 10)	Hospital C (N = 4)	Total (N = 23)
<i>Discipline, N (%)</i>				
Medical	4 (44)	3 (30)	3 (75)	10 (43)
Surgical	5 (56)	7 (70)	1 (25)	13 (57)
Median age, years (min, max)	42 (22, 69)	40.5 (25, 63)	48 (42, 55)	42 (22, 69)
<i>Gender, N (%)</i>				
Male	4 (44)	6 (60)	4 (100)	14 (61)
<i>Ethnic group, N (%)</i>				
Chinese	6 (67)	6 (60)	1 (25)	13 (57)
Malay	1 (11)	3 (30)	1 (25)	5 (22)
Indian	2 (22)	1 (10)	2 (50)	5 (22)
<i>Residency status, N (%)</i>				
Singapore citizen	9 (100)	7 (70)	2 (50)	18 (78)
Singapore permanent resident	0	2 (20)	1 (25)	3 (13)
Foreigner	0	1 (10)	1 (25)	2 (9)
<i>Educational level, N (%)</i>				
Lower educated (A-levels and below)	3 (33)	7 (70)	2 (50)	12 (52)
Higher educated (Diploma and above)	6 (67)	3 (30)	2 (50)	11 (48)

"Every time when antibiotics need to be eaten, I feel like rejecting... Because I feel like if I take too much antibiotics, I do not know if I will cause my own immune system to be bad...I complained to the doctor today that my stomach always feels uncomfortable after I eat [antibiotics]...I did ask the doctor why I need to take antibiotics." (IDI01, 59yo, Female, Lower educated).

"I remember a few years back, I had rashes. I was allergic to one of the antibiotics. So I will ask the doctor first. What is the purpose of it, and also if it is going to reoccur? Whatever happened in the past, you know?" (IDI15, 46yo, Female, Lower educated).

Not actively querying the clinical team on their antibiotic therapies if lack the motivation and opportunities to do so.

On the other hand, several reasons were uncovered as to why the majority of the participants did not engage in active conversations with their clinical team on the antibiotic therapy they received. A self-perceived paternalistic relationship with their clinical team could have diminished these hospitalised patients' motivation in questioning the antibiotic therapy received. Additionally, some patients also expressed that they were less likely to query if they trusted the doctors' professional know-how and mission to provide the best treatment for them.

"For me, if they [referring to the clinical team] give me antibiotics, then I guess I should just take it." (IDI06, 28yo, Male, Higher educated).

"I will eat what the doctor asks me to eat. It is only right to listen to the doctor's instructions. It is not as if the doctor will want to harm us, right?" (IDI08, 63yo, Male, Lower educated).

"From my point of view, I see it as the doctor has the final say. They know what is best for me. I should not need to question them whether this is effective for me or not. Whatever they prescribe, I will take it as it is going to help me. So I will not question further." (IDI23, 28yo, Male, Higher educated).

A self-perception of own knowledge of antibiotics (both good and poor knowledge) also seemed to demotivate these hospitalised patients in engaging in active conversations with their doctors. Patients, who felt that they had an inadequate knowledge of antibiotics and those who perceived that they had a relatively good knowledge about antibiotics (although not necessarily correct), had shared that they did not raise questions to their clinical teams about their antibiotic therapies.

"I just do not ask. Because I feel that the clinical team would know better than me...I feel like that they have more knowledge so I just follow their instructions." (IDI06, 28yo, Male, Higher educated).

"We do not go into the details actually. I know generally antibiotics are given to create an immune system in the body for the condition. So I normally do not ask any questions." (IDI05, 51yo, Male, Higher educated).

Hospitalised patients also opined that they were more likely to go with the suggested treatment plan decided by the clinical team due to their desire to recover quickly from their conditions. This was further

compounded by an absence of bad past experiences with antibiotics and a perception of good outcomes from the use of antibiotics, as highlighted by some patients. As a result, these participants were reportedly less likely to query the clinical team on the need for antibiotics.

"Because my skin has to take a longer time to heal. Hence, there is no choice but to use antibiotics." (IDI03, 55yo, Male, Lower educated).

"I can feel that my wound is healing faster. My wound dries faster. I like to keep getting antibiotics because I can heal faster." (IDI14, 27yo, Male, Lower educated).

"I am kind of ok with taking it [referring to antibiotics]. I have not had any bad experiences with antibiotics yet." (IDI17, 22yo, Male, Higher educated).

"If the antibiotic helps my body to get better, then I will just take it." (IDI19, 31yo, Female, Higher educated).

The perceived lack of capability to communicate effectively and absence of opportunities for interaction were potential barriers to hospitalised patients' engagement in antibiotic conversations with their doctors. Besides language barriers, a couple of participants shared that they would refrain from asking their doctors any questions due to their perceived lack of knowledge and the ability to properly articulate medical-related questions and to adequately understand the explanations given by their doctors. In addition, as observed and reported by participants, doctors would usually be in the wards for short periods of time and during busy ward rounds, limiting the opportunities for patients to interact with their doctors. On the contrary, some participants voiced that there were more instances to interact with nurses as the latter would usually be around in the ward caring for them. Notably, a few patients expressed concerns about burdening the medical team with their antibiotic questions, while observing the team's busy work schedules.

"I am afraid that I will not understand even after he tells [me about antibiotics]. Because that doctor is a Caucasian. But he knows how to speak some Mandarin...I do not know how to ask him those medical sort of questions." (IDI01, 59yo, Female, Lower educated).

"For questions on what medications to provide, it is always the staff nurses who will discuss with us actually...Maybe the doctors are busy...they spend not even one minute with the patients when they are around." (IDI05, 51yo, Male, Higher educated).

Despite so, some participants highlighted that they desired more opportunities to discuss with their doctors and receive information from them on their antibiotic therapies. They valued such interactions, as those could help them to understand the rationale for the antibiotics and learn how to use antibiotics properly.

"I think the doctor should be the one that tells you whether you have a bacterial or viral infection, and then explain the reasons why he will not be giving antibiotics...If it is bacterial infection, then he should explain like, 'I am giving you this antibiotic because it is

a bacterial infection.' And also explain why we should complete the full course." (IDI06, 28yo, Male, Higher educated).

"It will be best if he [referring to the doctor] can articulate it clearly to us, the patients. To explain the reasons to why an antibiotic is taken and how it helps with the wound. That will help us to understand better. We will not comprehend if the prescription was given without any explanation since it will be rare for us to ask the doctor too." (IDI08, 63yo, Male, Lower educated).

"Every time the doctor is to prescribe the antibiotics, if the nurse or the pharmacist is able to give a verbal explanation...I think that will help. I think that is the most effective way, because when you receive the medicine, obviously we need to know when we need it, how are we supposed to take it, and what are the precautions to take." (IDI23, 28yo, Male, Higher educated).

Limited knowledge of the antibiotic therapy prescribed.

Interviewed hospitalised patients seemed to have poor knowledge pertaining to the antibiotic therapy prescribed to them. Most of the participants highlighted that they were generally informed of their prescribed antibiotic therapy through nurses as it was a common practice to do so, prior to administration of any drug to patients. Through observing what the nurses did, almost all were aware of the administration route of the antibiotics prescribed to them and the dosing frequency of the antibiotic therapy.

"When it's time for medication, the nurses will come in, and they will tell me to take the medication. They generally do not describe what the antibiotics is for, although they do say that it is an antibiotic. They will read out the name too." (IDI23, 28yo, Male, Higher educated).

"Initially they [referring to the prescribed antibiotic] were given via drips. Then subsequently the clinical team changed it into tablets." (IDI19, 31yo, Female, Higher educated).

"Every 8 h, the nurse will come again and give me the antibiotic. Every day." (IDI18, 42yo, Male, Lower educated).

However, these patients could not share further information on the other details of their antibiotic therapy. While most knew that the prescribed antibiotics were used to treat their bacterial infections, the rest could not articulate the reasons why antibiotics were prescribed.

"Because of the wound, I was given antibiotics. If not, it will get infected." (IDI14, 27yo, Male, Lower educated).

"I think the antibiotics are for my...I do not know. For my inside? And the nebuliser is for my breathing." (IDI04, 45yo, Male, Lower educated).

"I was prescribed antibiotics maybe to protect my gastrointestinal system." (IDI10, 69yo, Female, Lower educated).

Furthermore, many interviewed patients were unaware of the duration of the antibiotic therapy they were on. It was notable that the majority could not name the antibiotics they were prescribed with as

well. These patients emphasised that they were either not given the information or they could not remember the names provided.

"I am not sure how long I need to be on the antibiotic, but I am guessing all the way till next week." (IDI17, 22yo, Male, Higher educated).

"I am not sure what kind of antibiotics are those. I just know it is an antibiotic...I do not know the name of it." (IDI19, 31yo, Female, Higher educated).

"They did tell me the name, but I do not study medicine. I do not remember them." (IDI23, 28yo, Male, Higher educated).

Last but not least, only one patient mentioned being aware of the potential side effects of antibiotics when consumed.

"The doctor told me about the side effects for the antibiotic. My doctor said that it is normal to...visit the toilet maybe about 3 or 4 times." (IDI07, 25yo, Male, Lower educated).

4 Discussion

This study has provided valuable insights on the facilitators and barriers influencing hospitalised patients' engagement in SDM with healthcare professionals on antibiotic use in an Asian context (Figure 1). Overall, we observed that patients admitted to tertiary hospitals in Singapore were generally apprehensive about being actively involved in SDM to discuss their prescribed antibiotic therapy with their doctors. This was similarly reported by Zanichelli et al. (21) where they found that a perceived lack of medical knowledge and a trust in doctors' professional know-how to provide the best treatment plan could impede SDM. In our study, the patients also shared that they were reluctant to initiate conversations with their doctors due to language barriers, and perceived lack of knowledge and the ability to engage in technical conversations surrounding medical science and terminology, which could have resulted in their increased tendency to trust their doctors. Together with the lack of opportunities to meet and communicate with their doctors, unanimously highlighted by our participants, and corroborated with previous literature (20, 21), it is therefore crucial to first tackle communication barriers faced by hospitalised patients. In order to enable and empower hospitalised patients to be more engaged in SDM with their doctors for their prescribed antibiotic therapy, a multi-pronged intervention would be needed.

Leveraging on the facilitators, barriers and desired states identified through the interviews, intervention strategies were recommended using the behaviour change wheel (BCW) (23) – an extension from the COM-B system – to encourage SDM between hospitalised patients and doctors for their antibiotic therapies (Table 2).

First and foremost, it was noted that side effects or allergic reactions experienced with antibiotics in the past had played an important role in motivating hospitalised patients to engage in SDM. The negative experiences, together with perceived negative outcomes from antibiotic use, had prompted these patients to proactively ask their doctors about their antibiotic therapies. However,

it is the opposite phenomenon — patients who had never experienced or never knew of the side effects of antibiotics and therefore were less likely to ask — that warrants attention. The techniques of persuasion can be used to develop relevant educational materials to highlight the potential side effects of antibiotics. It was suggested by a systematic review that such a multifaceted approach could provide better outcomes in behaviour change than providing education alone (28). Messages should relate to their desired state of fast recovery and relief of discomfort and how potential side effects of antibiotics could jeopardise that. This could thereafter spark interest amongst hospitalised patients to want to understand more about the antibiotics prescribed to them and their related side effects from their doctors.

Next, despite patients' desire for more opportunities to discuss and receive antibiotic-related information from their doctors (20, 21), a paradigm shift would be required to encourage open communications between patients and their clinical teams. For a start, the hospital environment needs to be restructured to create an enabling working culture for the clinical team to be receptive to receiving questions from their patients. Coupled with the receipt of patient educational materials, patients could be encouraged by the clinical team to raise any questions when they have doubts pertaining to their prescribed antibiotic therapy. Following the findings from a hand hygiene campaign organised in an acute care hospital in United Kingdom (29), empowering hospitalised patients to voice their concerns to healthcare professionals could result in positive effects on appropriate antibiotic prescribing. To reinforce these cultural changes, the creation of visual cues, such as a badge worn by members on the clinical team (for example, nurses), can be designed while serving two purposes. Firstly, by embedding 'Ask Me About Your Antibiotics' on the badge, it could help to nudge and create a channel for hospitalised

patients to approach and pose any antibiotic-related questions to any clinical team member who is wearing the badge. Secondly, by adding a secondary declaration that 'I Am An Antibiotic Steward' on the same badge, it could remind and motivate clinical team members to be committed towards having active discussions with patients around antibiotic therapies (30). However, even though patients desire a two-way conversation with their doctors (31), the paternalistic model of care is deeply ingrained and adopted by doctors in the Southeast Asian cultural context (32). Furthermore, patients often express their respect and trust for their attending doctors by complying with their recommendations without openly questioning their treatment decisions. As a result, such paradigm shift could be difficult to implement from a ground-up approach. To effect change at the clinical care team level, engagement of the hospital's management to provide top-down guidance and strategic directions on patient activation would be necessary.

In particular, nurses, by nature of their job, have a high frequency of interaction with hospitalised patients and they are valuable members of the clinical team that should be counted on to improve antibiotic stewardship in tertiary hospitals. The expansion of nursing roles was first introduced by the US Centers for Disease Control and Prevention (33) and broadly discussed by several international studies (34). Involving nurses as champions to don the badge to advocate for patient empowerment in SDM engagement would therefore be ideal. Even though nurses are not empowered to provide clinical advice pertaining to antibiotic choices (35), they are exemplary antibiotic administrators who are well aware of the name, and correct antibiotic dose and duration to be given to the patients for patient safety reasons (36, 37). Once doctors have selected the antibiotics for treatment, nurses could provide patients with information on the indication,

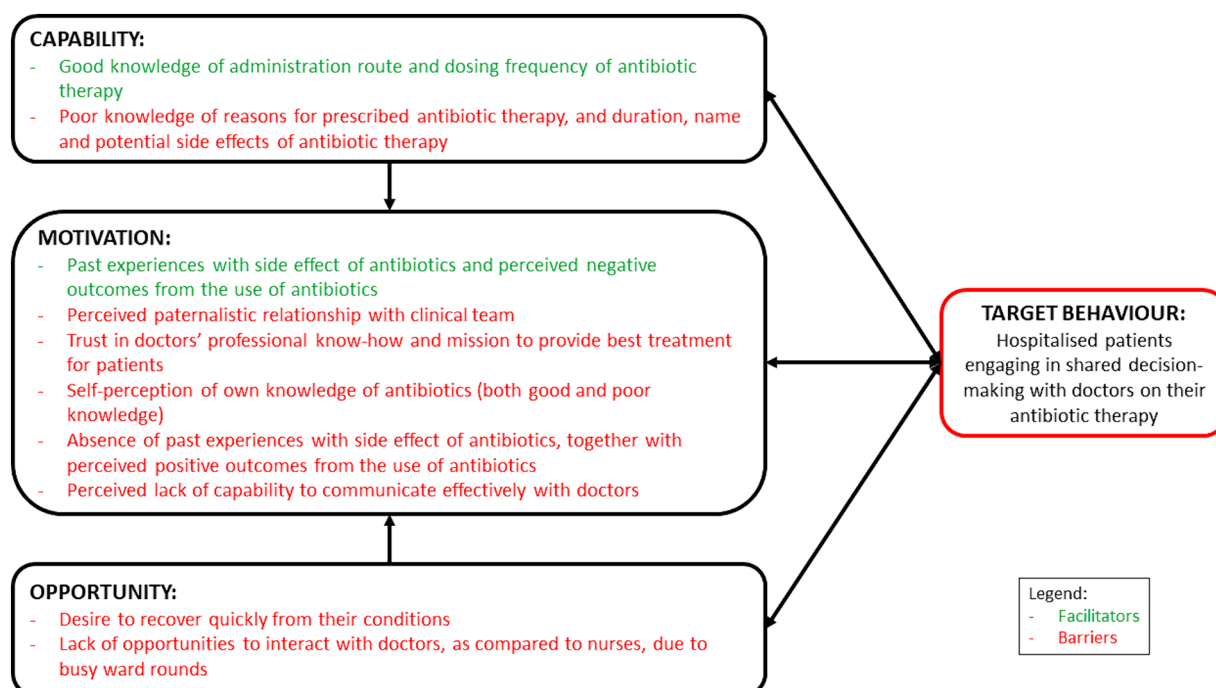


FIGURE 1

Schematic representation of the facilitators and barriers, mapped on the COM-B model, influencing hospitalised patients to engage in shared decision-making with healthcare professionals on antibiotic use.

TABLE 2 Recommended strategies to engage hospitalised patients in SDM for their prescribed antibiotic therapy.

Desired state by hospitalised patients	Facilitators and barriers influencing engagement in SDM	Recommended strategies to improve patients' engagement in SDM
Fast recovery and relief of discomfort	- Past experiences with side effects of antibiotics - Perceived outcomes from the use of antibiotics	Intervention function: Education and persuasion To design patient educational materials to highlight the potential side effects of antibiotic use with specific emphasis on: 1. The potential extension of recovery period due to the additional side effects of antibiotic use; and 2. The undesirable physical discomforts that would accompany with the side effects
More opportunities to discuss and receive information from doctors	- Paternalistic relationship with clinical team - Trust in doctors' professional know-how and mission to provide best treatment - Afraid of being burdensome by querying clinical team	Intervention function: Enablement, persuasion and environmental restructuring To encourage open communication between the clinical team and patients by: 1. Introducing a change in work culture for clinical team to expect and receive queries from patients when they are in doubt of their antibiotic therapy; 2. Having the clinical team to encourage patients to ask questions when they have doubts on their antibiotic therapy; 3. Designing and creating visual cues to assure and nudge patients to actively approach the clinical team to clarify any doubts pertaining to their antibiotic therapy; and 4. Measuring patient satisfaction and related indicators to encourage active discussions between the clinical team and patients around their antibiotic therapy
	- Lack of capability and opportunities to communicate effectively with doctors - More opportunities to interact with nurses	Intervention function: Environmental restructuring To expand nurses' roles to become antibiotic stewards and upskill them to: 1. Receive and relay queries on antibiotic use from patients to their attending doctors; 2. Provide accurate information to the patients for basic questions related to antibiotic administration; and 3. Ensure patients' queries are answered during hospital rounds by the doctors
Good knowledge of antibiotic use	- Good knowledge of administration route and dosing frequency of prescribed antibiotics - Poor knowledge of reasons for use, duration, name and associated side effects of prescribed antibiotics	Intervention function: Education To provide patients with bedside patient information leaflets delivered through offline or online modalities on the details of the antibiotic therapy prescribed, which include: 1. Name of antibiotic prescribed; 2. Route of administration; 3. Duration and dosing frequency; 4. Reason(s) for prescribing the antibiotic; and 5. The side effect(s) of the antibiotic prescribed

choice, dosing frequency, duration and route of administration of the antibiotics. Where further clarifications are required, nurses could direct the patients' queries to the attending doctors and remind doctors to provide their responses during the next consultation round. Already, nurses are acting as information brokers who actively relay patient's clinical status to doctors (38). Hence, as supported by previous findings, nurses can potentially take on an expanded role in antibiotic stewardship if they were provided with a formal recognition, proper education and clear guidelines (38, 39).

Last but not least, as recommended by Elwyn et al. (40) and supported by Waddell et al. (41), it is essential to provide hospitalised

patients with high quality information before engaging them in SDM. The timely provision of patient information leaflets, through both offline and online modalities, upon prescribing the antibiotic therapy at the bedside would be useful to increase their awareness and knowledge of the antibiotics administered. The availability of both offline and online resources is important to ensure sufficient reach to the intended audience due to differential acceptance for digital materials by different age groups (42, 43). The sharing of the patient information leaflets should also be accompanied by a brief explanation given by a doctor or a trained nurse on the key messages, as provider-facilitated education is likely to be more effective than self-directed

learning (44–46). Information pertaining to the antibiotic therapy can include the name of the antibiotic, route of administration, duration of therapy, dosing frequency, reasons for prescribing, and potential side effects, as recommended by the US CDC in its 2019 updated guidance document (47). This information can also help hospitalised patients to be more involved in their prescribed antibiotic therapy by being mindful of the specific antibiotics administered, and be watchful of the potential side effects expected and highlight them to the clinical team when needed. Nonetheless, the fidelity of this strategy depends on the clinical and cognitive statuses of the hospitalised patients to be able to receive and process the educational messages and visual nudges (i.e., the badge).

There are several strengths to this study. To our knowledge, this is the first qualitative study conducted with hospitalised patients to explore the facilitators and barriers influencing their engagement in SDM on antibiotic therapy. The purposive sampling of patients from both medical and surgical wards had ensured a good representation of voices from the two predominant disciplines within the tertiary hospital setting, where antibiotic prescribing behaviours can be very different. Social desirability bias was highly unlikely as the participants were assured that their participation would not influence the standard of care they received. However, there was a smaller proportion of participants recruited from Hospital C. This was due to unforeseen circumstances that prevented the study team from crossing between institutions and entering a different hospital during the COVID-19 pandemic. Nonetheless, data saturation was achieved despite this shortcoming. Lastly, there is a paucity of evidence-based effective strategies for engaging hospitalised patients in antibiotic stewardship (48). Further efficacy and effectiveness studies should be conducted to assess the feasibility of the proposed strategies. Also, since the recommended strategies are based on the study's findings in our hospital, further research is warranted to understand the contextual factors in other institutions, for the successful implementation of these interventions.

5 Conclusion

Hospitalised patients could be part of the antibiotic stewardship efforts in tertiary hospitals. Multi-pronged strategies focusing on creating an enabling hospital environment and culture, expanding nurses' role in antibiotic stewardship and dissemination of antibiotic-related educational messages valued by patients, could help to empower patients to be involved in SDM surrounding their prescribed antibiotic therapy.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the National Healthcare Group Domain Specific Review Board, 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

HG: Formal analysis, Methodology, Project administration, Writing – original draft. DCL: Writing – review & editing. TMN: Writing – review & editing. JS: Writing – review & editing. ALHK: Writing – review & editing. SJC: Writing – review & editing. AC: Conceptualization, Funding acquisition, Methodology, Supervision, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2024.1347764/full#supplementary-material>

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A real-world analysis of pharmacotherapy adherence and the factors influencing it in Serbia: a nationwide, population-based, cross-sectional study

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Introduction: Monitoring the pharmacotherapy adherence in society is crucial for identifying occurrence and causes of potential inadequate use of drugs and inform providers about the need for better customer counseling. It is necessary component of the strategic planning of the quality of healthcare services. This population-based study aimed to assess the medication intake adherence in the Republic of Serbia and the individual factors and health system variables influencing its pattern.

Methods: We applied a cross-sectional approach to study medication intake adherence using a secondary analysis of the latest 2019 Serbian National Health Survey data. The statistical modeling of the pharmacotherapy adherence incorporated sociodemographic data, self-reported disease, and lifestyle behavior.

Results: In 2019, in the representative sample of 12,066 adults in Serbia, requiring prescribed medicine, 49.8% did comply with the prescribed drugs, and 50.2% do not. Participants who adhered to prescribed medication were significantly ($p < 0.001$) older (62.4 ± 14 years), predominantly female (55.3%), had secondary education (48.5%), resided in southern and eastern parts of Serbia (55.5%), and belonged to the lowest income quintile (21.4%). The participants most often take prescribed drugs for hypertension (64.1%) and lower back pain (30.5%), while around 20% take medication for coronary disease, diabetes mellitus, and high blood cholesterol. About 85–92% of participants with financial or general difficulties using prescribed medication.

Conclusion: There is poor medication intake adherence to prescribed medication in Serbia. Gender, age, and region determine the adherence. Also, health-related and healthcare system-related factors impact the use of prescribed medication. Study findings can inform planning the counseling interventions in the target groups where improving medication adherence is necessary, as well as to enhance training of healthcare providers about pharmacotherapy adherence.

KEYWORDS

compliance, adults, population study, chronic disease, pharmacotherapy, medication intake

Introduction

Pharmacotherapy adherence refers to the extent to which a patient's behavior in taking medication aligns with a doctor's advice regarding timing, dosage, and frequency of use (1, 2). According to the International Society for Pharmacoeconomics and Outcome Research (ISPOR) Medication Compliance and Persistence Working Group, adherence is a synonym for compliance (2). Full adherence is when a patient follows a doctor's instructions completely. Although opinions vary, non-adherence is generally defined as taking medications less than 80% of the time (3, 4). Consequently, the incidence of suboptimal adherence may vary based on different definitions. In our study, the prevalence with 95% CI of medication intake adherence was studied not among the patients but at the population level.

Healthcare providers may have difficulties in routine control of adherence with prescribed therapy due to various reasons including of patients' absenteeism on scheduled follow-ups, loss of memory among older adult, multimorbidity induced polypharmacy, etc. Non-adherence to the medication can be intentional or unintentional. In unintentional non-adherence, patients typically forget to take their medication, or were unaware of its relevance. With regard to adherence of prescribed generic drugs, which are often less expensive than branded drugs, many authors found out that among demographic, social, psychological and clinical factors a lower income of patients is significant for optimal adherence, regardless of their gender or age (5). There are many reasons for intentional non-adherence, including experiencing or anticipating side effects, disagreement with the pharmacological treatment, and an inability to pay for the medications (6). According to data from the World Health Organization, about 50% of patients with chronic diseases do not consistently follow the treatment recommendations of pharmacists and doctors, resulting in a lack of the expected response to proven effective medications (7). It is estimated that up to 30% of prescribed drugs never reach the pharmacist for dispensation (3). As a result of insufficient patient adherence, treatment success is questioned, leading to unwanted quality of life and clinical outcomes, unnecessary healthcare utilization and treatment costs, which ultimately may additionally strain the healthcare budget (8–10). It has been estimated that in the US, of all medication-related hospital admissions, about 33–70% result from poor adherence, which represents an annual cost of US\$ 100 billion (11).

Adherence can be measured by subjective (patient self-reports, family interviews, and questionnaires) and objective methods (prescription refill rates, residual pill counting, and electronic measurement devices), and there is no specific measure of medication adherence. In our study focus was on self-reports of pharmacotherapy adherence, which allowed us also exploring the respondents' awareness and reasons of the applied adherence patterns.

Many studies explore medication adherence for specific disease (12–14), factors (15), or drugs (16), and most of these investigations

are within specific healthcare institutions or regions (13, 15). In Serbia, patients with Parkinson's disease and depression compared to Parkinson's disease patients without depression have a three times higher risk for lower adherence to pharmacotherapy (12). A recently published study conducted in one of the larger cities in Serbia showed that adherence to hypertension medications is poor and that non-adherence is more common when taking more medications and a more complicated dosing regimen, and among residents of the urban settlements (13). Additionally, in Serbia, one study has investigated self-medication (the use of medicine without a doctor's prescription) at the population level (17). The prevalence of self-medication was 27.1%, and was associated with sociodemographic and health-related factors, as well as unmet healthcare needs (17). To the best of our knowledge, no studies have assessed nationwide medication intake adherence to prescribed medication within the Serbian population.

This study aimed to assess the prevalence of medication intake adherence to prescribed medication among adults in Serbia and identify factors that might affect adherence. We focused on examining the impact of social and demographic factors, disease-related factors, and barriers within the healthcare system.

Methods

Study design

This is a population-based, cross-sectional study. The study is a secondary analysis of the Serbian National Health Survey data conducted between October and December 2019 by the Institute of Statistics of the Republic of Serbia in cooperation with the Institute of Public Health of Serbia "Dr. Milan Jovanovic Batut" and the Ministry of Health of the Republic of Serbia.

The study followed the Declaration of Helsinki (1964 and 2013) and was approved by the Ethics Committee of Faculty of Medicine University of Belgrade, Belgrade, Serbia (permit number 1322/IX-9). To safeguard the privacy of survey participants and collected data, all procedures were in accordance with the General Data Protection Regulation and the National Personal Data Protection Act.

Sample

Subject sampling and the study protocol were implemented in accordance with the guidelines of the European Health Interview Survey-third wave (EHIS wave 3) (18). In brief, a two-stage stratified sample was used based on the type of settlement (urban or other) and geographical areas (four statistical regions: Vojvodina, Belgrade, Šumadija, and western Serbia, southern and eastern Serbia). The survey included a representative sample size of the population, based on Eurostat recommendations for conducting population health

surveys. From 13,589 participants, based to the focus of this research and after adjust for age (18 years and older) and answers from the use of drugs prescribed by a doctor (yes/no) the total number of participants was 12,066.

Research instrument and variables and setting

For data collection, a face-to-face self-reported questionnaire was used. The questionnaire was standardized and based on Eurostat (19). Trained interviewers gave the participants a printed document with information about the survey.

The sociodemographic characteristics, health status (disease and chronic conditions), use of prescribed medicines by participants, and information regarding the healthcare system were extracted from the National Health Survey 2019 database. The following specific questions about regular use of the medication were used: *“During the past two weeks, have you used any drugs prescribed by your doctor?”* (yes or no), *“Do you have difficulty performing certain activities - taking medication?”* (yes or no) and *“In the past 12 months, have you needed prescribed medication but could not afford it due to financial reasons?”* (yes or no).

The variable *“During the past two weeks, have you used any drugs prescribed by your doctor?”* was dichotomized into a variable with two modalities, “no” and “yes,” where “yes” was the reference category and represented the dependent variable. That variable (medication intake adherence) is related to the use of drugs prescribed by a doctor (relevant to study the adherence, as proposed by EHIS wave 3 Methodological Manual instructions) served as the basis for creating the database in this study. The analysis included pharmaceutical drugs, while vitamins, minerals, herbal medicines, and herbal teas (unless classified as medicines) were excluded. The analysis included the following independent variables: (1) sociodemographic (age years as continuous variable 18+), gender (male/female), level of education (no formal education/primary school/secondary school/ undergraduate studies/postgraduate studies), socioeconomic status (income quintile, five categories from lowest to highest); (2) health-related including the presence of a chronic disease from a list of most common diseases in the population (yes/no). The following question was used: *“Have you had any of the above diseases or conditions in the past 12 months?”* with answers yes or no; (3) healthcare system-related, such as unmet healthcare needs (medication intake) in the past 12 months due to either inability to afford prescribed medication for financial reasons, or difficulty in taking medication. The following questions were used: *“In the past 12 months, have you needed prescribed medication but could not afford it due to financial reasons?”* with answers yes or no and *“Do you have difficulty performing certain activities - taking medication?”* with answers yes or no.

The outcome of interest was medication intake adherence to prescribed medication. In most studies, adherence is defined as taking 80% or more of the prescribed medication doses (3, 4). However, depending on the outcome, that threshold of 80% can be different and cannot be confirmed or rejected (20). It is estimated that adherence to chronic medications is around 50%. Based on the literature data, adherence to medication is considered low or suboptimal if greater

than 20–50% of participants are non-adherent to prescribed medications (12, 21–25).

Statistical analysis

The data are presented with descriptive statistics as n (%), arithmetic mean $\pm$ standard deviation or median (min-max), depending on the type of variables and the normality of the distribution. The t - and chi-square tests were used to test statistical hypotheses. Logistic regression was used to model the relationship between the dependent variable (medication intake adherence with prescribed pharmacotherapy) and potential predictors. Variables significantly associated with the prescribed drugs in the univariate analysis were included in the multivariate logistic regression models. Statistical significance in all analyses was considered if the computed probability value was ≤ 0.05 . The results are presented in tabular and graphic form. All data were processed in the IBM SPSS Statistics 24 (SPSS Inc., Chicago, IL, United States) software package or the R programming environment.

Results

Adherence with prescribed pharmacotherapy

The final total sample included 12,066 adult participants requiring medicine 2 weeks before survey. The prevalence of prescribed medicine use is 49.8% (95% CI 48.9–50.1). Participants who used and did not use prescribed medications in the previous 2 weeks were almost equally distributed in the study sample, 6,005 (49.8%), and 6,061 (50.2%), respectively (Table 1).

Adherence and association with sociodemographic characteristics

Participants who adhered, take prescribed medication in the last 2 weeks, were statistically significant ($p < 0.001$), predominantly female (55.3%), had secondary education (48.5%), resided in southern and eastern Serbia (55.5%), and belonged to the lowest income quintile (21.4%). Older participants (average age 62.4 ± 14) used prescribed drugs more often than younger participants (average age 41.8 ± 15.5). Participants who not take the prescribed medication were predominantly men, participants with secondary school and higher income quintile, resided in Belgrade region. Detailed sociodemographic characteristics of the study sample are shown in Table 1.

Use of prescribed medications associated with disease

The use of prescribed medicines for certain diseases is significantly ($p < 0.001$) higher than non-use. Participants who reported taking prescribed medicines significantly ($p < 0.001$) suffered from hypertension (64.1%) and lower back pain (30.5%),

TABLE 1 The use of prescribed medications and sociodemographic characteristics.

Variable	Total <i>n</i> (%)	Yes <i>n</i> (%)	No <i>n</i> (%)	<i>p</i> -value
Use of prescribed medications in the previous 2 weeks	12,066	6,005 (49.8%)	6,061 (50.2%)	
Gender, <i>n</i> (%)	12,066	2,558 (43.8%)	3,276 (56.2%)	<0.001
Male	5,834 (48.4%)	3,447 (55.3%)	2,785 (44.7%)	
Female	6,232 (51.6%)			
Age, median, min-max		64 (18–99)	40 (18–99)	<0.001
Region	12,066	6,005 (49.8%)	6,061 (50.2%)	<0.001
Belgrade Region	2,847 (23.6%)	1,294 (45.5%)	1,553 (54.5%)	
Vojvodina Region	2,665 (22.1%)	1,403 (52.6%)	1,262 (47.4%)	
Šumadija and western Serbia	3,934 (32.6%)	1853 (47.1%)	2081 (52.9%)	
Southern and eastern Serbia	2,620 (21.7%)	1,455 (55.5%)	1,165 (44.5%)	
Education level	12,062	6,003 (49.8)	6,059 (50.2%)	<0.001
No formal education	924 (7.7%)	731 (12.2%)	193 (3.2%)	
Primary school	2030 (16.8%)	1,322 (22%)	708 (11.7%)	
Secondary school	6,736 (55.8%)	2,912 (48.5%)	3,824 (63.1%)	
Undergraduate (vocational studies)	889 (7.4%)	46 (7.7%)	422 (7%)	
Undergraduate (academic studies)	1,243 (10%)	482 (8%)	761 (12.6%)	
Postgraduate studies (MSc, PhD)	240 (2%)	89 (1.5%)	151 (2.5%)	
Socioeconomic status	12,066	6,005 (49.8%)	6,061 (50.2%)	<0.001
1- lowest income quintile	2,432 (20.2%)	1,287 (21.4%)	1,145 (18.9%)	
2	2,437 (20.2%)	1,265 (21.1%)	1,172 (19.3%)	
3	2,471 (20.5%)	1,230 (20.5%)	1,241 (20.5%)	
4	2,434 (20.2%)	1,176 (19.6%)	1,258 (20.8%)	
5 – highest income quintile	2,292 (19.0%)	1,047 (17.4%)	1,245 (20.5%)	

while about 20% of the respondents had coronary disease, cervical spine pain, diabetes mellitus and high blood cholesterol (Table 2). Our results indicated that there are probably respondents with more than one specific disease, since that the total number of respondents who have specific disease (16.187) is higher than total number of participants (12.066) (Table 2).

Adherence with prescribed pharmacotherapy associated with the healthcare system

Analysis showed that prescribed medication use was significantly ($p < 0.001$) associated with unmet healthcare needs in the past 12 months (Table 3). There is a statistically significant ($p < 0.001$) difference in the frequency of respondents who have financial difficulties in obtaining medication (728/12050) in relation to whether or not the respondents take prescribed medicine (Table 3). However, the respondents with financial difficult to afford medications still in a high extent (85%) intake prescribed medications. The respondents with financial difficulties were more prevalent in the Belgrade region, as well as eastern and southern Serbia. There is a statistically significant difference ($p < 0.001$) in the frequency of difficulty taking medication in relation to whether or not the respondents are taking the prescribed medication. About 91% of respondents who used medication typically had difficulty (minor, major difficulties or total incapacity) in taking prescribed medication (Table 3). Difficulties in performing daily activities, particularly in adhering to therapy (medicines), are significantly ($p < 0.001$) higher in older people

(78 ± 8 –9 years old), women compared to men (62% vs. 37.7%), and in the regions of Šumadija, western, southern, and eastern Serbia.

Predictors of adherence with prescribed pharmacotherapy

In a multivariate logistic regression model, medication intake adherence with prescribed pharmacotherapy was the dependent variable, while social and demographic independent variables were included as adjusted for the existence of a disease or condition in the last 12 months. There was no significant multicollinearity between the social and demographic variables.

In the multivariate logistic regression model, statistically significant potential predictors of medication intake adherence with prescribed pharmacotherapy were female gender ($B = 0.460$; $p < 0.001$), older age ($B = 0.060$; $p < 0.001$), and the region [Belgrade ($B = -0.344$; $p < 0.001$)], with southern and eastern Serbia as a reference category (Figure 1).

Discussion

This was the first study to investigate the characteristics and predictors of prescribed medication usage at the national level in Serbia, allowing to understand the frequency and reasons for non-adherence with prescribed pharmacotherapy. The prevalence of prescribed medicine use is 49.8% (95% CI 48.9–50.1). The study

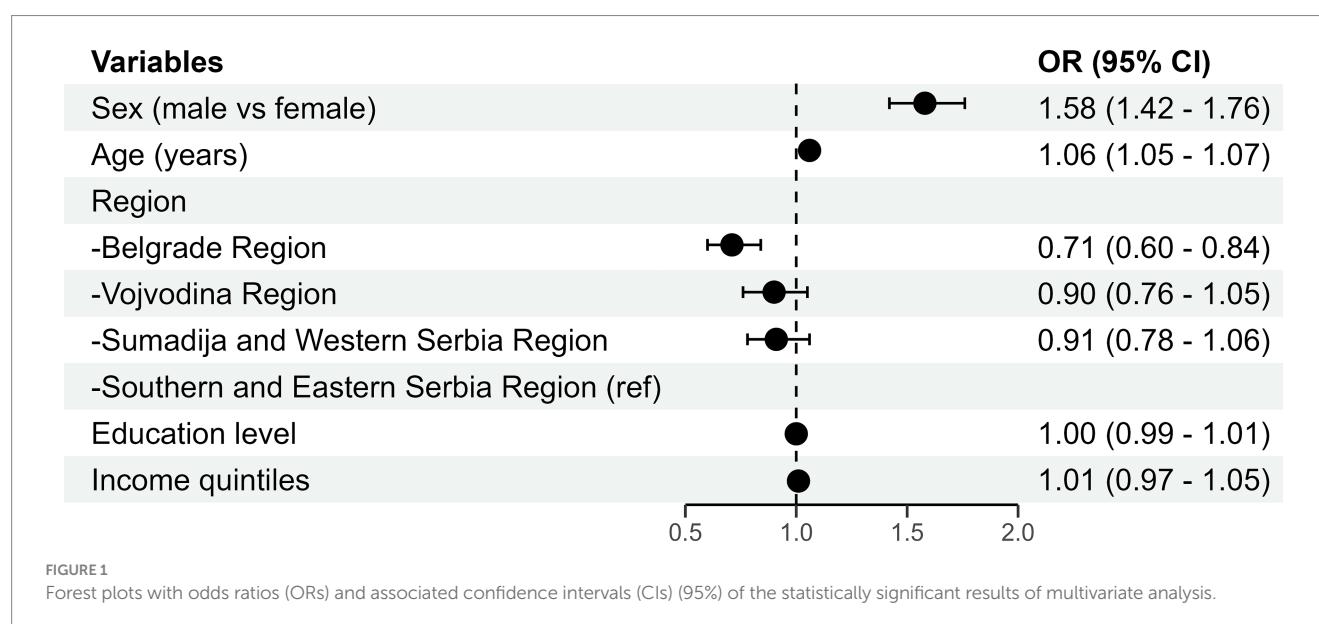
TABLE 2 Use of prescribed medications and presence of disease.

Variable	Total <i>n</i> (%)	Yes <i>n</i> (%)	No <i>n</i> (%)	<i>p</i> -value
Use of prescribed medications in the previous 2 weeks	12,066	6,005 (49.8%)	6,061 (50.2%)	
Asthma	462 (3.8%)	394 (6.6%)	68 (1.1%)	<0.001
COPD*	447 (3.7%)	388 (6.5%)	59 (1.0%)	<0.001
AIM*	255 (2.1%)	244 (4.1%)	11 (0.2%)	<0.001
Coronary disease	1,240 (10.3%)	1,192 (19.9%)	48 (0.8%)	<0.001
Hypertension	4,100 (34%)	3,849 (64.1%)	251 (4.2%)	<0.001
Stroke	160 (1.3%)	153 (2.5%)	7 (0.1%)	<0.001
Arthrosis	971 (8.1%)	860 (14.3%)	111 (1.8%)	<0.001
Lower back pain	2,318 (19.2%)	1833 (30.5%)	485 (8%)	<0.001
Cervical spine pain	1,633 (13.5%)	1,317 (21.9%)	316 (5.2%)	<0.001
Diabetes mellitus	1,087 (9%)	1,049 (17.5%)	38 (0.6%)	<0.001
Allergy	863 (7.2%)	579 (9.6%)	284 (4.7%)	<0.001
Inability to hold urine	450 (3.7%)	405 (6.7)	45 (0.7%)	<0.001
Depression	548 (4.5%)	492 (8.2%)	56 (0.9%)	<0.001
High blood cholesterol	1,428 (11.9%)	1,287 (21.6%)	141 (2.3%)	<0.001
Cancer	225 (1.9%)	202 (3.4%)	23 (0.4%)	<0.001

*COPD, chronic obstructive pulmonary disease; AIM, acute myocardial infarction.

TABLE 3 Use of prescribed medications and healthcare system related characteristics.

Variable	Total <i>n</i> (%)	Yes <i>n</i> (%)	No <i>n</i> (%)	<i>p</i> -value
Difficulty taking medication	335/5675 (5.9%)	307 (91.6%)	28 (8.4%)	<0.001
Cannot afford the medicine for financial reasons – in the last 12 months	728/12050 (6%)	622 (85.4%)	106 (14.6%)	<0.001



revealed a significantly high frequency (about 50%) of non-use of prescribed medicines in the population and poor adherence. Significant associations between the sociodemographic factors and

prescribed medication use among adults in Serbia were observed, as well as the impact of health- and healthcare system-related factors and prescribed medication use. Based on these findings, gender, age,

and region are factors determining the use of prescribed medication in Serbia. Among the adult population, female gender, older age, and residence in the regions of southern and eastern Serbia are significantly associated with higher use of prescribed medicines. Also, individuals with secondary school education and belonging to the lowest income quintile were not associated with medication intake adherence to the prescribed medication. In Serbia, the population primarily takes prescribed drugs to treat conditions such as hypertension, lower back pain, coronary disease, cervical spine pain, diabetes mellitus, and high blood cholesterol. Our results indicate that the population have unmet healthcare needs due to financial constraints or difficulties in adhering to medication therapy which significantly affect the use of medicines, but on the other hand people have high prevalence (85–92%) of medication intake adherence to prescribed medications.

Between the two national surveys, a slight increase in the frequency of prescribed drug use in the population was observed. In 2013, about 44% of the population reported using medicines prescribed by a doctor within the 2 weeks before the survey (26); results of this study show that this frequency was 49% in 2019. However, our results indicate that around 50% of the participants did not use prescribed medicines, which points a high prevalence of nonintake of medication and to poor adherence. Since 98% of the population has obligatory social health insurance, in the Serbian predominant Bismarck social model (income-based and financed jointly by employers) of health system, a burden of costs for prescribed pharmacotherapy is shared between the Health Insurance Fund (27) and out-of-pocket copayment. In 2013, female gender, middle age, and chronic disease were sociodemographic factors associated with self-medication in Serbia (17). Similarly, in our study conducted in 2019, these same factors were found to be associated with the adherence with prescribed pharmacotherapy. As this is about the use of prescribed and non-prescribed drugs, it is possible that a certain degree of self-medication is also present in our respondents.

Our results are in the accordance with the results from population-based study in Germany (21). In Germany at least 33% of population does not use prescribed medication and nonadherence to medication occurs more often in younger populations with higher socioeconomic status than in older populations with chronic conditions (21). Results of the National Health Survey in Spain showed that about 92% of participants take some medication prescribed by a doctor, but unlike Serbia, they included only respondents aged 65+ which does not allow the comparisons (28). For instance, in our research, the age was important from two distinct perspectives. Middle aged individuals (about 40 years and older) were more prone to be non-adherent, and older individuals (about 78 years and above) had difficulties in medicine use. The majority of participants use prescribed medicines for specific diseases, a factor that positively influences treatment outcomes and health preservation. Similar to our results, in Spain women take prescribed drugs significantly more often than men, and these drugs are mostly used for the treatment of hypertension, diabetes, and other chronic diseases (28). Our results agree with data obtained from the adult population in Austria, indicating that individuals from less educated and lower socioeconomic groups were more likely to use prescribed medicines (29). One of the most important predictor of nonadherence may be out-of-pocket costs for medication (30). Our results suggest

that although participants have difficulties in taking and buying medications, there is still a high prevalence (85–92%) of the intake of prescribed medication and good adherence in relation to the general intake medication adherence. In some patients (both low-income and high-income, depression and asthma), it is possible that cost associated nonadherence with may be influenced by patient beliefs, about the perceived value of prescription medication (31). In general, it is difficult to directly compare our results to results of other studies due to methodological differences in sample selection, assessment of adherence, type of drugs and disease and differences between countries.

Study about medication intake adherence was performing in Albania in similar period. As in Serbia and in Albania, there is a high incidence of nonintake of prescribed medication (about 20%) in adult population (23). Factors associated with nonintake of medication in Albania included rural residence, low educational level and low economic level, while in Serbia were residence in capital region, lower educational and higher economic level. In both countries, financial difficulties have a significant impact on the use of prescribed medication, however, in Serbia the frequency of medication intake is still high (85%) compared to Albania (9%). As Albania is a country in transition in Southeast Europe, just like Serbia, the results are interesting to compare because both studies included intake medication adherence and associated factors. However, the results cannot be fully compared, given that the study was in Albania included primary users health care, not general population.

The descriptive data on the extent of medication intake (non-) adherence in the Serbian population are highly informative. The present real-world analysis included 12,066 adults (18+ years) who need prescribed medicine and was not limited by selection bias. The study findings can inform stakeholders efforts toward improving medication adherence in the population and enhancing implementation of national healthcare programs in specific population groups. Moreover, a recommendation is to add more specific questions for polipharmacy into the questionnaire for the next National Health Survey. Pharmacoepidemiologic data from the Health Surveys may be limited due to self-reporting if understanding of the adherence with prescribed pharmacotherapy differs among respondents. The suboptimal level of pharmacotherapy adherences indicates a necessity for rising awareness and counseling of its relevance among people as well as its control among pharmacotherapy prescribers.

This study has some limitations. For monitoring the medication adherence we used subjective method - patient interview and self-report assessments. This is simple to use and less expensive method for measure of medication adherence, but respondents may be biased and give expected answers. However, this is a one of the most common tool for measure of medication adherence, especially useful for nationwide study. As the study was conducted in the population and as a part of a national investigation, the results are useful in the future investigations of medication adherence and in acting on critical factors for a patient's non-adherence and in developing policy to improve adherence in improving treatment outcomes and reducing overall health costs. Next, this survey include medication intake adherence but not determine adherence using another tools. It would be more appropriate, if the survey determine taking medication with more questions and only for specific disease and determine which number taking medication for more than one disease etc. Furthermore, in this study we controlled only the use of prescribed drugs, while the use of drugs on its own was

not included. Also, self-medication in these patients can affect the adherence in prescribed pharmacotherapy. In addition language barriers in some regions (e.g., Vojvodina) should be assessed.

Conclusion

This study gives evidence about the extent and sociodemographic determinants of medication intake adherence among adult population in a transitional South Eastern European region. Our research shows that lower adherence to prescribed medicines is common in the adult population in Serbia. Gender, age and geographic region are the main social and demographic predictors for prescription medicine use. In Serbia, the population most often takes prescribed drugs for the treatment of chronic disease such as hypertension, coronary disease, diabetes mellitus and high blood cholesterol. Although population has difficulties in taking and buying medicines, there is still a high prevalence of the use of prescribed medicines and good adherence in relation to the general intake medication adherence. The priority population groups for improving adherence with prescribed pharmacotherapy includes men, young adults, individuals with higher levels of education and income, and the population residing in the Belgrade region. Study findings can inform for planning health politics because there are determinants that can be influenced by improving health regulations and training health service providers on the necessity of monitoring and advising patients on pharmacotherapy adherence. Decision-makers and policy-makers in Serbia should consider these findings because the costs of noncompliance may be higher.

Data availability statement

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

Ethics statement

Ethical review and approval was not required for the study on human participants in accordance with the local legislation and institutional requirements. Written informed consent from the [patients/ participants OR patients/participants legal guardian/next of kin] was not required to participate in this study in accordance with the national legislation and the institutional requirements.

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Author contributions

DS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Supervision, Visualization, Writing – original draft, Writing – review & editing. ZB: Software, Visualization, Writing – review & editing. MŠM: Data curation, 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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A real-world, cross-sectional, and longitudinal study on high-risk human papillomavirus genotype distribution in 31,942 women in Dongguan, China

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Background: Persistent human papillomavirus (HPV) infection remains a key risk factor for cervical cancer. HPV-based primary screening is widely recommended in clinical guidelines, and further longitudinal studies are needed to optimize strategies for detecting high-grade cervical lesions compared to cytology.

Methods: From November 2015 to December 2023, 31,942 participants were included in the real-world observational study. Among those, 4,219 participants underwent at least two rounds of HPV tests, and 397 completed three rounds of HPV tests. All participants were tested for high-risk types of HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68 (hrHPV) and low-risk types of HPV6/11 genotyping. Some participants also received cytology or colposcopy with pathology.

Results: In the cross-sectional cohort, the prevalence of hrHPV and all HPV subtypes was 6.6% (2,108/31,942) and 6.8% (2,177/31,942), respectively. The three top hrHPV genotypes were HPV52 (1.9%), HPV58 (0.9%), and HPV16 (0.9%). Age distributions showed two peaks at 45–49 and 60–65 years. For the primary screening cohort, the hrHPV prevalence rate increased from 4.8% in 2015–2017 to 7.0% in 2020–2020 and finally reached 7.2% in 2023. For the longitudinal cohort study, the hrHPV prevalence rates in the repeated population (3.9, 5.3, and 6.0%) were lower than the primary hrHPV screening rates (6.6%), which indicated that repeated screening might decrease the prevalence rate. Methodologically, the hrHPV (89.5%) and the screening group of 16 subtypes (92.3%) demonstrated superior sensitivity than the cytology group (54.4%). Moreover, the longitudinal study indicated that the persistent hrHPV subgroup had a significantly higher ($p = 0.04$) incidence of high-grade squamous intraepithelial lesions and more histology progression events (7/17 vs. 0/5) than the reinfection group.

Conclusion: The study indicates a rising high-risk HPV prevalence in Dongguan, with repeated screening reducing this trend. The findings support HPV-based

primary screening and might guide HPV vaccination and cervical cancer prevention in South China.

KEYWORDS

screening, high-risk human papillomavirus, cervical cytology, genotype distribution, infection rate, China, Dongguan

Introduction

According to the Global Cancer Observatory (GLOBOCAN) 2020 estimates, there were approximately 604,127 new cervical cancer cases and 341,831 deaths globally (1). The annual report of the China Cancer Registry indicated that both the incidence and mortality rates of cervical cancer increased in China in 2020, with an incidence rate of 11.35 and a mortality rate of 3.42 per 100,000 women (2). Further socioeconomic analysis indicates that as the human development index (HDI) increases, the incidence rates of cervical cancer tend to decrease (3). Additionally, various risk factors such as human papillomavirus (HPV) have also been associated with cervical cancer, especially the high-risk HPV (hrHPV) strains (4). There are more than 200 types of HPV, and approximately 14 hrHPV (HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68) strains are considered high risk for cervical cancer (4, 5).

The European Research Organization on Genital Infection and Neoplasia (EUROGIN) has recommended HPV testing as a primary screening approach for cervical cancer (6). The Addressing the Need for Advanced HPV Diagnostics (ATHENA) study, the largest prospective clinical study in the United States, assesses the use of HPV testing as the primary screening method for cervical cancer in women aged 25 years and older, which includes testing for hrHPV (14 pooled types) and genotyping for HPV 16 and HPV 18 (5, 7). Moreover, the American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines also suggest that primary HPV testing screening should be considered as an alternative to the current cytology-based cervical cancer screening methods (8).

The HPV for cervical cancer (HPV FOCAL) trial reveals that multiple rounds of cytology missed more participants with cervical intraepithelial neoplasia grade 2 or higher (CIN2+) than HPV-based screening (9). The ATHENA study also confirms that primary HPV testing screening is safer and more effective than primary cytology-based screening (5, 7). In a real-world comparison conducted in Denmark, HPV-based screening demonstrated a 90% increase in the detection of cervical intraepithelial neoplasia grade 3 or higher (CIN3+) (10). Additionally, a cross-sectional study involving 11,064 Chinese women found that HPV-based screening with type 16/18 genotyping is more suitable for China due to a lack of cytologists in the country (11).

To address the high burden of cervical cancer in China, this longitude observational study aimed to evaluate the effectiveness of HPV testing with 14 high-risk genotyping and HPV6/11 types, alongside the ThinPrep cytologic test and colposcopy at the baseline or after repeat screening within 9 years. The goal was to provide evidence for the efficacy of repeated HPV-based screening in addressing the cervical cancer burden.

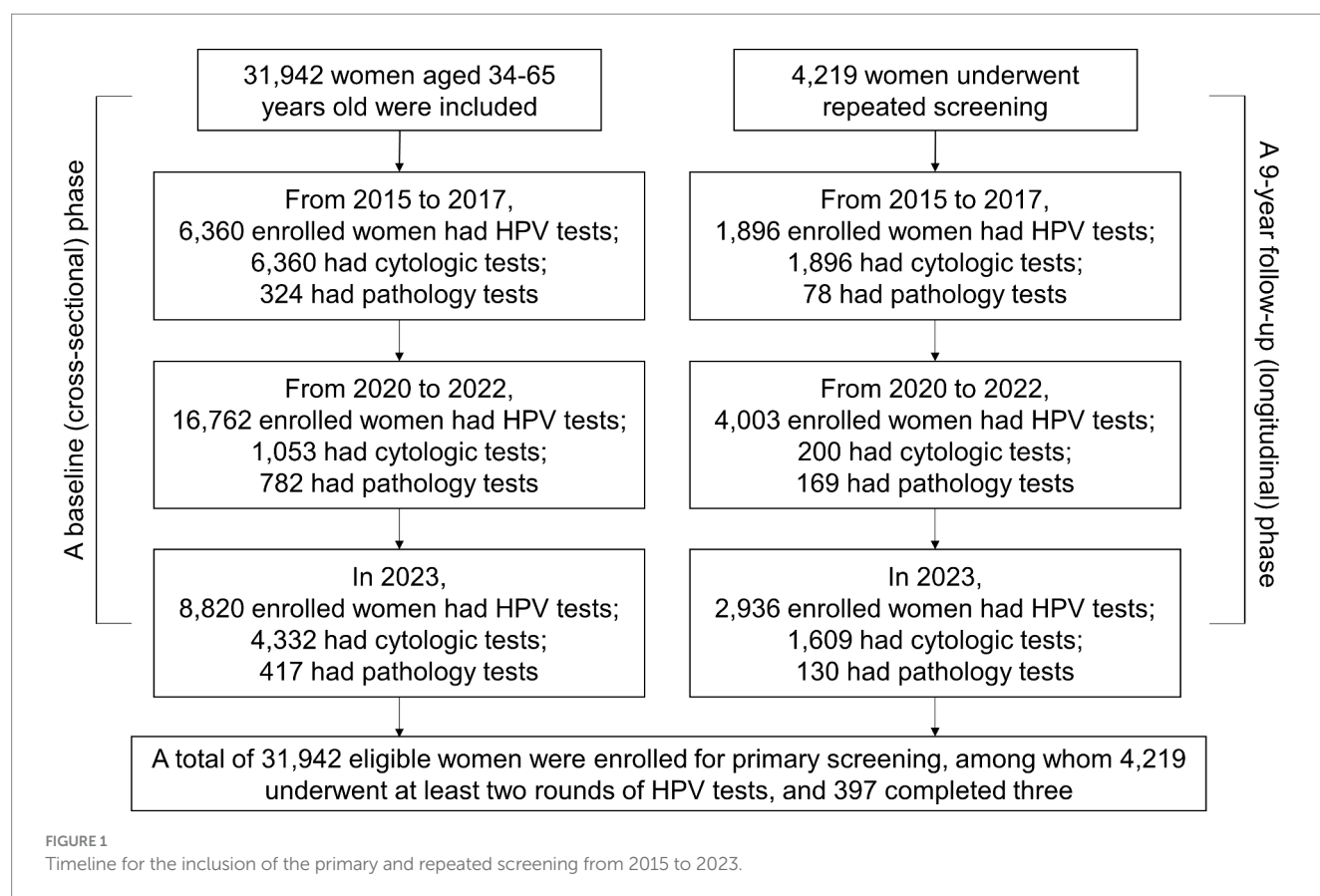
Materials and methods

Patients' inclusion/exclusion criteria and relevant clinical data

A total of 31,942 women aged from 34 to 65 years old (y.o.) were finally enrolled in this observational real-world cervical cancer screening trial from November 2015 to December 2023. It is noteworthy that all of the data including the HPV test results and clinical information were collected in February 2024. Due to the policy support, all the clinical samples were collected by the clinician in the Dongguan Maternal and Child Health Care Hospital. Moreover, in this real-world single-center setting, the primary inclusion criteria included women aged between 34 and 65 years with a history of sexual activity. Those individuals with a history of hysterectomy or being pregnant or menstruating at the time of recruitment were excluded. Women of childbearing age must have adopted reliable contraceptive measures or have undergone a pregnancy test within 7 days before enrollment. The Dongguan Maternal and Child Health Care Hospital in Guangdong Province, China, was the candidate screening locale. Women who underwent the HPV test would receive the ThinPrep cytologic test and colposcopy examination based on individual preferences. Patients with cervical lesions were able to select the treatment options based on their actual clinical condition and personal preference. In our study, patients with cervical lesions were treated with physical therapy and excisional treatment. All collected clinical data at baseline and follow-up phases including screening date, patient age, HPV test results, cytology result, and pathology results are shown in [Supplementary Table S1](#). Among those, the SeqHPV assay (BGI Shenzhen, PRC) on clinician-collected cervical samples was used for screening, and the detailed operating procedures were also shown in a previous publication (12).

Study design

The screening was conducted for three cycles from November 2015 to December 2023. However, the screening was temporarily stopped from 2018 to 2019 due to the shortage of medical resources and the outbreak of the Coronavirus disease 2019 (COVID-19) pandemic. The whole period was divided into three cycles: 2015–2017, 2020–2022, and 2023, with approximately 10,000 participants in each cycle. The timeline for the inclusion of the primary screening is shown in [Figure 1](#). The study was conducted in two phases: a baseline (cross-sectional) phase and a 9-year follow-up (longitudinal) phase. For the baseline phase (cross-sectional phase), participating women were selected for primary screening as the baseline. For the follow-up phase (9-year longitudinal follow-up), women were scheduled for further



second or third follow-up examinations from November 2015 to December 2023. It is noteworthy that all of the data including the HPV test results and clinical information were collected in February 2024.

Statistical analysis

The prevalence of 14 hrHPV types (HPV 16/18/31/33/35/39/45/51/52/56/58/59/66/68) and all 16 HPV subtypes (14 hrHPV types plus low-risk types of HPV6/11) was indicated by positivity rates. The HPV infection and hrHPV infection in this study were defined as positive for all 16 HPV subtypes or 14 hrHPV subtypes, respectively. Abnormal rates of cytology and histology were calculated based on all participants with cytology and pathology testing results. The effectiveness of detecting abnormal histology was assessed by using the sensitivity and specificity between the three strategy groups (HPV, hrHPV, and cytology). All data were analyzed by SPSS 19.0 software or GraphPad Prism 9 software. A *p*-value of less than 0.05 (two-sided) was considered statistically significant.

Results

Clinical data and HPV prevalence among all eligible women

From November 2015 to December 2023, 31,942 women with a median age of 41 y.o. [aged 34–65 years, interquartile range (IQR):

37–46 years] were finally included in this trial. The detailed information of the clinical data is shown in Table 1. Most populations were within the 34–39 y.o. (41.9%) and 40–49 y.o. (41.8%) subgroups. For the 50–59 y.o. and 60–65 y.o. subgroups, the percentages were 14.1 and 2.2%, respectively. The overall positivity rate for the 16 HPV subtypes was 6.8%, with 2,177 out of 31,942 individuals testing positive. For hrHPV types, the positivity rate was slightly lower at 6.6% (2,108/31,942). In Table 2, the HPV prevalence results within different age groups presented two peaks, namely, a higher (45–49 y.o.) and a lower one (60–65 y.o.) both for the hrHPV and the HPV of 16 subtypes.

In Table 1, both the cytology and pathological tests were also performed for the included cohorts. In all, 11,745 individuals with ThinPrep cytologic test results and 1,523 participants with pathological biopsy results were recorded. From the cytological and pathological statuses, the recorded patients showed an abnormal cytology rate of 8.8% (1,029/11,745) and an abnormal histology rate of 46.7% (712/1,523).

Human papillomavirus prevalence, cytology, and pathological status in three cycles of primary screening

The participants for primary HPV screening were 6,360, 16,762, and 8,820 within three cycles of 2015–2017, 2020–2022, and 2023, respectively (Figure 1; Table 3). A relatively small proportion with 29.8% (1,896/6,360), 23.9% (4,003/16,762), and 33.3% (2,936/8,820) of individuals took repeated screening during the 1st, 2nd, and 3rd

TABLE 1 Clinical data for all eligible women.

Characteristics	Eligible women (n = 31,942)
Age, y	
Mean ± SD	42.6 ± 6.7
34–39, n (%)	13,373 (41.9%)
40–49, n (%)	13,362 (41.8%)
50–59, n (%)	4,514 (14.1%)
60–65, n (%)	693 (2.2%)
HPV prevalence results	
HPV52	614 (1.9%)
HPV58	290 (0.9%)
HPV16	282 (0.9%)
hrHPV positive, n (%)	2,108 (6.6%)
HPV positive, n (%)	2,177 (6.8%)
Thinprep cytologic test	
NILM	10,716
ASC-US	674
ASC-H	48
LSIL	219
HSIL	74
SCC	3
AGC	8
AGC, favor neoplastic	3
Pathological biopsy	
Cervicitis	811
LSIL	496
HSIL	198
AIS	3
Carcinoma	15

AGC, atypical glandular cells; AIS, adenocarcinoma in situ; ASC-US, atypical squamous cells of uncertain significance; ASC-H, atypical squamous cells, cannot rule out HSIL; HPV: human papillomavirus; hrHPV: high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesions or malignancies; SCC, squamous cell carcinoma; SD, standard deviation.

TABLE 2 HPV prevalence was identified using the BGI HPV test.

		HPV positive, n (%)	
Age group, y	Total, n	HPV	hrHPV
34–39	13,373	878 (6.6%)	844 (6.3%)
40–44	8,441	593 (7.0%)	577 (6.8%)
45–49	4,921	353 (7.2%)	343 (7.0%)
50–54	2,836	172 (6.1%)	166 (5.9%)
55–59	1,678	122 (7.3%)	119 (7.1%)
60–65	693	59 (8.5%)	59 (8.5%)

HPV, human papillomavirus.

rounds, respectively. Moreover, in Table 3, both the HPV and hrHPV-positive rates gradually increased over time. For hrHPV types, the prevalence increased from 4.8% in 2015–2017 to 7.0% in 2020–2022

TABLE 3 HPV prevalence within the screening year.

Screening year	Total, n	HPV positive, n (%)	
		HPV	hrHPV
2015–2017	6,360	323 (5.1%)	308 (4.8%)
2020–2022	16,762	1,198 (7.1%)	1,166 (7.0%)
2023	8,820	656 (7.4%)	634 (7.2%)

HPV, human papillomavirus.

and finally increased to 7.2% in 2023. Similarly, the prevalence of 16 types of HPV increased from 5.1% in 2015–2017 to 7.1% in 2020–2022 and finally increased to 7.4% in 2023. In Table 4, the number of participants undergoing primary cytology screening was 6,360, 1,053, and 4,332 within three cycles of 2015–2017, 2020–2022, and 2023, respectively. The abnormal cytology rates were not gradually increased over time, with 5.5% in 2015–2017, 36.0% in 2020–2022, and 6.9% in 2023, which might be affected by the low population compared to HPV cohorts during COVID-19 (2020–2022). In Table 5, the number of patients with histology results was 324, 782, and 417 within three cycles of 2015–2017, 2020–2022, and 2023, respectively. For the pathology cohorts, the trends were similar to HPV cohorts, with the positive histological rates gradually increasing from 31.5% in 2015–2017 to 49.0% in 2020–2022 and finally increasing to 55.4% in 2023.

Human papillomavirus infection risk, cytology, and pathological results within three rounds of repeated screening

Except for the primary screening cohorts, 4,219 patients participated in at least two rounds of screening and were further analyzed as repeated screening subgroups (Table 6). In general, 4,219 individuals received two cycles and 397 individuals received three cycles of HPV screening. The prevalence of HPV in repeated subgroups demonstrated a trend similar to that observed in the primary screening cohort, with both HPV and hrHPV positivity rates exhibiting a gradual increase over time (Table 1; Figure 2A). For hrHPV, the positivity increased from 3.9% in the 1st screening to 5.3% in the 2nd screening and finally increased to 6.0% in the 3rd screening; for 16 types of HPV, the prevalence was also increased from 4.0 to 5.6% and finally increased to 6.2% for the 1st, 2nd, and 3rd rounds of screening, respectively. Interestingly, in Figure 2A, compared to primary screening cohorts, we observed that the hrHPV prevalence was slightly lower in repeated populations during the whole study. In addition, we also compared the hrHPV prevalence and cytologic abnormality in different age groups, as shown in Figure 2B. The results showed that the rate of cytology abnormalities was higher than hrHPV positivity in all age groups. Moreover, the two peaks for cytology abnormalities occurred at 60–65 y.o., with the lowest rate at 45–49 y.o., which was the same pattern observed with hrHPV. The study also compared the proportion of women referred for the ThinPrep cytologic test and colposcopy between HPV-based and cytology-based screening methods. We pooled the patients with histology results and compared the methodology of three screening strategies (HPV, hrHPV, and cytology), as shown in Figure 2C. The methodology analysis showed that the sensitivity for HPV (in blue), hrHPV (in yellow), and cytology (in green) strategies was 92.3, 89.5, and 54.4%,

TABLE 4 Cytology results within the screening year.

Screening year	Total, <i>n</i>	Thinprep cytologic test							
		NILM	ASC-US	ASC-H	LSIL	HSIL	SCC	AGC	AGC, favor neoplastic
2015–2017	6,360	6,011	264	15	51	12	3	4	0
2020–2022	1,053	674	202	22	115	39	0	0	1
2023	4,332	4,031	208	11	53	23	0	4	2

AGC, atypical glandular cells; ASC-US, atypical squamous cells of uncertain significance; ASC-H, atypical squamous cells, cannot rule out HSIL; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesions or malignancies; SCC, squamous cell carcinoma.

TABLE 5 Pathological results within the screening year.

Screening year	Total, <i>n</i>	Cervicitis	Pathological biopsy, <i>n</i> (%)			
			LSIL	HSIL	AIS	Carcinoma
2015–2017	324	222	55	37	0	10
2020–2022	782	399	275	102	3	3
2023	417	190	166	59	0	2

AIS, adenocarcinoma in situ; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion.

respectively. The specificity for HPV, hrHPV, and cytology cohorts was 18.9, 20.7, and 65.1%, respectively.

Persistent type-specific HPV infection was defined as the detection of the same hrHPV type at baseline and the follow-up study (13). In our study, 60 individuals had positive hrHPV results within two cycles of screening, 83.3% (50/60) of the hrHPV-positive individuals presented with the same hrHPV subtype and were identified as persistent hrHPV infection within a screening interval of more than 3 years. The remaining 16.7% (10/60) of the reinfection group had a 28.6% (2/7) histology-positive rate in the 1st screening round, and this percentage decreased to 0% (0/6) in the 2nd screening round. For the persistent hrHPV infection subgroup, the histology-positive rate increased from 38.7% (12/31) to 52.6% (10/19), especially for the percentage of the high-grade squamous intraepithelial lesion (HSIL) percentage group, and this percentage significantly increased ($p=0.04$) from 3.2% (1/31) to 21.1% (4/19), as shown in Figure 2D. According to the real-world hrHPV and histology data, the increased histology events only occurred (5/14) in the persistent infection group during a follow-up of more than 2000 days, and no events (0/5) occurred in the reinfection group (Figure 2E). More attention should be paid to the persistent hrHPV infection subgroup.

Discussion

The prevalence of 14 hrHPVs and low-risk HPV types 6 and 11 was analyzed in 31,942 Chinese women. Here, in our study, the prevalence of hrHPV was 6.6%, which was lower than the 12.6% hrHPV positivity rate reported in the ATHENA trial (5), as well as the 18.63% hrHPV prevalence documented in a separate study conducted in Guangzhou (14). This might be due to our limited inclusion criteria for participants of 34–65 y.o. in only one center. Moreover, we included individuals with a median age of 41 y.o. (IQR: 37–46), which was higher than these two studies. Actually, in our cohort, the hrHPV positivity of 6.6% was similar to another study with an overall HPV infection prevalence of 7.3% in the Guangdong province (15), which was lower than most other regions of China.

The prevalence of cytologic abnormalities was 8.8% in our study, which was higher than both the ATHENA study of 7.1% (5) and one multicenter randomized study of 5.9% with ASC-US+ (16). The elevated percentage observed may be influenced by the real-world data, which indicates that 93.2% (2029 out of 2,177) of HPV-positive patients underwent cytological examinations, in contrast to only 33.1% (9,860 out of 29,765) of HPV-negative individuals. Moreover, this phenomenon was more obvious in the subgroup with pathology results, in which 60.4% (1,315/2177) of HPV-positive and 0.7% (208/29765) of HPV-negative patients underwent pathology examination. The examination data were very low, especially during 2020–2022, which might be affected by both the COVID-19 pandemic and the shortage of medical resources. This data also reflected the real-world data that only the HPV-positive individuals would have further examinations. Moreover, due to the policy support (17), all participants underwent concurrent HPV and cytology tests during 2015–2017. There was a notable decline in the number of individuals receiving cytology tests during 2020–2022, which could be attributed to alterations in policy and the impact of the COVID-19 pandemic.

A previous study on Guangdong province has reported two peaks of HPV infection in the population of less than 25 y.o. (8.2%) and more than 50 y.o. (9.6%) (15). Another study in Guangzhou shows that two peaks were aged <21 and 46–50 years (14). A cross-sectional study presents two peaks: a higher (14–19 y.o.) and a lower one (30–34 y.o.) (18). In our cross-sectional cohort group, we only enrolled primary screening participants aged between 34 and 65 years, which might miss some crucial populations. Notably, we observed two age distribution peaks within the age ranges of 45–49 and 60–64 years, which was similar to previous reports of ages older than 46 years (14, 15). This pattern might be attributed to geographical variations, as our cohort was exclusively drawn from patients at a single hospital in Dongguan. It was also important to highlight that the representation at the extreme ends of our age spectrum was minimal, with only 1 patient aged 34 years and 3 patients aged 65 years. Such low numbers at these points were insufficient for statistical analysis to draw reliable conclusions. Moreover, the peaks for cytologic abnormalities were at 45–49 and 60–64 y.o.,

TABLE 6 HPV infection risk, cytology, and pathological results within three rounds of repeated screening.

		HPV positive, <i>n</i> (%)		Thinpre cytologic test								Pathological biopsy				
Screening round	Total, <i>n</i>	HPV	hrHPV	NILM	ASC-US	ASC-H	LSIL	HSIL	SCC	AGC	AGC, favor neoplastic	Cervicitis	LSIL	HSIL	Carcinoma	
1st (15–17)	1,896	76 (4.0%)	73 (3.9%)	1,800	80	6	9	1	0	0	0	55	14	8	1	
2nd (20–22)	4,003	223 (5.6%)	212 (5.3%)	127	42	2	24	4	0	0	1	90	64	15	0	
3rd (23)	2,936	182 (6.2%)	175 (6.0%)	1,513	77	3	13	2	1	0	0	72	52	6	0	

AGC, atypical glandular cells; ASC-US, atypical squamous cells of uncertain significance; ASC-H, atypical squamous cells, cannot rule out HSIL; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; HSIL, high-grade squamous intraepithelial lesion; LSIL, low-grade squamous intraepithelial lesion; NILM, negative for intraepithelial lesions or malignancies; SCC, squamous cell carcinoma.

which was the same as the phenomenon in the hrHPV group, confirming the real-world data of both hrHPV and the cytology procedure.

Our longitude cohort study had a follow-up of approximately 9 years for a repeated population of 4,219 participants. The hrHPV prevalence was also increased in this repeated screening subgroup over time. While compared to the primary screening cohort, the total percentage was lower than the average percentage of 6.6% hrHPV prevalence rate. One study shows that the negative conversion rate of hrHPV increased to 68.9% within half a year and increased most rapidly within the first 2 years after treatment (19). Another study shows that all subtypes of HPV-negative rates were 81.81, 85.71, and 90.91% at 6, 12, and 24 months, respectively (20). Factors such as age, HPV type, sexual behavior, and initial treatment can affect HPV clearance, yet most HPV-infected women typically remove the virus within 6 to 12 months (21). In our cohort, our hrHPV-negative rate was only 45.8% (50/76) between primary screening and the second screening rounds, which might be affected by our long follow-up of more than 3 years. Additionally, the histology-positive rate significantly increased from 38.7 to 52.6% in the persistent hrHPV infection group, especially for the percentage of HSIL, which significantly increased from 3.2 to 21.1%. The recent longitudinal study, including a multicenter cohort study, has demonstrated the reliable efficacy of a real-time polymerase chain reaction (PCR)-based assay in cervical screening (22). This assay differentiates between HPV16 and HPV18 and 12 other hrHPV types among 9,829 eligible women, with a follow-up of 3 years (22). Another 3-year longitudinal study of 10,186 women reveals that infections with hrHPV types other than HPV 16/18 are responsible for a large number of CIN2 and CIN3+ cases (23). In our study, we conducted a 9-year follow-up involving larger populations. Additionally, by utilizing next-generation sequencing (NGS) techniques, we achieved a high-throughput analysis of 16 specific HPV subtypes.

As this is a retrospective real-world study, certain limitations are inherent, including the use of specific inclusion and exclusion criteria. Patients could choose the treatment options based on their actual clinical condition and willingness. This approach slightly differs from that of the ATHENA study, which indicated that patients did not have prearranged plans for further HPV treatment (5). In our real-world cohort, patients with cervical lesions had physical therapy or excisional treatment. Figure 1 shows the number of participants screened during each cycle, with re-screening based on individual willingness. Consequently, a relatively small proportion of individuals of 29.8, 23.9, and 33.3% underwent repeated screening during the 1st, 2nd, and 3rd rounds, respectively. The slight decrease in participation during the second round from 2020 to 2022 might have been influenced by the COVID-19 pandemic. There is no definitive treatment for HPV infection to date; emerging immunotherapeutic approaches, such as toll-like receptor agonists, therapeutic HPV vaccines, and immune checkpoint inhibitors are under investigation (24). The routine treatment could not guarantee HPV clearance. Other studies on the gene polymorphisms of toll-like receptors and cyclooxygenase-2 in relation to cervical cancer risk hold promise for advancing our understanding of predictive biomarkers, which could significantly enhance the risk stratification for hrHPV (25, 26). Further studies could explore future predictive biomarker development, as well as the pathological outcomes and treatment responses associated with these HPV subtypes, monitored over the course of the follow-up period.

A recent study focusing on primary cervical cancer screening in Latin America has shown that the sensitivity of CIN3+ using cytology and HPV tests was 48.5 and 98.1%, respectively (27). The results were similar to our

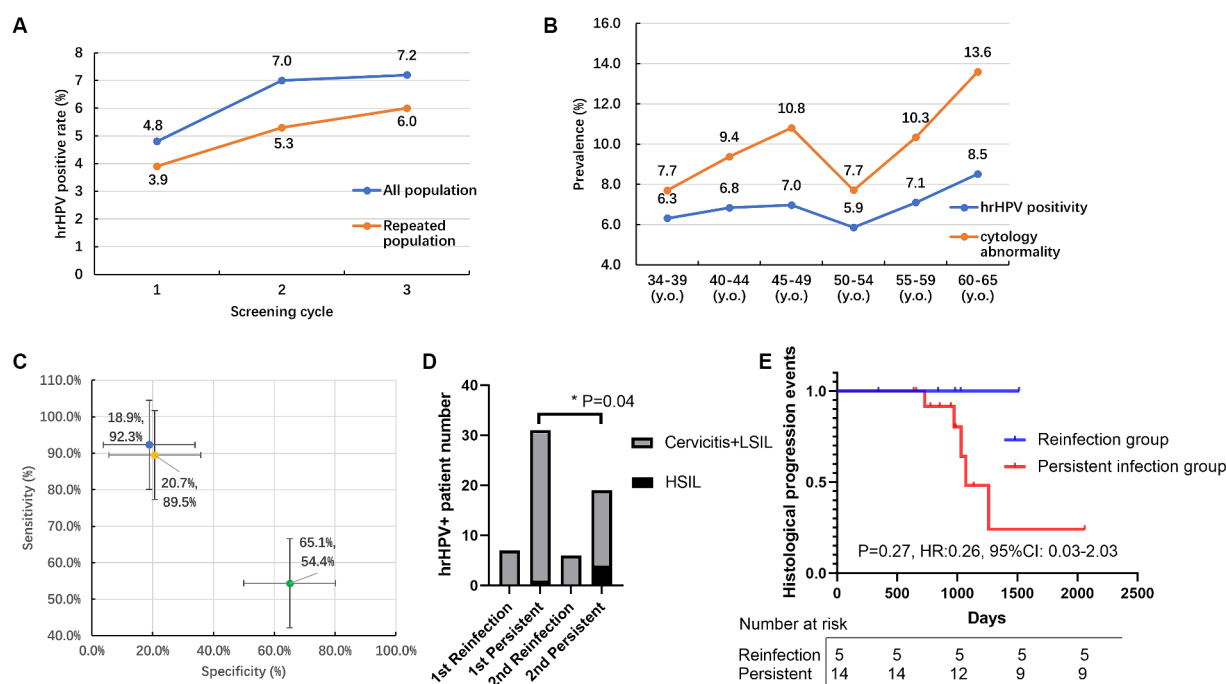


FIGURE 2

The HPV prevalence, cytology, and pathological results in the primary and repeated screening cohorts. (A) Prevalence of hrHPV positivity for all populations and repeated populations within three cycles of screening; (B) Prevalence of hrHPV positivity and cytology abnormality in different age groups; (C) Sensitivity and specificity of HPV (in blue), hrHPV (in yellow), cytology (in green) strategies to detect pathology abnormalities; (D) The number of normal/abnormal histology patients for HSIL subtypes in persistent hrHPV infection versus reinfestation groups within 1st and 2nd rounds of repeated screening. (E) The follow-up of increased histology events in persistent hrHPV infection and reinfestation groups during screening. HSIL: high-grade squamous intraepithelial lesion. y.o., years old. * $p < 0.05$.

subgroup with histology results; we concluded that the sensitivity was very high in both the hrHPV screening group (89.5%) and the 16 subtypes group (92.3%) compared to the cytology group (54.4%). The specificities observed in our three groups were notably lower than the reported 96.5% for cytology and 88.7% for HPV testing (27), with our figures being 20.7% for the hrHPV group, 18.9% for the 16 HPV subtypes group, and 65.1% for the cytology group. This discrepancy may be due to the high proportion of HPV-positive individuals in our study cohort, where 93.2 and 60.4% of HPV-positive patients underwent cytological and pathological examinations, respectively, compared to only 33.1 and 0.7% of HPV-negative individuals. In our study, the rapid and low-cost isothermal amplification HPV assay (12), which is priced at half the cost of the ThinPrep cytologic test, could also be recommended because of its high sensitivity and high-throughput characteristics. The findings from this study have the potential to guide future HPV immunization strategies and the development of policies aimed at preventing cervical cancer in Dongguan.

Conclusion

In this real-world study for hrHPV screening among 31,942 Chinese women, the prevalence of hrHPV was 6.6% in the cross-sectional cohort. We also observed two age distribution peaks within the age ranges of 45–49 and 60–64 years. For the longitude cohort study, a repeated population of 4,219 participants with a follow-up of nearly 9 years was investigated. The hrHPV prevalence was decreased in the repeated screening cohort than

in the primary screening cohort. It is noteworthy that within the cohort subjected to repeated screening, the subgroup with persistent hrHPV infections exhibited a higher HSIL-positive rate and more histological progression events than the hrHPV reinfestation subgroups. Furthermore, the sensitivity of the hrHPV screening group (89.5%) and the 16 subtypes group (92.3%) surpassed that of the cytology group (54.4%). Despite the HPV test having a lower specificity and higher cost compared to cytology, the HPV test should also be considered due to its superior sensitivity and high-throughput capability.

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/s.

Ethics statement

This study was approved by the Ethics Committee of Dongguan Maternal and Child Health Care Hospital (Ethics approval number: Dongguan Maternal and Child Health Care Hospital-2022-103) and the BGI Genomics (grant no. BGI-IRB 24029). 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

HZ: Data curation, Formal analysis, Funding acquisition, Writing – original draft, Writing – review & editing. WP: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. BC: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. JG: Data curation, Validation, Writing – original draft, Writing – review & editing. YL: Data curation, Writing – original draft, Writing – review & editing. GS: Data curation, Writing – original draft, Writing – review & editing. XH: Data curation, Writing – original draft, Writing – review & editing. HY: Data curation, Writing – original draft, Writing – review & editing. HG: Data curation, Writing – original draft, Writing – review & editing. LZ: Data curation, Writing – original draft, Writing – review & editing. ZL: Data curation, Writing – original draft, Writing – review & editing. PZ: Data curation, Supervision, Writing – original draft, Writing – review & editing. SZ: Data curation, Supervision, Writing – original draft, Writing – review & editing. LT: Data curation, Supervision, Writing – original draft, Writing – review & editing.

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Conflict of interest

Author BC was employed by company BGI Genomics.

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

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2024.1409030/full#supplementary-material>

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Can periodontitis affect the COVID-19 severity, symptoms, hospital stay, and mortality? A case-control study

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Background/purpose: Poor oral hygiene and periodontal disease have been identified as potential risk factors for the coronavirus disease 2019 (COVID-19). The present study aimed to determine the association between periodontitis and COVID-19 severity, nature of symptoms, mortality, and hospital stay.

Methods: In total, 163 COVID-19-positive patients (men: 93; women: 70) were categorized into two groups: the control group, consisting of 120 patients with asymptomatic or mild symptoms, and the case group, consisting of 43 patients with moderate-to-severe symptoms. The severity of periodontal disease, oral hygiene status (OHI), pocket depth (PD), bleeding on probing (BOP), number of decayed/missing/filled teeth, mortality, duration of stay in the hospital, oxygen requirement, and nature of COVID-19 symptoms were assessed in both groups. The association between periodontitis and COVID-19 was analyzed with other confounding factors such as age, sex, comorbidities, oral hygiene, and smoking status.

Results: The presence of periodontitis increases the severity of COVID-19 by 3.7 times ($p = 0.002$). A statistically significant difference was noted for symptoms such as dizziness ($p = 0.036$), running nose/cold ($p = 0.009$), and headache ($p = 0.005$) in the presence of periodontitis. The risk estimate for death associated with periodontitis was 1.03. Additionally, the average duration of stay was longer for individuals with periodontitis than for those in the control group.

Conclusion: There is a positive association between periodontal disease and COVID-19. Periodontitis increases the severity of COVID-19 and alters the symptoms. Hence, periodontal disease management should be an integral part of managing patients with coronavirus infection.

KEYWORDS

coronavirus disease, COVID-19, periodontitis, periodontal disease, SARS-CoV-2

Introduction

The coronavirus disease 2019, referred to as COVID-19, is caused by SARS-CoV-2. SARS-CoV-2 is a zoonotic virus, similar to the Middle East respiratory syndrome coronavirus (MERS-CoV) and the SARS-CoV-2 (1, 2). The human-to-human transmission primarily occurred via oral and respiratory droplets, through both direct contact and non-contact modes (2–7). As of 14 May 2023, over 766 million confirmed cases and

over 6.9 million deaths have been reported globally. Many variants of SARS-CoV-2 have been discovered across the globe causing myriads of symptoms (8, 9). Some common symptoms of SARS-CoV-2 infection are fever, sore throat, fatigue, body aches, and loss of taste and smell. In severe cases of COVID-19, pneumonia, shortness of breath, need for oxygen or ventilation, and multi-organ failure have been reported (10, 11). Many studies have found that the severity of COVID-19 increases in individuals with comorbidities such as hypertension, diabetes mellitus, cardiovascular disease, respiratory disorders, immunocompromised conditions, and in individuals undergoing radiotherapy and chemotherapy (12–14).

Poor oral hygiene and periodontal disease, an inflammatory condition affecting the soft tissues around the teeth, have recently been associated with many respiratory diseases, including COVID-19 infection (15–25). This association is attributed to the influx of periodontal pathogens and proinflammatory cytokines into the systemic circulation during periodontitis (21–23). Additionally, inflamed periodontal tissues have been identified as a source of increased presence of angiotensin-converting enzyme 2 (ACE2). The increased ACE2 allows the attachment of viral particles, promoting their spread from the oral cavity into the systemic circulation (22). Many studies have established the association between periodontitis and COVID-19 and confirmed that the probability of acquiring COVID-19 is 2.81 times higher in patients with periodontitis (18–25). The risk of COVID-19 also increases as periodontal inflammation increases with an OR of 11.75 for severe periodontitis and an OR of 17.65 for gingivitis (18). Periodontal disease has also been linked to many COVID-19 complications, including death, increased ICU admission, and the need for assisted ventilation (25). Periodontitis has been associated with a 4-fold increase in hospitalization (OR = 4.72); a 6-fold increase in the need for assisted breathing (OR = 6.24); and a more than 7-fold increase in death (OR = 7.51) (20). However, the association between the severity of periodontitis to the nature and severity of COVID-19 has not been explored. With this background, the present study aims to determine the association of periodontitis with the severity of COVID-19. The main objectives of the study are as follows:

1. To correlate and compare the severity of periodontitis with the severity of COVID-19 symptoms, ranging from asymptomatic to mild, and moderate-to-severe symptoms.
2. To assess the relationship between oral hygiene status (plaque and calculus scores), bleeding on probing (BOP), and alveolar bone loss in individuals with mild, moderate, and severe COVID-19 symptoms.
3. To evaluate the effect of age, sex, smoking, and the presence of any comorbidities on the association between COVID-19 and periodontitis.
4. To determine the association between periodontitis, mortality rates, and length of hospital stay for COVID-19 patients.

Materials and methods

This case-control study was conducted at Manipal College of Dental Sciences, Manipal, Karnataka, India, from December

2019 to January 2022 following the Declaration of Helsinki (as revised in 2013), STROBE, and SAGER guidelines. The study was initiated after approval from the institutional review committee of Kasturba Medical College and Kasturba Medical Hospital (IEC no: IEC2-107/2022; Clinical Trial Registry India registration no: CTRI/2022/08/045088).

Sample size

The sample size was determined based on a previous study by Mouraf et al. aiming for 80% power (25). Based on the above calculations, a total sample size of 144 was required (case group = 24 and control group = 120).

Screening, eligibility criteria for recruitment, and grouping

The medical and dental records of COVID-19-positive patients were obtained from the Office of Medical Superintendent, Kasturba Medical College, Manipal, from the year 2019 to 2021. A total of 3,392 medical records were screened to note the presence and absence of COVID-19 based on positive reverse transcription-polymerase chain reaction (RT-PCR) reports. Both verbal consent and written informed consent were taken. All the COVID-19-positive patients were further screened based on specific inclusion and exclusion criteria as follows:

Inclusion criteria

1. Both male and female participants aged above 18 years with a history of COVID-19 infection and having a previous dental record were included in the study.

Exclusion criteria

1. Subjects <18 years of age.
2. Edentulous patients (with complete loss of teeth).
3. COVID-19-positive patients who were pregnant and lactating at the time of coronavirus infection.

The following data were retrieved from the medical records of each patient who fulfilled the eligibility criteria: the date of the RT-PCR test; the date of onset of COVID-19 infection; and the signs and symptoms of COVID-19. Based on the signs and symptoms, all participants were categorized into asymptomatic, mild, moderate, and severe as per the ICMR guidelines into two groups: case (participants with moderate-to-severe symptoms of COVID-19) and control groups (participants with mild-to-asymptomatic symptoms of COVID-19) (26, 27).

The following data were recorded for both groups: age (in years), sex (male/female), weight (in kilograms) and height (in centimeters), smoking status (yes/no), frequency of brushing, use of interdental aids or mouthwash (yes/no), presence of any comorbidities, hospital stay after COVID-19, and partial pressure of oxygen (PO₂). The dental records of all the participants were

checked by another investigator (SM) who was blinded to the COVID-19 severity and grouping of participants. The dental records were checked for the following data: presence or absence of bleeding on probing (BOP) or percentage of site with BOP; amount of plaque and calculus scores; mean pocket depth (PPD) (in millimeters); and clinical attachment loss (CAL) (in millimeters). The radiographic assessment of bone loss (in millimeters) and the presence/absence of decayed, missing, and filled teeth was noted. The extent of bone loss was measured from the cemento-enamel junction (CEJ) to the apex to the root (in millimeters) using ImageJ software (online version) (28, 29). Based on the average PPD mentioned in the dental records, participants with an average PPD of <4 mm and no interdental bone loss (BL) were considered as the non-periodontitis group ($N = 63$). Those with an average PPD of more than 4 mm and having two or more teeth with interdental/interproximal bone loss were classified as the case group (periodontitis group; $n = 100$). The periodontitis group was further divided based on the extent of interdental bone loss according to the 2017 periodontal disease classification as Stage I/Stage II/Stage III/Stage IV (28, 29).

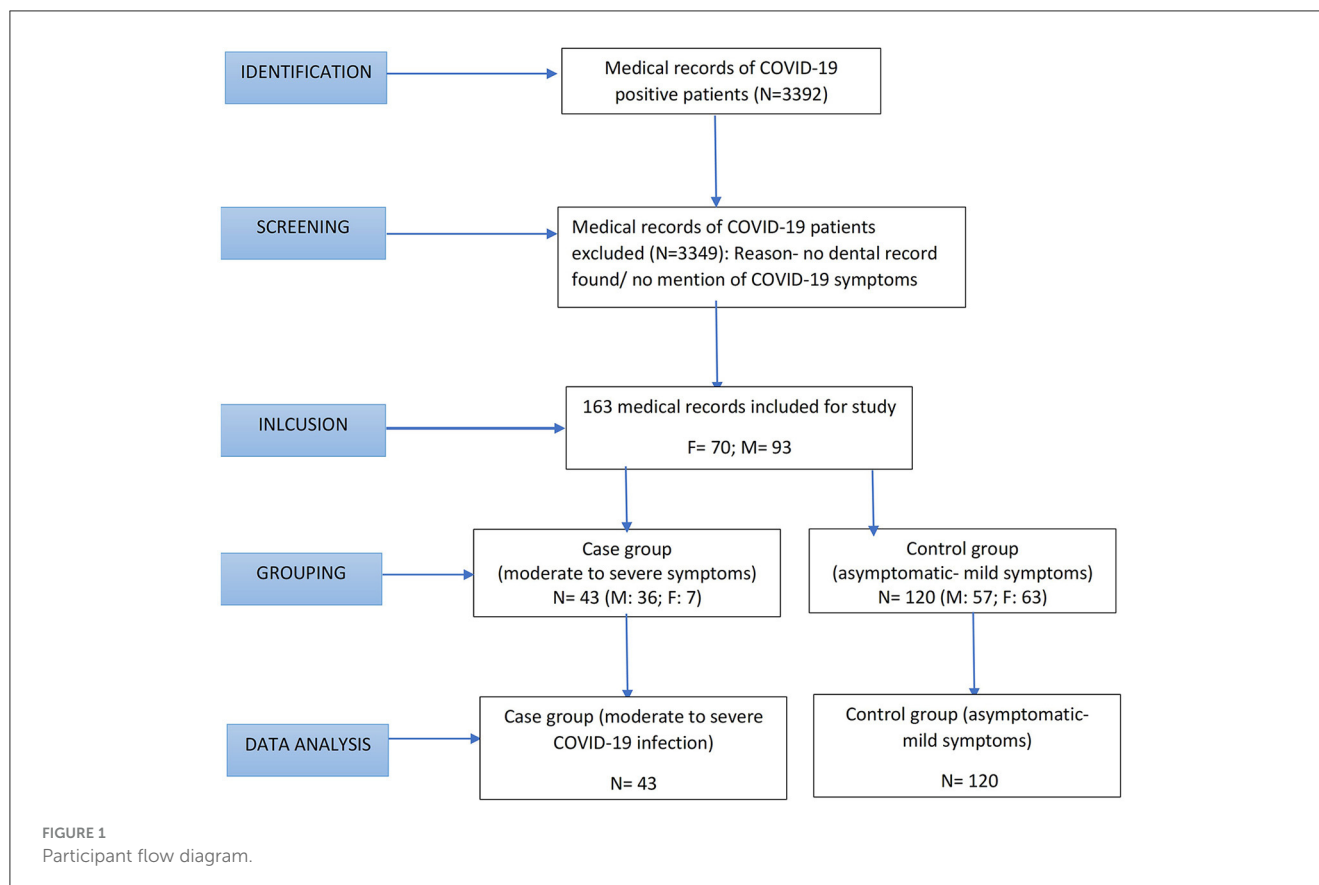
Statistical analysis

All data were entered manually, coded, and proofread for entry errors by two investigators (AC and SM). The data were subjected to statistical analysis using Statistical Package for Social Sciences (SPSS v 20.0, IBM). Descriptive statistics such as frequencies and

percentages for categorical variables and the mean and standard deviation (SD) for continuous variables were calculated. To assess the relationship between exposure (severity of periodontitis) and outcome (severity of COVID-19), OR with 95% CI was calculated. Multiple linear regression was performed to predict the effect of the independent variables [age, sex, comorbidities, oral hygiene index (OHI), and smoking] on the association between periodontitis and COVID-19 severity. For all the statistical tests, a p -value of <0.05 was considered to be statistically significant, keeping α error at 5% and β error at 20%.

Results

A total of 3,992 medical records were initially screened for the presence of COVID-19 by checking for a positive RT-PCR report or signed medical diagnosis of COVID-19. Of this, 3,512 records were included and checked for the presence of dental records. Out of 3,512 records, 3,349 were excluded as their dental records were not available. Additionally, 32 records were excluded as the symptoms of COVID-19 or its severity were not mentioned. Finally, medical records of 163 patients were included in the study (Figure 1). Among the 163 participants, 120 participants were in the control group and 43 in the case group. The symptom-wise distribution was as follows: 16 were asymptomatic (13.3%), 104 showed mild symptoms of COVID-19 (86.7%), 21 had moderate symptoms of COVID-19 (48.8%), and 22 had severe (51.2%). The descriptive data and the results of the regression analysis



to assess the association between periodontitis and COVID-19 severity based on age, sex, comorbidities, and oral hygiene status are discussed in detail in the following paragraphs (Tables 1–4).

Sex and age

The group-wise distribution showed that 83.7% of the participants in the case group were men and 16.3% were women. The odds of having a higher severity of COVID-19 was 5.68 (p -value: 0.001) in men (Table 1, Supplementary Tables S1–S9). Upon comparison of sex with the presence or absence of periodontitis in the case and control groups, a statistically significant difference was seen between the men and women with a p -value of 0.053 (Table 1, Supplementary Table S1). Upon comparing the severity of periodontitis, a statistically significant difference was observed in age across different stages of periodontitis ($p < 0.001$).

Habits (smoking and smokeless tobacco use)

The percentage of smokers and non-smokers in the case group was 4.7% and 95.3%, respectively. In the control group, 8.3% were smokers, and 91.7% were non-smokers ($p = 0.428$) (Table 1). The association between periodontal status and COVID-19 severity, considering the use of smokeless tobacco and smoking, showed a significant association (OR: 5.85; p -value < 0.001) (Supplementary Table S1).

Endocrine diseases

As endocrine disorders (especially diabetes mellitus) are an established risk factor for periodontal disease and COVID-19 severity, the association was assessed. A statistically significant difference was noted between the groups and COVID-19 severity ($p = 0.036$) with 46.5% of moderate–severe COVID-19 patients reporting a history of endocrine disorders compared to 27.7% of asymptomatic–mild cases. The difference in the severity of periodontal diseases was statistically significant ($p < 0.001$), with 44.4% of periodontitis patients and 14.3% of no-periodontitis patients affected by endocrine disease (Table 1).

Comorbidities

A statistically significant difference was noted between periodontitis and non-periodontitis groups for all comorbidities except for neurological conditions (Supplementary Table S2). The association of comorbidities with the severity of COVID-19 showed an OR of 3.37 for cardiovascular diseases, an OR of 2.26 for endocrine disorders, an OR of 4.04 for respiratory diseases, and an OR of 6.32 for neurological diseases (Tables 2, 4, Supplementary Table S2). Respiratory (p -value: 0.009) and neurological conditions (p -value: 0.002) were shown to have a

significant influence on the association between periodontitis and COVID-19 with an OR of 4.125 and 5.778, respectively (Table 2).

Association of periodontitis and periodontal parameters in the case and control groups

In the case group, 100 participants (81.4%) had periodontitis; whereas 65 participants (54.2%) in the control group had periodontitis. In the case group, periodontitis was absent in 18.6% of participants (p -value: 0.002) (Table 3). The staging of periodontal disease showed that 9.8% of the individuals were in Stage I, 28.8% were in Stage II, 9.2% were in Stage III, and 13.5% were in Stage IV. A statistically significant association was observed between periodontitis and COVID-19 severity ($p = 0.002^*$, OR = 3.702) (Supplementary Tables S3, S6). A significant association was also noted between periodontal parameters, such as BOP, and COVID-19 severity ($p = 0.017$). The BOP in the case and control groups was 95.3% and 100%, respectively (Supplementary Tables S3, S4).

Association of oral hygiene practice and decayed/missing/filled teeth scores in the case and control groups

The oral hygiene status of the participants with regard to plaque ($p = 0.275$) and calculus scores ($p = 0.370$) showed no statistically significant difference between the case and the control groups (Supplementary Table S5). When the DMF scores were compared in the case and control groups, the number of decayed and missing teeth was higher in the case group than in the control group. The total DMF score for the case group was 6.72 ± 7.27 , and the control group was 4.90 ± 6.40 (p -value: 0.124) (Supplementary Table S6).

Nature of COVID-19 symptoms, mortality, days of hospital stay, and partial pressure of oxygen

Out of 163 individuals, the following COVID-19 symptoms were recorded: presence of fever ($n = 108$), loss of taste ($n = 8$), loss of smell ($n = 7$), dizziness ($n = 10$), presence of GIT disturbances ($n = 39$), breathlessness ($n = 35$), running nose ($n = 16$), cough ($n = 61$), and headache ($n = 25$). No difference was noted in the COVID-19 symptoms between those with periodontitis and non-periodontitis groups. Of those presenting with fever, 62% had periodontitis and 73% did not have periodontitis (OR = 0.60) (Table 3). Only 5% of individuals with periodontitis and 4.8% with non-periodontitis showed loss of taste; 4% of individuals with periodontitis and 4.8% without periodontitis had reported loss of smell. A statistically significant difference was noted only for dizziness ($p = 0.036$), running nose/cold ($p = 0.009$), and headache ($p = 0.005$) (Table 4).

TABLE 1 Demographic characteristics of study participants and their association with COVID-19 and periodontal disease severity.

Demographic variables		COVID-19 severity			Periodontal disease severity		
		Cases (moderate/severe COVID-19)	Controls (asymptomatic/mild COVID-19)	<i>p</i> -value	Periodontitis	Non-periodontitis	<i>p</i> -value
Age	Mean ± SD	53.70 ± 19.13	40.67 ± 17.34	<0.001*	Stage I (<i>n</i> = 16): 39.31 ± 16.74 Stage II (<i>n</i> = 47): 44.02 ± 15.96 Stage III (<i>n</i> = 15): 61.27 ± 16.89 Stage IV (<i>n</i> = 22): 69.00 ± 11.44	32.60 ± 10.72	0.044*
Sex	Males	36 (83.7%)	57 (47.5%)	<0.001*	63 (63.0%)	30 (47.6%)	0.053
	Females	7 (16.3%)	63 (52.5%)		37 (37.0%)	33 (52.4%)	
Smokeless tobacco	Present	6 (14.0%)	7 (5.8%)	0.092	9 (9.0%)	4 (6.3%)	0.5438
	Absent	37 (86.0%)	113 (94.2%)		91 (91.0%)	59 (93.7%)	
Smoking tobacco	Present	2(4.7%)	10 (8.3%)	0.428	8 (8.0%)	4 (6.3%)	0.694
	Absent	41 (95.3%)	110 (91.7%)		92 (92.0%)	59 (93.7%)	
Medical history—Endocrine (Diabetes Mellitus)	Present	20 (46.5%)	33 (27.7%)	0.036*	44 (44.4%)	9 (14.3%)	<0.001*
	Absent	23 (53.5%)	86 (72.3%)		55 (55.6%)	54 (85.7%)	

*Significant at a *p*-value of < 0.05. The *p*-value in bold indicates statistically significant results.

TABLE 2 Association of comorbidities with COVID-19 severity and periodontal status.

Periodontal status	Comorbidities		Cases (moderate/severe COVID-19)	Controls (asymptomatic/mild COVID-19)	OR (95% CI)	p-value
Periodontitis	H/o cardiovascular disease	Present	22 (62.9%)	27 (42.9%)	2.25 (0.96–5.26)	0.058
		Absent	13 (37.1%)	36 (57.1%)		
	H/o respiratory disease	Present	11 (31.4%)	6 (10.0%)	4.125 (1.36–12.45)	0.009*
		Absent	24 (68.6%)	54 (90.0%)		
	H/o endocrine disease	Present	18 (51.4%)	26 (40.6%)	2.256 (0.96–5.26)	0.058
		Absent	17 (48.6%)	38 (59.4%)		
	H/o neurological disease	Present	4 (11.4%)	0 (0%)	-	0.005*
		Absent	31 (88.6%)	65 (100%)		
	H/o other systemic diseases	Present	20 (57.1%)	17 (26.2%)	3.765 (1.58–8.97)	0.002*
		Absent	15 (42.9%)	48 (73.8%)		
Non-periodontitis	H/o cardiovascular disease	Present	1 (12.5%)	3 (5.5 %)	2.47 (0.22–27.20)	0.445
		Absent	7(87.5%)	52 (94.5%)		
	H/o respiratory disease	Present	0 (0%)	3 (5.5%)	-	0.498
		Absent	8 (100%)	52 (94.5%)		
	H/o endocrine disease	Present	2 (25.0%)	7 (12.7%)	2.47 (0.22–27.20)	0.445
		Absent	6 (75.0%)	48 (87.3%)		
	H/o neurological disease	Present	2 (25.0%)	3 (5.5%)	5.77 (0.79–41.79)	0.056
		Absent	6(75.0%)	52 (94.5%)		
	H/o other systemic diseases	Present	4 (50.0%)	7 (12.7%)	6.85 (1.38–33.85)	0.009*
		Absent	4 (50.0%)	48 (87.3%)		

*Significant at a p -value of <0.05 . The p -value in bold indicates statistically significant results.

TABLE 3 Association of periodontal status with COVID-19 severity.

Periodontal status	Cases (moderate/severe COVID-19)	Controls (asymptomatic/mild COVID-19)	OR (95% CI)	p-value
Periodontitis	35 (81.4%)	65 (54.2%)	3.7 (1.58–8.64)	0.002*
Non-periodontitis	8 (18.6%)	55 (45.8%)		

*Significant at a p -value of <0.05 . The p -value in bold indicates statistically significant results.

In participants with moderate-to-severe symptoms of COVID-19, 11 individuals died. Out of 11, 9 (81.8%) had periodontitis, and 2 (18.2%) were without periodontitis. In the case group, the risk estimation of death compared to survival for individuals with periodontitis was found to be 1.038. Similarly, among patients with asymptomatic to mild COVID-19, two deaths were reported: one individual with periodontitis and one without periodontitis. The OR was 0.84

in the control group (Table 4, Supplementary Table S7). The mean duration of stay was longer in the periodontitis group than in the non-periodontitis group. The duration of stay also increased as the stage of periodontitis increased. However, the results were not statistically significant (p -value: 0.094) (Supplementary Tables S7, S8). The Po2 was lower in individuals with severe COVID-19 symptoms (p -value: 0.007); however, no statistically significant difference was noted between the

TABLE 4 Association of COVID-19 symptoms with periodontal status for cases (moderate/severe COVID-19) and controls (asymptomatic/mild COVID-19).

Symptom	Group	Periodontal status	Symptom		p-value
			Present	Absent	
Fever	Cases	Periodontitis	24 (80.0%)	11 (84.6%)	0.721
		Non-periodontitis	6 (20.0%)	2 (15.4%)	
	Controls	Periodontitis	38 (48.7%)	27 (64.3%)	0.103
		Non-periodontitis	40 (51.3%)	15 (35.7%)	
Loss of taste	Cases	Periodontitis	1(100%)	34(81.0%)	0.629
		Non-periodontitis	0 (0%)	8(19.0%)	
	Controls	Periodontitis	4(57.1%)	61(54.0%)	0.871
		Non-periodontitis	3(42.9%)	52(46.0%)	
Loss of smell	Cases	Periodontitis	0 (0%)	35(81.4%)	<0.001*
		Non-periodontitis	0 (0%)	8(18.6%)	
	Controls	Periodontitis	4(57.1%)	61(54.0%)	0.871
		Non-periodontitis	3(42.9%)	52(46.0%)	
Dizziness	Cases	Periodontitis	0.00 (0%)	35(83.3%)	0.034
		Non-periodontitis	1(100%)	7(16.7%)	
	Controls	Periodontitis	3(33.3%)	62(55.9%)	0.192
		Non-periodontitis	6(66.7%)	49(44.1%)	
GI disturbances	Cases	Periodontitis	8(80.0%)	27(81.8%)	0.897
		Non-periodontitis	2(20.0%)	6(18.2%)	
	Controls	Periodontitis	16(55.2%)	49(53.8%)	0.901
		Non-periodontitis	13(44.8%)	42(46.2%)	
Breathlessness	Cases	Periodontitis	16(69.6%)	35(83.3%)	0.033*
		Non-periodontitis	7(30.4%)	7(16.7%)	
	Controls	Periodontitis	6(50.0%)	62(55.9%)	0.760
		Non-periodontitis	6(50.0%)	49(44.1%)	
Cold or running nose	Cases	Periodontitis	0(0%)	35(81.4%)	<0.001*
		Non-periodontitis	0(0%)	8(18.6%)	
	Controls	Periodontitis	5(31.3%)	60(57.7%)	0.048*
		Non-periodontitis	11(68.8%)	44(42.3%)	
Cough	Cases	Periodontitis	15(83.3%)	20(80.0%)	0.782
		Non-periodontitis	3(16.7%)	5(20.0%)	
	Controls	Periodontitis	22(51.2%)	43(55.8%)	0.622
		Non-periodontitis	21(48.8%)	34(44.2%)	
Headache	Cases	Periodontitis	2(66.7%)	33(82.5%)	0.497
		Non-periodontitis	1(33.3%)	7(17.5%)	
	Controls	Periodontitis	7(31.8%)	58(59.2%)	0.020*
		Non-periodontitis	15(68.2%)	40(40.8%)	
Mortality	Cases	Periodontitis	9 Deaths (81.8%)	26 Alive (81.3%)	0.967
		Non-periodontitis	2 Deaths (18.2%)	8 Alive (18.6%)	
	Controls	Periodontitis	1 Death (50.0%)	64 Alive (54.2%)	0.905
		Non-periodontitis	1 Death (50.0%)	54 Alive (45.8%)	

*Significant at a p-value of <0.05. The p-value in bold indicates statistically significant results.

periodontitis and non-periodontitis groups (p -value: 0.109) (Supplementary Tables S7, S8).

A multivariate analysis was conducted using three models. In the first model, the severity of COVID-19 was associated with age and periodontal disease status. The model explained 15.1% of the variance in COVID-19 severity. The risk of severe COVID-19 was doubled for patients with periodontitis; however, this finding was not statistically significant. Patients with periodontitis were 3.7 times more likely to exhibit severe COVID-19 ($p = 0.002$). However, there was no statistically significant association between COVID-19 severity and periodontitis in smokers and non-smokers (OR: 0.48). A third model with the most common risk factors (sex, age, periodontal status, smoking, history with cardiac diseases, respiratory diseases, and endocrine diseases) associated with COVID-19 showed a 33.2% variance. Significant variables contributing to the increased severity of COVID-19 were increased age (OR: 1.02), male sex (OR: 5.77), and the presence of respiratory diseases (OR: 3.00) (Supplementary Table S9).

Discussion

The present study aimed to assess the association of periodontitis with the severity of COVID-19. The study found a positive association between periodontitis and COVID-19 severity (OR = 3.70). We also noted that all patients with COVID-19 had some form of gingival inflammation and compromised oral hygiene. However, no significant association was observed between periodontal parameters, such as BOP and bone loss, between the case and control groups. Patients with moderate-to-severe forms of COVID-19 have shown more decayed and missing teeth compared to those with mild or asymptomatic COVID-19 patients. These results are similar to previous studies by Moradi et al., which found that poor oral hygiene and the presence of periodontitis were associated with increased severity of COVID-19 symptoms, increased admission to the ICU, and increased mortality (30, 31). However, a recent systematic review found that the symptoms of periodontitis, particularly BOP, were not associated with COVID-19 positivity (OR = 1.1) or mortality (OR = 2.71) but were associated with COVID-19 severity (OR = 3.18) (32). Few studies have also observed an increased risk of dizziness and headaches in patients with periodontitis (33–38). We also noted a statistically significant difference in symptoms such as dizziness, running nose, and headache in the periodontitis group compared to the non-periodontitis group. This could be attributed to the direct connection between the oral cavity to the nose, maxillary sinus, and the neurological system. A recent study by Huang et al. in the Taiwanese population also observed an increased risk of dizziness in patients with periodontitis (33). The authors retrieved data from 2008 to 2013 from the National Health Insurance Research of Taiwan. Patients diagnosed with periodontal disease or dizziness after at least one hospital admission or three outpatient visits were included in the study. In total, 445 patients with periodontal disease and 1,780 healthy controls were selected. Dizziness was significantly higher among the patients with periodontal disease relative to the controls. Periodontal disease is not only a risk factor for dizziness but also an independent risk factor for dizziness. Similarly, few studies and systematic reviews have found

a positive association between poor oral hygiene, periodontitis, and respiratory such as respiratory pneumonia, COPD, asthma, and headaches, thus justifying the results of the increased prevalence of respiratory and neurological symptoms in COVID-19 patients with periodontitis (34–38).

The correlation between periodontitis and COVID-19 is attributed to increased systemic oxidative stress and inflammatory burden with increasing severity of periodontitis. Additionally, the presence of ACE2 receptors in the inflamed periodontal tissues also contributes to the presence of coronavirus in the oral cavity and its invasion into the systemic circulation (39, 40). The local inflammatory burden and oxidative stress in the inflamed periodontal tissues must be prevented and managed effectively. This is crucial as the proinflammatory cytokines enter the systemic circulation and increase the inflammatory burden in the body, including the respiratory system (41).

Although our study did not evaluate the effect of oral hygiene maintenance in systemically compromised patients, it is crucial for reducing the severity of coronavirus infections. Therefore, the maintenance of good oral hygiene and the control of periodontal disease is crucial for the effective management of COVID-19. Simple measures such as practicing good oral hygiene and scheduling regular dental visits could help prevent or decrease the severity of COVID-19. Although our study found that increasing severity of periodontitis is associated with more severe COVID-19 symptoms, we would like to highlight some limitations of the study. One key limitation is that the data were collected from patients aged 18 years and older; therefore, the role of periodontitis in young individuals or the pediatric population cannot be commented upon in this study. Our study analyzed patients with asymptomatic and mild COVID-19 symptoms as well as those with moderate-to-severe forms of the disease. Hence, future research studies should compare symptomatic and asymptomatic individuals and their correlation to periodontal disease. Additionally, studies should investigate how COVID-19 affects periodontal outcomes and evaluate the effectiveness of periodontal therapy in COVID-19 patients.

Conclusion

A positive association exists between periodontitis and the severity of COVID-19. Respiratory and neurological disorders significantly influence the association between periodontitis and COVID-19 severity. Increased severity of periodontitis is associated with higher hospital stays and mortality. Individuals with periodontitis have a higher risk of experiencing dizziness, headaches, and running noses than those without periodontitis. An interdisciplinary approach involving the management of periodontal disease and maintaining good oral hygiene for the effective management of COVID-19 should be further explored.

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 Kasturba Medical College and Kasturba Medical Hospital. 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

SM: Conceptualization, Data curation, Formal analysis, Investigation, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. AC: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. VR: Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. RD: Data curation, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. RG: Data curation, Investigation, Methodology, 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.

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Supplementary material

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2024.1421380/full#supplementary-material>

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Women's self-reported symptoms of reproductive tract infection, medical consultations, and factors influencing them in less developed regions: perimenopausal and older women in need of urgent attention

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Background: Reproductive tract infections (RTIs) represent a critical public health concern impacting women's health, particularly pronounced in developing countries. We aim to investigate the prevalence of self-reported RTI symptoms in women aged 18 to 65 and the factors influencing gynecological examination behavior and associated risks are explored.

Methods: From May 6, 2022, to September 20, 2022, a comprehensive survey engaged 20,864 women aged 18 to 65 in 19 townships in Lueyang, Shaanxi. Each survey team, comprising a gynecologist, two female health center staff, and a master's student, conducted face-to-face questionnaire surveys in villages and households. The questionnaire featured three sections: prevalence of self-reported RTI symptoms, gynecological examination history, and factors influencing examination behavior.

Results: The high response rate of 98.69% yielded 20,590 valid questionnaires. Among surveyed women, 42.04% reported RTI symptoms, with a higher prevalence in those aged 30 and above, peaking at 44.88% in the 50–59 age group. Vulvar itching (24.73%), abnormal vaginal discharge (17.41%), and urinary tract irritation signs (11.73%) were most common. Older adult women (≥60) reported higher frequency and longer duration of symptoms. Only 9.88% of symptomatic women underwent gynecological examinations in 2022. Examination likelihood decreased with age, presenting a critical gap in healthcare utilization among older women. Reasons for checkups included routine checkups (47.02%), symptom concerns (41.29%), and the availability of free medical checkup programs (9.82%). Barriers included perceived lack of serious symptoms (23.7%), lack of necessity (4.41%) and lack of time (3.98%). Among those examined, 40.58% had a diagnosis of gynecological disorders,

including pelvic inflammatory disease, human papilloma virus infection and cervical cancer.

Conclusion: Widespread RTI symptoms, particularly in perimenopausal and older women, underscore the need for enhanced healthcare. Barriers to gynecological examinations include awareness, education, and accessibility issues. Strategies must prioritize health education, routine examinations, and improved healthcare infrastructure in underdeveloped regions.

KEYWORDS

reproductive tract infection (RTI), self-reported symptoms, gynecological examinations, women's health, underdeveloped regions

1 Introduction

Reproductive tract infections (RTIs) represent a critical public health concern impacting women's health, particularly pronounced in developing countries (1). Early prevention of RTI is paramount as they elevate the risk of severe reproductive diseases, including pelvic inflammatory disease, miscarriage, preterm birth, infertility, sexually transmitted infections, and cervical cancer (2). These infections not only inflict reproductive harm upon women of childbearing age but also pose a heightened risk of malignant tumors, such as cervical cancer, among perimenopausal and older adult women (3).

In underdeveloped regions, the prevalence of RTIs may be underestimated due to factors such as lower educational levels, limited autonomy in personal health decisions, lower economic status, inadequate healthcare facilities, insufficient medical resources, and cultural norms of silence concerning diseases (4). These factors contribute to the poor recognition and management of RTIs, leading to a cycle of poor health outcomes and further socio-economic decline. Approximately 38 to 85% of rural women in China are reported to exhibit at least one symptom indicative of RTIs; however, the utilization of healthcare services remains remarkably low (5).

Laboratory testing is considered the gold standard for accurately measuring RTI prevalence. However, the applicability of such tests is limited in rural areas due to insufficient healthcare facilities and a shortage of specialized gynecologists (6). Therefore, in cost-effective terms, self-reporting of RTI symptoms remains a crucial means of assessing the incidence and impact of RTIs, particularly in underdeveloped regions (7). Globally, most cancers (85–90%) are diagnosed on the basis of symptomatic manifestations, despite the availability of cancer screening programs (8), and self-reporting of RTI symptoms contributes to three-early prevention of the disease (9, 10).

Lueyang County in Shaanxi Province is recognized as one of the regions with the highest incidence of cervical cancer and the lowest average income nationwide (11). Therefore, the aim of this study was to investigate the self-reported RTI symptoms, gynecological examination, and related influencing factors of women in Lueyang County, Shaanxi Province, to clarify the relationship between self-reported RTI symptoms and gynecological disorders, and to provide a preventive strategy to improve the rate of gynecological examination and gynecological health of women in underdeveloped areas.

2 Methods

2.1 Study participants

From May 6, 2022, to September 20, 2022, we conducted a cross-sectional survey of women aged 18 to 65 years in 19 townships in Lueyang County, Shaanxi Province, regarding self-reported RTI symptoms, gynecological examination history, and related influencing factors. The sample for this study was selected based on a census approach, including all eligible women who met the inclusion criteria. The inclusion criteria were: (1) age between 18 and 65 years; (2) residency in Lueyang County for at least 6 months; and (3) complete understanding of the study.

2.2 Survey method

Each survey team comprised one gynecologist from Lueyang County Maternal and Child Health Hospital, two female staff members from township health centers, and one master's degree student from Chongqing Medical University. In total, six survey teams conducted face-to-face questionnaire surveys by visiting villages and households.

2.3 Questionnaire design

The survey questionnaire was designed based on a literature review and sought input from professional gynecologists and epidemiologists to ensure its alignment with the specific characteristics of Lueyang County (2, 5, 12–14). The questionnaire consisted of three main sections:

Prevalence of self-reported RTI symptoms in the past 6 months: RTI symptoms included vulvar itching, abnormal vaginal discharge (high volume, yellow color, odor, soybean dregs, bloody, pus, foamy), frequent urination, urgency, painful urination, non-menstrual lower back pain, non-menstrual lower abdominal pain, bleeding after sexual intercourse. Women reporting at least one of these symptoms were considered to have a RTI. The frequency of RTI symptoms was categorized as occasional, sometimes, often, and always. The duration of RTI symptoms was classified as <3 months, 3–6 months, 6 months–1 year, 1–3 years, and >3 years.

Gynecological examination history and gynecological diagnosis history: whether symptomatic women had a gynecological

Abbreviations: RTI, Reproductive tract infection; HPV, Human papillomavirus.

examination in 2022, reasons for having/not having a gynecological examination, and history of gynecological diagnosis.

Factors influencing women with RTI symptoms who did not undergo gynecological examinations: basic information, number of self-reported RTI symptoms in the past 6 months; menstrual history, marital and reproductive history, sexual behavior, hygiene practices, spouse or sexual partner information.

2.4 Statistical analysis

Statistical analyses were conducted using IBM SPSS (version 23.0), SAS (version 9.4), and R (version 4.2.1). Chi-square (χ^2) tests were employed for the analysis of count data rates (%), revealing differences in the prevalence of various RTI symptoms across different groups. The Cochran-Armitage trend test was utilized to assess the linear relationship between the prevalence of individual RTI symptoms and age. The Jonckheere-Terpstra trend test was applied to evaluate whether there was a linear relationship between the frequency and duration of various RTI symptoms and age.

Univariate and multivariate (Inverse stepwise likelihood ratio test) logistic regression analyses were employed to explore the correlation between women exhibiting RTI symptoms but not undergoing gynecological examinations and the influencing factors obtained from the questionnaire survey. Additionally, the Hosmer-Lemeshow test was conducted to assess the goodness of fit of the models. Risk factors with $p < 0.1$ in the univariate logistic regression analysis were included in the risk regression analysis. The statistical significance level was set at $p < 0.05$.

To address issues related to missing data, the mice package and VIM package in R software were employed to explore the proportion and types of missing data. The pattern of missing data was determined through a correlation matrix called a shadow matrix. Multiple imputation techniques were employed to handle missing data, followed by an assessment of the reliability of the imputation results. After multiple imputation, the interpolation results with the highest Cronbach's alpha coefficient were used for multifactor analysis.

3 Results

3.1 Prevalence of self-reported RTI symptoms

A total of 20,864 women were surveyed, and 20,590 valid questionnaires met the inclusion criteria, with a validity rate of 98.69%. A total of 8,657 women (42.04%) reported RTI symptoms in the past 6 months. The prevalence of RTI symptoms exceeded 40% in women aged 30 and above, peaking at 44.88% among those aged 50–59. Individuals with one, two, and multiple (three or more) symptoms constituted 22.93, 11.98, and 7.13% of the total population, respectively (Table 1).

The most common RTI symptoms were vulvar itching (24.73%), abnormal vaginal discharge (17.41%), and urinary symptoms including frequency, urgency, and pain (11.73%). The prevalence of non-menstrual lower back pain (8.6%), non-menstrual lower abdominal pain (8.16%), and post-coital bleeding (0.5%) was comparatively lower. Vulvar itching (26.3%),

abnormal vaginal discharge (20.21%), non-menstrual lower abdominal pain (9.21%), and post-coital bleeding (0.76%) were most common in the 40–49 age group. Urinary symptoms (16.85%) and non-menstrual lower back pain (11.98%) showed the highest prevalence in individuals aged 60 and above. The prevalence of most symptoms differed across age groups or showed a linear trend with increasing age.

3.2 Frequency of self-reported RTI symptoms

RTI symptoms in females aged 18–65 predominantly exhibit an occasional occurrence, surpassing 40%. Notably, in women aged 60 and above, the proportion of RTI symptoms occurring occasionally decreases, while the occurrence of these symptoms frequently significantly increases in comparison to women under 60 (Figure 1). The Jonckheere-Terpstra trend test showed that the frequency of vulvar itching, vaginal discharge that was heavy, yellowish in color, and foul smelling, urinary frequency, urgency, and pain of urination, non-menstrual lumbosacral pain, and non-menstrual lower abdominal pain increased with age ($p < 0.0001$; Supplementary Table S1).

3.3 Duration of self-reported RTI symptoms

The majority of individuals experiencing self-reported RTI symptoms reported a duration of less than 3 months (>30%). However, a notable proportion of individuals with non-menstrual lower back pain reported a duration exceeding 3 years (33.37%; Figure 2). In the age group of 60 years and above, non-menstrual lower abdominal pain (30.89%), non-menstrual lower back pain (50.3%), increased vaginal discharge (28.38%), yellow-colored discharge (33.33%), malodorous discharge (48.39%), and discharge resembling soybean dregs (40%) had the highest proportions of durations exceeding 3 years.

According to the Jonckheere-Terpstra trend test, the duration of symptoms for vulvar itching, increased vaginal discharge, yellow-colored discharge, odor, discharge resembling soybean dregs, frequent urination, urgency, painful urination, and non-menstrual lower abdominal pain increased with age ($p < 0.05$; Supplementary Table S2).

3.4 Gynecological examination status of patients with self-reported RTI symptoms

Among the 8,657 women who self-reported RTI symptoms in the past 6 months, only 855 individuals (9.88%) underwent gynecological examinations in 2022. Notably, no women under the age of 20 chose to undergo gynecological examinations, only 2.43% of women aged ≥ 60 years chose to have a gynecological examination. According to the Cochran-Armitage trend test, the likelihood of undergoing gynecological examinations gradually decreased with increasing age ($p < 0.0001$; Table 2). Patients reported that the main reasons for undergoing gynecological examinations in the current year were routine check-ups (47.02%), the presence of symptoms

TABLE 1 Prevalence of self-reported symptoms of RTI in the last 6 months among women of different ages.

RTI symptoms in the last six months	Total(% ^a) (N = 20,590)	<20(% ^b) (n = 142)	20–29(% ^b) (n = 1,599)	30–39(% ^b) (n = 4,407)	40–49(% ^b) (n = 6,289)	50–59(% ^b) (n = 6,776)	≥60(% ^b) (n = 1,377)	χ ^{2c}	p-value ^c	χ ^{2d}	p-value ^d
Self-reported symptoms of RTI								285.407	<0.0001	133.6405	<0.0001
Yes	8,657(42.04)	9(6.34)	413(25.83)	1821(41.32)	2,797(44.47)	3,041(44.88)	576(41.83)				
No	11,933(57.96)	133(93.66)	1,186(74.17)	2,586(58.68)	3,492(55.53)	3,735(55.12)	801(58.17)				
Number of symptoms											
1	4,722(22.93)	5(3.52)	234(14.63)	985(22.35)	1,479(23.52)	1709(25.22)	310(22.51)	114.8581	<0.0001	62.4037	<0.0001
2	2,466(11.98)	2(1.41)	109(6.82)	546(12.39)	822(13.07)	820(12.1)	167(12.13)	63.4056	<0.0001	16.3499	<0.0001
≥3	1,469(7.13)	2(1.41)	70(4.37)	290(6.59)	496(7.89)	512(7.56)	99(7.19)	34.6061	<0.0001	17.3647	<0.0001
Types of symptoms											
1. Vulvar itching	5,091(24.73)	5(3.52)	246(15.38)	1,158(26.28)	1,654(26.3)	1702(25.12)	326(23.67)	124.7129	<0.0001	22.4505	<0.0001
2. Abnormal vaginal discharge	3,584(17.41)	4(2.82)	224(14.01)	884(20.06)	1,271(20.21)	1,059(15.63)	142(10.31)	152.9114	<0.0001	19.6728	<0.0001
High volume	1901(9.23)	3(2.11)	116(7.25)	465(10.55)	711(11.31)	532(7.85)	74(5.37)	97.3403	<0.0001	13.2955	0.0003
Yellow color	1,635(7.94)	1(0.7)	92(5.75)	387(8.78)	563(8.95)	526(7.76)	66(4.79)	52.6547	<0.0001	0.6921	0.4054
Odor	1,354(6.58)	1(0.7)	57(3.56)	278(6.31)	510(8.11)	446(6.58)	62(4.5)	65.7908	<0.0001	3.9196	0.0477
Soybean dregs	278(1.35)	0(0)	26(1.63)	104(2.36)	98(1.56)	45(0.66)	5(0.36)	72.6522	<0.0001	45.4977	<0.0001
Bloody	107(0.52)	0(0)	10(0.63)	27(0.61)	39(0.62)	29(0.43)	2(0.15)	7.8885	0.1625	3.7865	0.0517
Pus	102(0.5)	1(0.7)	3(0.19)	34(0.77)	39(0.62)	22(0.32)	3(0.22)	18.1571	0.0028	4.2062	0.0403
Foamy	58(0.28)	0(0)	2(0.13)	13(0.29)	25(0.4)	15(0.22)	3(0.22)	-	0.4012(Fisher)	0.0007	0.9785
3. Frequent urination, urgency, painful urination	2,416(11.73)	1(0.7)	63(3.94)	330(7.49)	731(11.62)	1,059(15.63)	232(16.85)	321.2634	<0.0001	313.9665	<0.0001
4. Non-menstrual lower back pain	1771(8.6)	0(0)	50(3.13)	317(7.19)	539(8.57)	700(10.33)	165(11.98)	131.2436	<0.0001	122.8839	<0.0001
5. Non-menstrual lower abdominal pain	1,681(8.16)	5(3.52)	92(5.75)	356(8.08)	579(9.21)	526(7.76)	123(8.93)	28.174	<0.0001	4.9231	0.0265
6. Bleeding after sexual intercourse	103(0.5)	0(0)	10(0.63)	23(0.52)	48(0.76)	19(0.28)	3(0.22)	18.7832	0.0021	5.3852	0.0203

^aPercentage of all women in study.
^bPercentage of women in each age group.
^cChi-square test between age groups.
^dCochran-Armitage trend test.

TABLE 2 Gynecological examination status of patients with self-reported RTI symptoms.

Gynecological examination (Missing = 246)	Total(N = 8,657)	<20(n = 9)	20–29(n = 413)	30–39(n = 1821)	40–49(n = 2,797)	50–59(n = 3,041)	≥60(n = 576)	χ^2_{2a}	p-value ^a	χ^2_{2b}	p-value ^b
Yes	855(9.88)	0(0)	76(18.4)	269(14.77)	317(11.33)	179(5.89)	14(2.43)	177.0236	<0.0001	168.7479	<0.0001
No	7,556(87.28)	9(100)	328(79.42)	1,513(83.09)	2,406(86.02)	2,755(90.6)	545(94.62)				

^aChi-square test between age groups.
^bCochran-Armitage trend test.

(41.29%), and the availability of free medical check-up programs (9.82%; [Table 3](#)).

Patients who did not undergo gynecological examinations in the current year provided various reasons, with the primary factors being the absence of severe symptoms (23.7%), perceived lack of necessity (4.41%), and time constraints (3.98%). Reasons for not undergoing examinations varied across different age groups. For those under 20 years old, the main reason was being young or unmarried (22.22%). Notably, a significant factor for not undergoing examinations in the 20–29 age group (5.79%), 40–49 age group (2.62%), and those aged 60 and above (6.24%) was a lack of awareness about the need for regular gynecological check-ups ([Table 4](#)).

Out of the 855 symptomatic women who underwent gynecological examinations in 2022, a total of 347 individuals (40.58%) had a documented history of gynecological diagnoses. There were no significant differences in the distribution of gynecological diagnostic history across various age groups ([Table 5](#)). The top three diagnosed gynecological conditions were vaginitis (75.22%), cervical erosion (38.04%), and uterine fibroids (30.55%). There were differences in gynecological disorders in various age groups, the important gynecological disorders in 20–29, 30–39, 40–49, and 50–59 years of age were: pelvic inflammatory disease (17.24%), pelvic inflammatory disease (30.10%), cervical cysts (30.94%), and uterine polyps (30.00%), respectively ([Table 6](#)).

3.5 Univariate logistic regression analysis

An exploration of factors influencing the reluctance of patients, reporting symptoms of RTI, to undergo gynecological examinations was conducted through univariate logistic regression analysis. In total, 38 variables were initially subjected to univariate analysis, and subsequently, 23 variables, exhibiting significance at $p < 0.10$, were selected for inclusion in the multivariate analysis ([Supplementary Table S3](#)).

3.6 Proportion and type of missing data

The missing data type was missing at random. [Supplementary Figure S1](#) delineates the pattern and proportion of missing data for variables included in the multifactorial analysis. Specifically, variables such as household annual income (14.39%) and spouse circumcision history (12.01%) exhibited higher rates of missing data.

3.7 Multivariate logistic regression analysis

The multiple imputation for random missing data is detailed in [Supplementary Table S4](#), and the results of the multivariate logistic regression analysis are presented in [Table 7](#). For reference, the analysis without multiple imputation is available in [Supplementary Table S5](#). Women with two or more symptoms, those with two or more marriages, and those with a history of miscarriage showed a lower

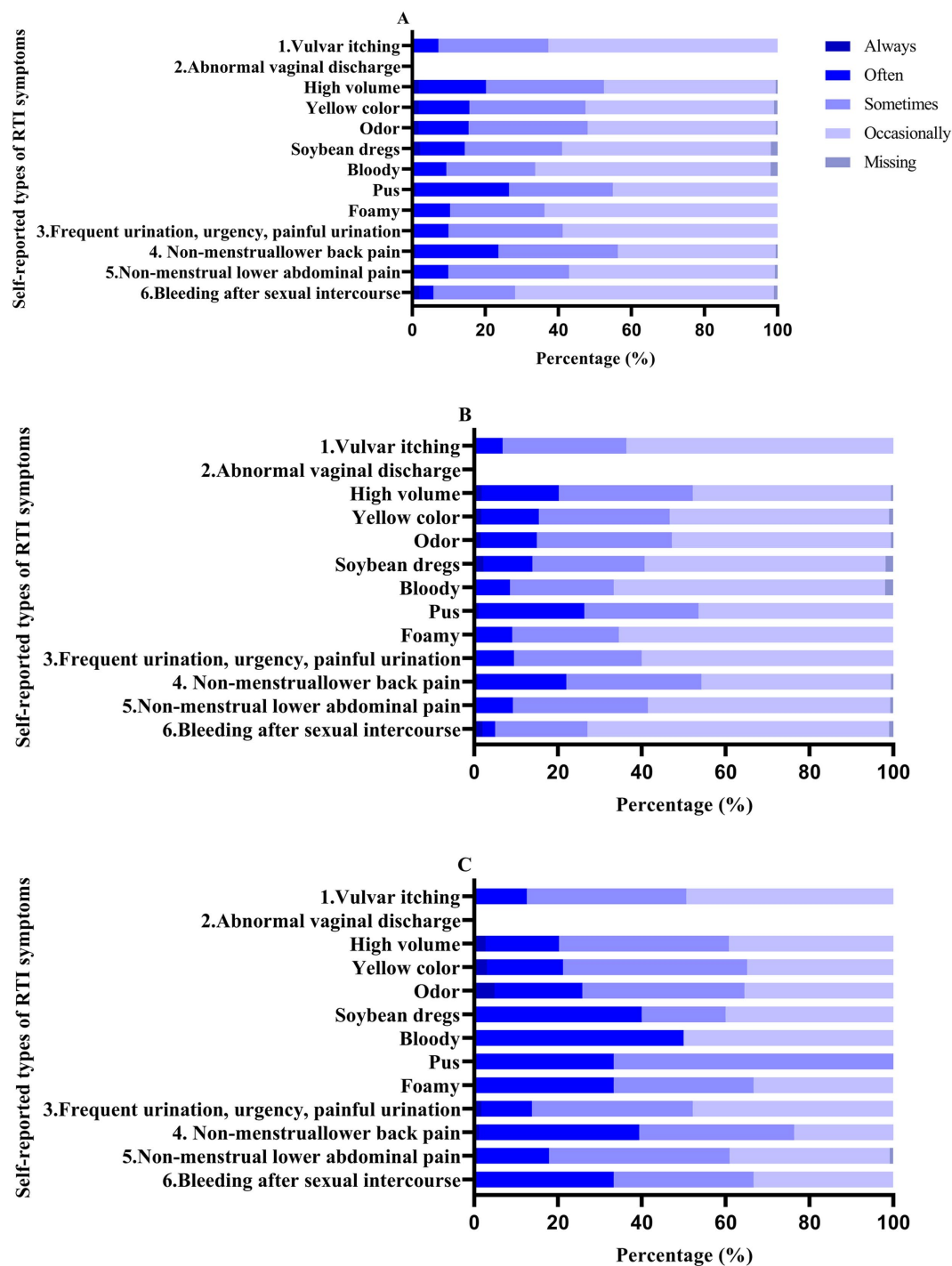


FIGURE 1
Percentage of self-reported RTI symptom frequencies in patients. A: 18–65 years; b: <60 years; c: ≥60 years.

likelihood of refraining from gynecological examinations after experiencing RTI symptoms. Conversely, factors such as rural residency, infrequent underwear changes (4–7 days per change), low bathing frequency in winter (>7 days/times), and a spouse aged 40 or older were associated with a higher likelihood of non-attendance for gynecological examinations. Moreover, the results from the multivariate logistic regression analysis without multiple imputation indicated that individuals with a BMI of 32 or higher and

postmenopausal women were less likely to undergo gynecological examinations.

4 Discussion

This study reveals the widespread prevalence of self-reported RTI symptoms among women. Women aged 60 and above experience a

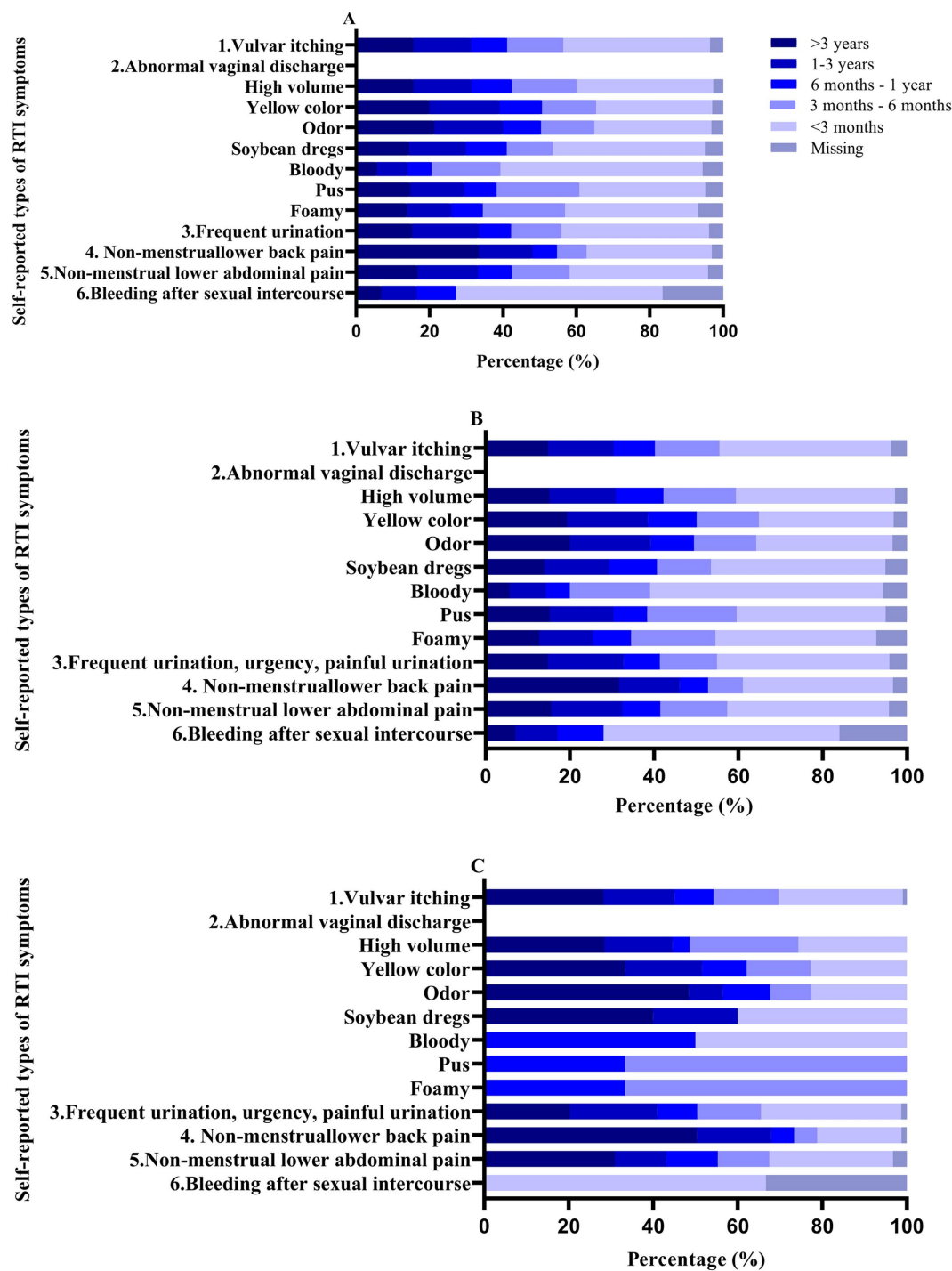


FIGURE 2
Percentage of duration of self-reported RTI symptoms in patients. A: 18–65 years; b: <60 years; c: ≥60 years.

higher frequency and longer duration of RTI symptoms compared to those under 60. Only a small proportion of women opt for examination when symptomatic. We investigated their reasons for seeking or avoiding gynecological examinations, which are primarily associated with lower levels of awareness, education, economic status,

inadequate promotion by healthcare institutions, and difficulties in accessing medical resources.

In this study, the prevalence of self-reported RTI symptoms in women aged 30 and above over the past 6 months exceeds 40%, closely aligning with the national level in India (40%) (15) and surpassing

TABLE 3 Reasons for undergoing gynecological examinations among patients with self-reported RTI symptoms (multiple selections allowed).

Reasons for gynecological examinations (Missing = 47)	Total(<i>N</i> = 855)	20–29(<i>n</i> = 76)	30–39(<i>n</i> = 269)	40–49(<i>n</i> = 317)	50–59(<i>n</i> = 179)	≥60(<i>n</i> = 14)
Routine gynecological examination	402(47.02)	37(48.68)	137(50.93)	149(47)	72(40.22)	7(50)
With gynecological symptoms	353(41.29)	24(31.58)	100(37.17)	139(43.85)	87(48.6)	3(21.43)
Free gynecological check-up program	84(9.82)	5(6.58)	24(8.92)	27(8.52)	25(13.97)	3(21.43)
Doctor's recommendation	52(6.08)	6(7.89)	17(6.32)	17(5.36)	11(6.15)	1(7.14)
Thinking that gynecological examination can solve all gynecological problems	8(0.94)	1(1.32)	2(0.74)	4(1.26)	1(0.56)	0(0)
Pregnancy test/pregnancy preparation	4(0.47)	2(2.63)	2(0.74)	0(0)	0(0)	0(0)
Other reasons	1(0.12)	0(0)	1(0.37)	0(0)	0(0)	0(0)

TABLE 4 Reasons for not undergoing gynecological examinations among patients with self-reported RTI symptoms (multiple selections allowed).

Reasons for without gynecological examinations (Missing = 5,025)	Total(<i>N</i> = 7,556)	<20(<i>n</i> = 9)	20–29(<i>n</i> = 328)	30–39(<i>n</i> = 1,513)	40–49(<i>n</i> = 2,406)	50–59(<i>n</i> = 2,755)	≥60(<i>n</i> = 545)
No serious gynecological symptoms	1791(23.7)	2(22.22)	116(35.37)	361(23.86)	456(18.95)	700(25.41)	156(28.62)
No need	333(4.41)	3(33.33)	20(6.1)	48(3.17)	66(2.74)	161(5.84)	35(6.42)
No time	301(3.98)	1(11.11)	14(4.27)	53(3.5)	63(2.62)	144(5.23)	26(4.77)
Unaware of the need for regular gynecological examinations	264(3.49)	0(0)	19(5.79)	43(2.84)	63(2.62)	105(3.81)	34(6.24)
Do not know the significance	213(2.82)	0(0)	6(1.83)	24(1.59)	52(2.16)	109(3.96)	22(4.04)
Inconvenient transportation	54(0.71)	0(0)	1(0.3)	3(0.2)	8(0.33)	27(0.98)	15(2.75)
Poor economic conditions	38(0.5)	1(11.11)	1(0.3)	4(0.26)	6(0.25)	17(0.62)	9(1.65)
Fear of pain	14(0.19)	1(11.11)	2(0.61)	6(0.4)	2(0.08)	2(0.07)	1(0.18)
Embarrassed	10(0.13)	1(11.11)	2(0.61)	3(0.2)	1(0.04)	1(0.04)	2(0.37)
Inconvenient to move around	4(0.05)	0(0)	0(0)	0(0)	2(0.08)	0(0)	2(0.37)
Young/unmarried	3(0.04)	2(22.22)	0(0)	1(0.07)	0(0)	0(0)	0(0)
Other reasons	30(0.4)	0(0)	3(0.91)	5(0.33)	9(0.37)	11(0.4)	2(0.37)

that of rural women in Sichuan Province, China (30.80%) (16) but falling below that of married women in rural Anhui Province, China (58.10%) (17). The most common RTI symptoms in the population are related to urinary tract infections, namely, external genital itching, abnormal vaginal discharge, and urinary irritation, mirroring results from other regions such as India (18, 19). Symptoms linked to cervical

TABLE 5 Gynecological diagnostic history among patients with self-reported RTI symptoms.

Diagnostic gynecological history (Missing = 8)	Total(N = 855)	20– 29(n = 76)	30– 39(n = 269)	40– 49(n = 317)	50– 59(n = 179)	≥60(n = 14)	χ^2_{2a}	p-value ^a	χ^2_{2b}	p-value ^b
Yes	347(40.58)	29(38.16)	103(38.29)	139(43.85)	70(39.11)	6(42.86)	2.3773	0.6667	0.3413	0.5591
No	500(58.48)	46(60.53)	164(60.97)	175(55.21)	107(59.78)	8(57.14)				

^aChi-square test between age groups.
^bCochran-Armitage trend test.

cancer, such as non-menstrual lower back pain, non-menstrual lower abdominal pain, and post-coital bleeding, are more prevalent in individuals over 40, emphasizing the need for attention (20). This study suggests that perimenopausal and older women have a higher burden of such symptoms. Women entering menopause and the menopausal period face risks of hormonal changes, gynecological malignancies, and various urogenital system diseases (21). We emphasize the necessity to extend healthcare focus beyond the reproductive period, ensuring perimenopausal and older adult women receive due attention (22, 23).

According to the American College of Obstetricians and Gynecologists (ACOG), all women over the age of 18 are recommended to have annual gynecological exams (24). Similar to other underdeveloped regions, despite the high prevalence of self-reported RTI symptoms among women, the proportion of those seeking gynecological examinations is low (25). Only 2.43% of women over 60 with more severe symptoms choose to undergo gynecological examinations. The primary reasons for older adult women not seeking such examinations are the perception of symptom severity, a lack of necessity, and unawareness of the need for regular gynecological check-ups. Similar reasons have been reported in other studies, where many perceive these symptoms as normal occurrences (26). The fundamental reasons may be attributed to lower levels of awareness and education, insufficient promotion by healthcare institutions, and difficulties in accessing medical resources (4). Conversely, women choosing gynecological examinations report routine check-ups, symptomatic concerns, and access to free medical check-up programs as their main reasons. This suggests that to increase the rate of gynecological examinations among women, efforts should focus on raising awareness and providing free gynecological check-up programs, as demonstrated by China's initiation of the "two-cancer" free screening program for rural women in 2009, effectively reducing mortality from breast and cervical cancers (27).

In this study, the lower rate of gynecological examinations among symptomatic women with BMI ≥ 32, poor personal hygiene habits, menopausal status, and spouse age ≥ 40 may be attributed to lower levels of education, lower cognitive levels, and poorer health awareness. After we initially identify the reasons why women with RTI symptoms do not undergo gynecological examinations, we can subsequently implement health education measures for these patients to raise awareness, encourage regular gynecological examinations, and promote good hygiene habits. Women residing in rural and communal areas may face lower economic status and unequal distribution of medical resources. Consistent with the results of a review from India (25). Symptomatic women with two or more symptoms, married more than once, or with a history of abortion may be more likely to undergo gynecological examinations, possibly due to the severity of their symptoms. Studies have indicated that health services and delayed treatment are influenced by the severity of symptoms (28).

Among women who underwent gynecological examinations, 40.58% were diagnosed with gynecological diseases, including pelvic inflammatory disease, HPV infection, and cervical cancer, all confirmed to be related to RTIs (29, 30). This indicates that while self-reported symptoms may not be the gold standard for disease diagnosis, it remains a rapid, convenient, and cost-effective choice,

TABLE 6 Specific diagnoses of gynecological diseases.

Gynecologic history diagnosis	Total(N = 347)	20–29(n = 29)	30–39(n = 103)	40–49(n = 139)	50–59(n = 70)	≥60(n = 6)
Vaginitis	261(75.22)	19(65.52)	79(76.70)	103(74.10)	55(78.57)	5(83.33)
Cervical erosion	132(38.04)	15(51.72)	62(60.19)	35(25.18)	19(27.14)	1(16.67)
Uterine fibroids	106(30.55)	1(3.45)	18(17.48)	50(35.97)	33(47.14)	4(66.67)
Pelvic inflammatory disease	80(23.05)	5(17.24)	31(30.10)	33(23.74)	11(15.71)	0(0)
Cervical cysts	78(22.48)	1(3.45)	19(18.45)	43(30.94)	15(21.43)	0(0)
Uterine polyps	66(19.02)	1(3.45)	10(9.71)	33(23.74)	21(30.00)	1(16.67)
Cervical polyps	65(18.73)	1(3.45)	10(9.71)	33(23.74)	20(28.57)	1(16.67)
History of ovarian abnormalities	40(11.53)	1(3.45)	14(13.59)	17(12.23)	8(11.43)	0(0)
Inflammation of the cervix	38(10.95)	3(10.34)	15(14.56)	13(9.35)	6(8.57)	1(16.67)
Vulvovaginitis	15(4.32)	1(3.45)	5(4.85)	5(3.6)	4(5.71)	0(0)
Endometriosis	14(4.03)	0(0)	5(4.85)	6(4.32)	3(4.29)	0(0)
Adenomyosis	12(3.46)	0(0)	4(3.88)	6(4.32)	2(2.86)	0(0)
HPV infection	11(3.17)	0(0)	1(0.97)	4(2.88)	5(7.14)	1(16.67)
History of ectopic pregnancy	7(2.02)	0(0)	2(1.94)	2(1.44)	3(4.29)	0(0)
History of tubal abnormalities	5(1.44)	1(3.45)	0(0)	3(2.16)	1(1.43)	0(0)
Pre-cancerous cervical lesions	4(1.15)	0(0)	1(0.97)	2(1.44)	1(1.43)	0(0)
Endometrial cancer	3(0.86)	0(0)	0(0)	0(0)	3(4.29)	0(0)
Cervical cancer	3(0.86)	0(0)	0(0)	0(0)	2(2.86)	1(16.67)
Sexually transmitted diseases	3(0.86)	0(0)	1(0.97)	1(0.72)	0(0)	1(16.67)
Adnexitis	2(0.58)	0(0)	0(0)	1(0.72)	1(1.43)	0(0)
Other gynecologic history	30(8.65)	1(3.45)	9(8.74)	14(13.59)	5(7.14)	1(16.67)

aiding in the identification of patients requiring follow-up management, especially in resource-limited regions (5).

The strengths of this study include comprehensive coverage of a large population in Lueyang County using a census approach, which minimizes selection bias. This study also fills a gap in research on RTI symptoms in specific age groups and identifies reasons for delayed gynecological examinations and the actual needs of patients. It provides a scientific basis for improving gynecological health strategies for women in less developed regions. However, this study has limitations. High rates of missing data on household income and sexual behavior were addressed using multiple imputation, highlighting the need for more private and sensitive survey methods in future research. The cross-sectional design also limits the ability to establish causal relationships or track symptom changes over time. Future longitudinal studies are needed to better understand the temporal dynamics of RTI symptoms and their impact on women's health.

5 Conclusion

The study found high self-reported RTI symptoms but limited gynecological examination rates among women in underdeveloped regions. There was a correlation between reported RTI symptoms and gynecological disorders. Targeted health education campaigns focusing on raising awareness of RTI symptoms and the importance of timely gynecological examination, especially for older women and those with lower levels of education, are necessary to improve healthcare utilization in less developed regions. At the same time, health-care providers should strengthen primary health-care infrastructure in rural areas by

TABLE 7 Multivariate logistic regression analysis of patients with self-reported RTI symptoms who did not undergo gynecological exams.

Characteristics	Classification	SE	p-value	OR[95% CI]
Number of symptoms	–	–	0	–
	1	–	–	1(Reference)
	2	0.085	0.01	0.803[0.679–0.949]
	≥3	0.098	0	0.671[0.554–0.813]
Place of residence(residency >6 months)	–	–	0.032	–
	County	–	–	1(Reference)
	Townships	0.126	0.047	1.285[1.004–1.646]
	Rural	0.096	0.016	1.261[1.044–1.523]
Number of marriages	≤1	–	–	1(Reference)
	≥2	0.163	0.029	0.7[0.508–0.964]
	–	–	0.001	–
Number of miscarriages	0	–	–	1(Reference)
	1–2	0.079	0.002	0.787[0.674–0.919]
	≥3	0.171	0.008	0.634[0.454–0.886]
Menopausal status	menopausal	–	–	1(Reference)
	non-menopausal	0.114	0	0.522[0.417–0.654]
Frequency of changing underwear	–	–	0.01	–
	1–3 days/times	–	–	1(Reference)
	4–7 days/times	0.212	0.003	1.897[1.252–2.875]
	>7 days/times	8653.978	0.998	94081343.43[0–.]
Age of spouse/sexual partner	–	–	0	–
	<30	–	–	1(Reference)
	30–39	0.218	0.11	1.418[0.924–2.176]
	40–49	0.22	0.002	1.974[1.282–3.039]
	50–59	0.226	0.001	2.101[1.35–3.27]
	≥60	0.286	0	3.547[2.025–6.213]
Frequency of bathing in winter	–	–	0.015	–
	1–3 days/times	–	–	1(Reference)
	4–7 days	0.114	0.053	1.247[0.997–1.558]
	8 days-2 weeks/times	0.136	0.005	1.471[1.126–1.922]
	>2 weeks/times	0.169	0.008	1.572[1.128–2.19]

expanding free gynecological screening programs and increasing the number of trained gynecologists and other necessary medical resources.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Maternity Service Center of Lueyang Maternal and Child Health Hospital. The studies were conducted in accordance with the local legislation and institutional requirements. All participants or their legal guardians/next of kin provided written/electronic informed consent.

Author contributions

RZ: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. DK: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft, Writing – review & editing. SY: Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – review & editing. DH: Methodology, Project administration, Resources, Supervision, Validation, Visualization, Writing – review & editing. LB: Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing. YA: Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – review & editing. JC: Investigation, Methodology, Project administration, Software, Supervision, Validation, Writing – review & editing. YN: Project administration, Resources, Supervision, Validation, Writing – review & editing. XZ: Project administration, Resources, Supervision, Validation, Writing – review & editing. WX: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software,

Supervision, Validation, Writing – review & editing. QS: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2024.1401474/full#supplementary-material>

SUPPLEMENTARY FIGURE S1

Types and proportions of missing data for variables included in the multifactor analysis.

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Association between cardiovascular health and human papillomavirus infection: analysis from NHANES 2005–2016

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Background: Given the strong association between cardiovascular disease and human papillomavirus (HPV) infection, this study aimed to assess the correlation between HPV infection and cardiovascular health (CVH) as represented by the Life's Essential 8 (LE8) score.

Methods: This study employed analysis of data obtained from the National Health and Nutrition Examination Survey covering the period from 2005 to 2016. To examine the correlation between the CVH score and both HPV and high-risk HPV (HR-HPV) infections, this research utilized a combination of multivariable regression analysis, smooth curve fitting, and subgroup analysis, following adjustment for pertinent covariates.

Results: This study included a total of 8,264 women, with an average age of 39.53 ± 11.24 years. The HPV prevalence was 43.43% overall, while the HR-HPV prevalence was 17.36%. In the fully adjusted model, an augmentation of 10 points in the CVH score correlated with an 8% reduction in the HPV infection rate [0.92 (0.88–0.96)], and a similar 8% decrease in the HR-HPV infection rate [0.92 (0.87–0.97)].

Conclusion: Our findings indicate that elevated CVH, as denoted by higher LE8 scores, correlates with a decreased HPV infection rate among U.S. females. The LE8 score shows potential as a shared predictive biomarker for both CVH and HPV infection.

KEYWORDS

HPV infection, HR-HPV infection, Life's Essential 8, cardiovascular health, NHANES

1 Introduction

Human papillomavirus (HPV) belongs to the Papovaviridae family, characterized by its double-stranded, circular DNA structure. With over 200 identified types to date (1), is one of the most common pathogens contributing to sexually transmitted infections globally. Transmitted through contact with skin or mucous membranes, HPV manifests a spectrum of clinical diseases in both genders (2, 3). While many HPV-induced conditions are benign, such as various skin warts, persistent infection can precipitate malignancies, notably cervical cancer (4). Moreover, among HPV-infected women, the risk of malignancies affecting extra-cervical organs (e.g., anus, vagina, vulva, oropharynx) is significantly elevated (5). Among women, cervical cancer is the fourth most frequently occurring cancer, trailing breast, colorectal, and lung cancers. Its global prevalence exhibits substantial variability across nations, with incidence

rates spanning from under 2–75 cases per 100,000 women (6). During the year 2018, there were 570,000 fresh instances of cervical cancer documented globally, and 311,000 fatalities attributed to the illness (6). Cervical cancer presents a significant public health concern around the world, so it is necessary to investigate cervical cancer-related or HPV infection-related indicators.

Recent research indicates a potential association between cardiovascular diseases (CVD) and HPV. Women infected with HPV exhibit a higher odds ratio (OR) for CVD compared to their non-infected counterparts, with high-risk HPV (HR-HPV) infection further elevating this risk (7, 8). Cervical cancer patients undergoing radiotherapy face higher risks of ischemic stroke and myocardial infarction compared to patients receiving radiotherapy for other diseases (9). Even among patients after radiotherapy for head and neck malignancies, compared with HPV-negative patients, HPV-positive patients had a more than fourfold increased risk of cerebrovascular events (10). On the contrary, for some conventional CVD risk factors, such as age, exercise, smoking, and diet, also exhibit strong correlations with HPV infection (11). These findings emphasize the significance of risk management for patients with HPV infection.

The concept of cardiovascular health (CVH) was introduced by the American Heart Association (AHA) in the year 2010 to assess CVD risk. Initially, CVH was evaluated using the Life's Simple 7 (LS7) score, comprising seven fundamental health behaviors and factors (12). With advancements in CVH research, scholars' understanding evolved over time. Consequently, the AHA enhanced LS7 into Life's Essential 8 (LE8), adding sleep health and improving its evaluation calculations in 2022 (13, 14). LE8 demonstrates notable efficacy in predicting outcomes related to CVD and other major chronic conditions, including life expectancy (14).

Considering the association between HPV infection and CVD itself as well as its risk factors, promoting CVH could potentially help prevent and manage HPV infection. To our knowledge, the correlation between HPV infection and CVH quantized by LE8 score has not been evaluated in research before. Therefore, we investigate the potential correlation between LE8 scores and both HPV infection and HR-HPV infection statuses in this study. Our findings aim to offer novel insights into the management of women's health.

2 Methods

2.1 Survey description and participants

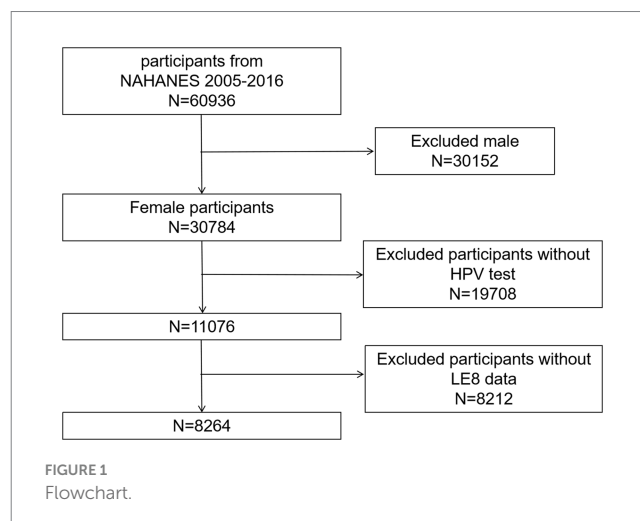
The National Center for Health Statistics (NCHS), oversees a large national program called the National Health and Nutrition Examination Survey (NHANES) database (15). Employing a sophisticated multi-stage stratified sampling approach, the survey aims to provide a detailed characterization of the broader U.S. population. The database offers a comprehensive repository comprising sociodemographic profiles, interview transcripts, and health screening results. Leveraging this resource, the NCHS can employ diverse methods to assess the health and nutritional condition of the population in the United States. Moreover, it facilitates the calculation and examination of disease prevalence, along with associated risk factors or indicators. The ethical guidelines were followed during the participant selection process, and the NCHS

Research Ethics Review Committee approved the research protocol. Every participant furnished written consent after being fully informed. All research conducted adhered to the principles outlined in the Declaration of Helsinki.

The data utilized in our study were sourced from NHANES spanning the years 2005 to 2016, encompassing both HPV testing results and all components of the LE8 assessment. The total cohort comprised 60,936 research participants. From this, we excluded 30,152 male participants, 19,708 individuals lacking HPV test results, and 8,212 participants with incomplete LE8 score data. Ultimately, our analysis included 8,264 participants (Figure 1).

2.2 Study variables

In this study, the independent variable was the LE8 score, wherein higher scores indicated better CVH. The LE8 score encompassed four health behaviors (diet, physical activity, nicotine exposure, and sleeping health) and four health factors [body mass index (BMI), blood lipids, blood glucose, and blood pressure (BP)]. Dietary indicators were assessed based on participants' two 24-h dietary assessments, utilizing the Healthy Eating Index (HEI) 2015 (refer to [Supplementary Table S1](#)) (16). To gather data on physical activity, nicotine exposure, sleep patterns, medication and diabetes history, self-report questionnaires were employed. Physical examinations were conducted to measure BP, weight, and height. The calculation of body mass index (BMI) involved dividing weight in kilograms by the square of height in meters. For the determination of serum cholesterol, plasma glucose, and hemoglobin A1c levels, blood samples were collected. Each of the eight components of CVH is scored on a scale ranging from 0 to 100 points. To calculate the total LE8 score, the scores of the eight components are summed and then divided by eight, yielding a maximum score of 100 (17). Based on the standards set by the AHA, a LE8 score of 80–100 suggests a high level of CVH, while 50–79 indicates a moderate level, and 0–49 denotes a low level (17). Instructions detailing the computation of LE8 scores using all variables found in NHANES data are provided in [Supplementary Table S2](#) (17, 18).



The dependent variables were the status of HPV infection and HR-HPV infection in this study, assessed through genotyping of DNA obtained from vaginal swabs. The HPV assay employed the Roche Linear Array HPV Genotyping test, while the HR-HPV assay utilized either Hybrid Capture 2 technology or the Cobas test.

Additionally, we investigated potential covariates that might impact the correlation between HPV infection and CVH, comprising age, race, BMI, marital status, education level, family income-to-poverty ratio (PIR), smoking history, age at first sexual intercourse, health insurance coverage, and history of oral contraceptive (OC) use. Data regarding these variables were extracted from the NHANES database, encompassing demographic information, examination records, and self-report questionnaire section.

2.3 Statistical analysis

The measurement and demographic characteristics of the study population underwent descriptive analysis. These indicators were stratified into three groups based on varying levels of CVH. Continuous variables were presented as mean $\pm$ standard (Mean $\pm$ SD), and between-group comparisons were conducted via the *t*-test. Categorical variables were expressed as frequency [n (%)], and compared across groups using the chi-square test. Logistic regression models were used to examine the relationship between LE8 scores and HPV infection rates. The results are presented as odds ratios (OR) with 95% confidence intervals (CI). A univariate logistic regression analysis was performed initially for the crude model. Models 2 and 3 involved multifactorial logistic regression analyses. Specifically, Model 1 remained unadjusted, while Model 2 adjusted for age and race. Moreover, model 3 adjusted for all covariates included in this study. Smoothed curve fitting and threshold effect analysis were employed to further explore the relationship between LE8 scores and HPV infection. Subgroup analyses were conducted based on age, race, education level, marital status, BMI, health insurance status, or smoking history. At last, logistic regression models were employed to investigate the relationship between BP and HPV infection. Model 1 was unadjusted, Model 2 was adjusted for age and race, and Model 3 was adjusted for age, race, BMI, and smoking history. Data were analyzed using Empower software (v.2.0) and the R statistical package (v.3.4.3). Statistical significance was defined as a two-sided *P*-value <0.05 .

3 Results

3.1 Baseline characteristics

Totally, 8,264 women aged 20–59 years were included in this study with an average age 39.53 ± 11.24 years. The overall HPV prevalence was 43.43%, while HR-HPV prevalence was 17.36%. The different CVH subgroups were significantly different with regard to age, race, marital status, education level, PIR, BMI, smoking history, age of first sexual intercourse, and health insurance status (all $P < 0.05$). Nevertheless, CVH subgroups did not exhibit any statistically significant difference in terms of OC using history ($P > 0.05$). As CVH levels rose, there was a corresponding decline in the HPV prevalence, a trend also observed in HR-HPV infections (all $P < 0.05$) (Table 1).

3.2 Relationship between CVH and HPV infection

The results of multivariate logistic regression analysis indicated a significant negative correlation between LE8 score and both HPV and HR-HPV infection. In the fully adjusted model, 10 points increase in LE8 score associated with an 8% lower in the odds of HPV infection (OR = 0.92, 95% CI 0.88–0.96); individuals with high levels of CVH exhibited 33% lower HPV infection rates in contrast to individuals exhibiting low levels (OR = 0.67, 95% CI 0.55–0.82). Similarly, 10 points increase in LE8 score was linked to an 8% lower in the odds of HR-HPV infection (OR = 0.92, 95% CI 0.87–0.97) (Table 2). This study confirmed the negative correlation between CVH and HPV infection using a smooth curve fitting approach. This correlation was consistently observed for both total HPV infection (Figure 2A) and HR-HPV infection (Figure 2B).

3.3 Relationship between BP and HPV infection

The logistic regression analysis revealed no correlation between BP and HPV infection in both the partially adjusted and fully adjusted models ($P > 0.05$). Only in the unadjusted model, a negative correlation was observed between BP and HR-HPV infection (Supplementary Table S3).

3.4 Subgroup analysis

To delve into the potential impact of diverse factors on the correlation between CVH and HPV infection, subgroup analyses were carried out. Upon inclusion of all covariates, subgroup analyses revealed that the relationship between CVH and HPV infection was influenced by marital status and smoking history (P for interaction <0.05). Married or cohabiting participants, and those with smoking history, exhibit a stronger negative relationship between CVH and HPV infection (separately OR = 0.87, 95% CI 0.83–0.93; OR = 0.87, 95% CI 0.81–0.93). Furthermore, concerning HR-HPV infection, the correlation with CVH was influenced by marital status and education level (P for interaction <0.05). Married or cohabiting participants, as well as those with a high school or General Educational Development (GED) education, displayed a stronger negative relationship between CVH and HR-HPV infection (separately OR = 0.85, 95% CI 0.79–0.92; OR = 0.83, 95% CI 0.74–0.93) (Table 3).

4 Discussion

Utilizing the NHANES database, we performed a cross-sectional investigation to explore the correlation between LE8 scores representing CVH and HPV infection. Our results suggest that individuals with elevated LE8 scores exhibited a reduced odds of HPV and HR-HPV infection. Subgroup analysis revealed that marital status influenced the relationship between CVH and HPV as well as HR-HPV infections, with married or cohabiting individuals experiencing greater benefits from higher CVH scores. Smoking history also influenced the relationship

TABLE 1 Baseline characteristics of the study population according to CVH levels.

Characteristics	CVH level			P-value
	Low level (LE8: 0–49) N = 1,294	Moderate level (LE8: 50–79) N = 5,134	High level (LE8: 80–100) N = 1836	
Age, years	45.10 ± 9.92	39.44 ± 11.20	35.85 ± 10.64	<0.001
Race, n (%)				<0.001
Mexican American	188 (14.53%)	958 (18.66%)	258 (14.05%)	
Other Hispanic	97 (7.50%)	540 (10.52%)	190 (10.35%)	
Non-Hispanic White	565 (43.66%)	1988 (38.72%)	872 (47.49%)	
Non-Hispanic Black	383 (29.60%)	1,168 (22.75%)	211 (11.49%)	
Other Races	61 (4.71%)	480 (9.35%)	305 (16.61%)	
Marital status, n (%)				<0.001
Married or living with partner	661 (51.08%)	3,044 (59.29%)	1,148 (62.53%)	
Widowed, divorced, or separated	387 (29.91%)	958 (18.66%)	193 (10.51%)	
Never married	246 (19.01%)	1,132 (22.05%)	495 (26.96%)	
Education level, n (%)				<0.001
Less than high school	414 (31.99%)	1,050 (20.45%)	151 (8.22%)	
High school or GED	341 (26.35%)	1,107 (21.56%)	211 (11.49%)	
More than high school	539 (41.65%)	2,977 (57.99%)	1,474 (80.28%)	
PIR	1.79 ± 1.42	2.43 ± 1.62	3.17 ± 1.69	<0.001
BMI, kg/m ²	36.02 ± 8.29	29.91 ± 7.31	24.08 ± 4.15	<0.001
Age of first sexual intercourse, years	16.89 ± 3.75	17.41 ± 3.44	18.29 ± 3.58	<0.001
Health insurance status, n (%)				<0.001
Has	917 (70.87%)	3,737 (72.79%)	1,499 (81.64%)	
Non	377 (29.13%)	1,397 (27.21%)	337 (18.36%)	
Oral contraceptive taking history, n (%)				0.133
Yes	933 (72.10%)	3,555 (69.24%)	1,285 (69.99%)	
No	361 (27.90%)	1,579 (30.76%)	551 (30.01%)	
Smoked at least 100 cigarettes, n (%)				<0.001
Yes	901 (69.63%)	1848 (36.00%)	267 (14.54%)	
No	393 (30.37%)	3,286 (64.00%)	1,569 (85.46%)	
HPV infection, n (%)				<0.001

(Continued)

TABLE 1 (Continued)

Characteristics	CVH level			P-value
	Low level (LE8: 0–49) N = 1,294	Moderate level (LE8: 50–79) N = 5,134	High level (LE8: 80–100) N = 1836	
Yes	679 (52.47%)	2,257 (43.96%)	653 (35.57%)	
No	615 (47.53%)	2,877 (56.04%)	1,183 (64.43%)	
HR-HPV infection, n (%)				0.002
Yes	269 (20.79%)	860 (16.75%)	306 (16.67%)	
No	1,025 (79.21%)	4,274 (83.25%)	1,530 (83.33%)	

CVH, cardiovascular health; GED, General educational development; PIR, Ratio of family income to poverty; BMI, Body mass index; HPV, Human papillomavirus; HR-HPV, High risk human papillomavirus. Continuous variables were indicated by mean ± SD, assessed via t-tests. Categorical variables were indicated by frequencies [n (%)] and assessed via chi-square tests.

between CVH and HPV infection, with smokers benefiting more from higher LE8 scores. Furthermore, in the case of HR-HPV infection, individuals with tertiary education rarely benefit from higher LE8 scores.

To the best of our knowledge, this research represents the first attempt to investigate the association between CVH as quantified by LE8 score and HPV infection. The LE8 score encompasses eight health behaviors and factors impacting CVH, providing an intuitive assessment of peoples’ cardiovascular well-being. Previous researches had predominantly focused on exploring the correlation between CVD and HPV infection and suggested that HPV infection is positively associated with CVD (7, 8). In a large-scale study conducted in Korea, 63,411 Korean women without pre-existing CVD were enrolled. Researchers found a strong link between HR-HPV infection and a higher incidence of CVD in women. Those who tested positive had hazard ratios (HR) compared to those who tested negative (HR = 1.25). Furthermore, the presence of obesity was found to elevate the risk of developing CVD (HR = 1.73) (11). In a 17-year follow-up study, researchers found that women with HR-HPV infection had a higher mortality risk after developing CVD compared to those who were not infected. What is more, obesity reinforces this risk (19). However, a study on head and neck squamous cell carcinoma patients found that HPV-negative patients had a higher 5-year cumulative incidence of CVD compared to HPV-positive patients (20). In another study, HPV DNA was detected in the atherosclerotic coronary arteries of 20 deceased patients who had suffered from myocardial infarction, with 55% of the samples testing positive for HR-HPV (21). In our study, it was observed that a 10 points enhance in CVH score was correlated with a 8% decrease in the positivity rates of HPV and HR-HPV, after adjusting for all covariates. Furthermore, it was noted that BMI did not exert any influence on this inverse relationship, which was different from previous studies. We also investigated the relationship between BP and HPV infection, given that atherosclerosis directly affects BP. Our results indicated a negative correlation between BP and HR-HPV infection only in the unadjusted model; however, this correlation disappeared after adjusting for covariates. This negative correlation observed in the unadjusted model may be influenced by confounding factors. The relationship between CVH and HPV infection appears to be more complex, as BP could be associated with other underlying variables, such as age, race, BMI and smoking history.

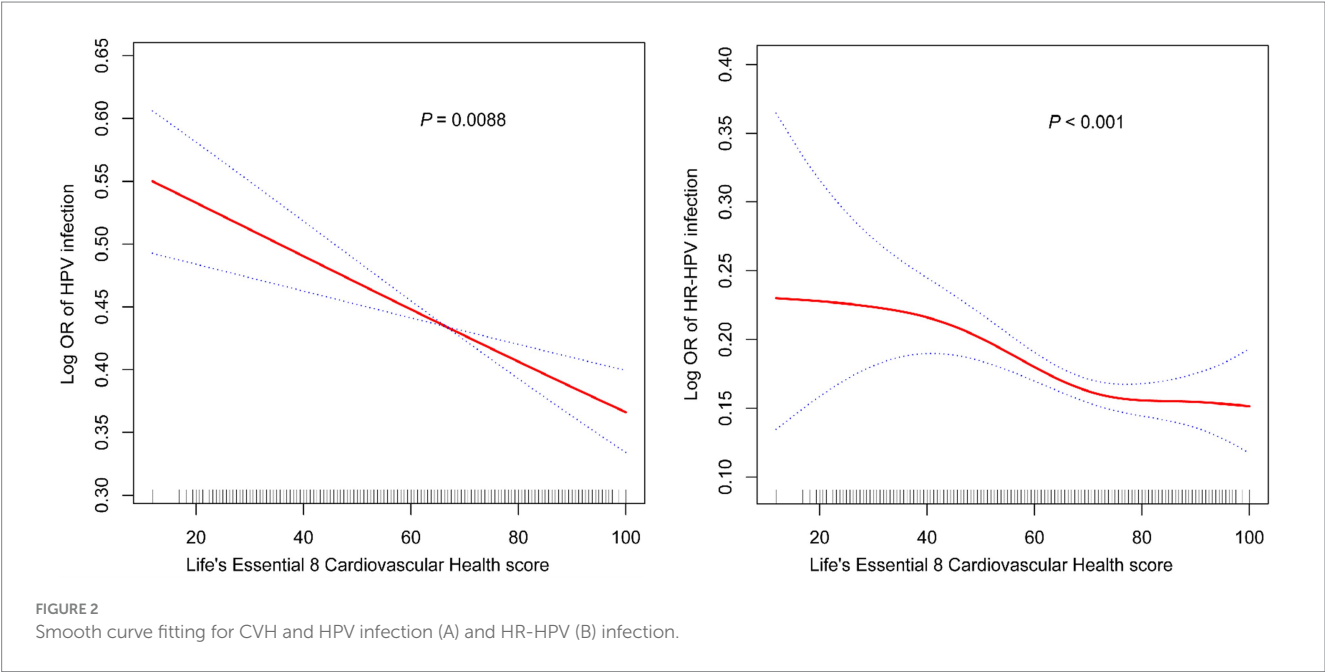
The mechanism behind the negative correlation between CVH and HPV infection is complex and currently lacks consensus. It is mainly believed to be the result of chronic infection. HPV infection can induce systemic inflammation and lead to an increase in inflammatory mediators in circulation, thereby fostering the development of atherosclerosis (19, 22). In addition, HPV, as a virus that primarily infects epithelial cells, may also directly infect vascular endothelial cells (23). Some studies have demonstrated that HPV can potentially access arterial tissues via the systemic circulation (21, 24, 25). The expression of vascular endothelial growth factor was controlled by the HPV through its oncoprotein E6, thus participating in the process of atherosclerosis (26). Meanwhile, the HPV oncoproteins E6 and E7 can degrade the tumor suppressor protein P53, which accelerates atherosclerosis, as P53 plays a crucial role in regulating this process (22, 27).

Furthermore, HPV infection is correlated with several shared risk factors with CVD. As widely recognized, tobacco exposure

TABLE 2 The association between CVH and HPV infection.

	Model 1 OR (95% CI) <i>P</i> -value	Model 2 OR (95% CI) <i>P</i> -value	Model 3 OR (95% CI) <i>P</i> -value
HPV infection status			
LE8 scores (per 10 scores)	0.87 (0.84, 0.89) <0.0001	0.85 (0.83, 0.88) <0.0001	0.92 (0.88, 0.96) <0.0001
Low CVH level	Reference	Reference	Reference
Moderate CVH level	0.71 (0.63, 0.80) <0.0001	0.66 (0.58, 0.75) <0.0001	0.80 (0.70, 0.93) 0.0032
High CVH level	0.50 (0.43, 0.58) <0.0001	0.47 (0.40, 0.55) <0.0001	0.67 (0.55, 0.82) <0.0001
<i>P</i> for trend	< 0.0001	< 0.0001	< 0.0001
HR-HPV infection status			
LE8 scores (per 10 scores)	0.94 (0.90, 0.97) 0.0003	0.89 (0.86, 0.93) <0.0001	0.92 (0.87, 0.97) 0.0017
Low CVH level	Reference	Reference	Reference
Moderate CVH level	0.77 (0.66, 0.89) 0.0007	0.66 (0.56, 0.77) <0.0001	0.74 (0.62, 0.89) 0.0012
High level	0.76 (0.64, 0.91) 0.0034	0.63 (0.52, 0.76) <0.0001	0.77 (0.60, 0.98) 0.0322
<i>P</i> for trend	0.0067	< 0.0001	0.0557

Model 1 took no adjustments; Model 2 adjusted for age and race; Model 3 adjusted for all covariates specified. CI, confidence interval; OR, odds ratio.



poses a significant risk for CVD and is related to the occurrence of severe cardiovascular events (28). Concurrently, previous studies have shown that smoking impairs the body’s ability to eliminate the HPV virus. This leads to persistent infection and ultimately contributes to a poor prognosis for cervical cancer (29–31). In our study, participants who had a history of smoking, exhibit a stronger negative correlation between CVH and HPV infection. At the same time, the socioeconomic status of individuals also exerts an influence on the prevalence of both CVD and HPV infection (32, 33). We also found that, the level of education influenced the relationship between CVD and HR-HPV infection. There are several other common influences, including hyperlipidemia, obesity, physical activity, etc. (34–36). These findings suggested the

potential effectiveness of employing the LE8 score as an indicator for assessing CVH and HPV infection. And the observed correlation between CVH and HPV infection may stem from common risk factors.

This study has several advantages. Firstly, the data employed in this research was obtained from the nationally conducted survey NHANES, thereby affording a substantial sample size and a degree of representativeness. Secondly, this study controlled for a series of covariates associated with HPV infection and CVH, enhancing the credibility of the fully adjusted model. Thirdly, we investigated the impact of various factors on the correlation between CVH and HPV infection through subgroup analysis. This study is subject to several limitations. First, the cross-sectional design of the study does not

TABLE 3 Subgroups analyses for the association between CVH and HPV infection.

Subgroup	HPV infection status		HR-HPV infection status	
	OR (95% CI)	<i>P</i> for interaction	OR (95% CI)	<i>P</i> for interaction
Age, years		0.2224		0.9977
Age < 25	1.01 (0.87, 1.16)		0.93 (0.81, 1.08)	
25 ≤ Age < 50	0.91 (0.87, 0.96)		0.94 (0.88, 1.00)	
Age ≥ 50	0.98 (0.90, 1.06)		0.94 (0.84, 1.05)	
Race		0.8261		0.4006
Mexican American	0.92 (0.83, 1.03)		0.92 (0.81, 1.06)	
Other Hispanic	0.96 (0.84, 1.09)		0.98 (0.83, 1.17)	
Non-Hispanic White	0.91 (0.85, 0.97)		0.88 (0.81, 0.96)	
Non-Hispanic Black	0.90 (0.82, 0.98)		0.91 (0.82, 1.01)	
Other Races	0.99 (0.85, 1.15)		1.08 (0.89, 1.32)	
Marital status		0.0309		0.0164
Married or living with partner	0.87 (0.83, 0.93)		0.85 (0.79, 0.92)	
Widowed, divorced, or separated	0.95 (0.87, 1.04)		1.03 (0.93, 1.15)	
Never married	1.00 (0.91, 1.09)		0.94 (0.85, 1.04)	
Education level		0.1559		0.0180
Less than high school	0.97 (0.88, 1.07)		0.85 (0.75, 0.97)	
High school or GED	0.85 (0.78, 0.94)		0.83 (0.74, 0.93)	
More than high school	0.93 (0.88, 0.99)		0.98 (0.92, 1.05)	
BMI, kg/m ²		0.0539		0.5765
BMI < 25	0.97 (0.90, 1.05)		0.95 (0.86, 1.05)	
25 ≤ BMI < 30	0.85 (0.78, 0.92)		0.95 (0.86, 1.06)	
BMI ≥ 30	0.91 (0.85, 0.96)		0.90 (0.83, 0.97)	
Health insurance status		0.7769		0.3497
Has	0.92 (0.88, 0.96)		0.93 (0.88, 0.98)	
Non	0.91 (0.85, 0.98)		0.89 (0.81, 0.97)	
Smoked at least 100 cigarettes		0.0118		0.4950
Yes	0.87 (0.81, 0.93)		0.90 (0.83, 0.98)	
No	0.97 (0.91, 1.02)		0.94 (0.87, 1.01)	

ORs were calculated as per 10 scores increase in LE8 score.

allow for the determination of a causal relationship between CVH and HPV infection. Whether CVH and HPV infection are causally related or their correlation stems from common risk factors needs to be exposed in future in-depth prospective studies. Second, while several covariates were incorporated into the analysis, the possibility remains that pertinent covariates were overlooked. Finally, owing to restrictions inherent in the NHANES database, the generalizability of the study’s findings may be constrained. Therefore, future comparative studies in other countries and regions are necessary.

5 Conclusion

In summary, the findings of this study illustrated a negative association between CVH and HPV infection, as well as HR-HPV infection. The LE8 score shows potential as a shared predictive

biomarker for both CVH and HPV infection. More prospective studies are needed to delve deeper into the causal association between CVH and HPV infection, as well as the underlying mechanisms.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found at: <https://www.cdc.gov/nchs/nhanes/>.

Ethics statement

The National Center for Health Statistics Research Ethics Review Committee approved the research protocol. 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

ML: Data curation, Methodology, Software, Writing – original draft. MS: Supervision, Writing – review & editing.

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Conflict of interest

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

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2024.1501409/full#supplementary-material>

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Outcomes and associated factors of cervical human papillomavirus infection among 608 women in Shenzhen, China, 2018–2023

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Objective: This study aimed to uncover the patterns of Human papillomavirus (HPV) infection outcomes in women and assess the risk factors that may affect these outcomes.

Methods: A retrospective study was conducted on 608 women who tested positive for HPV-DNA during their initial visit to the outpatient department of Shenzhen Longgang Central Hospital from 2018 to 2023 and who had subsequent HPV-DNA testing as part of their post-visit monitoring. The monitoring intervals were every 6 months. The rank sum test was used to analyze ranked data. The Kaplan–Meier method was used to analyze the turning negative time. Univariate analysis was performed using the log-rank test, and multivariate analysis was performed using the Cox model to analyze independent risk factors.

Results: The results showed that the median age was 40.00 years (interquartile 33.00–47.00 years), the total conversion rate of the HPV-negative patients was 38.98%, and the median conversion time of the HPV-negative patients was 8.95 months (interquartile 4.20–16.175 months). Age, infection status and type of health insurance were significantly correlated with HPV outcome ($p < 0.05$).

Conclusion: Among women infected with HPV, the overall rate of negative HPV infection was 38.93%, and the duration of negative conversion was 8.95 months. The study revealed that age, HPV infection status, and type of medical insurance are independent predictors of the persistence of negative HPV test outcomes.

KEYWORDS

human papillomavirus, outcome, prognosis, clearance rate, cervical lesions, negative conversion

1 Introduction

Human papillomavirus (HPV) is the most prevalent sexually transmitted disease, affecting more than 70% of sexually active women throughout their lifetime (1–3). A meta-analysis (4) reported a total HPV infection rate of 14.3% among women with a normal cervix in China, with a prevalence rate of 12.1% in South China. Further studies (5, 6) revealed HPV infection rates of 21.66 and 19.33% in Guangzhou and Shenzhen, respectively. Genital warts are associated primarily with low-risk human papillomavirus (LR-HPV) types 6 and 11, whereas high-risk human papillomavirus (HR-HPV) types 16, 18, 31, 33, 45, 52, and 58 are linked to

cervical cancer (7–10). According to GLOBOCAN 2022 statistics, about 660,000 individuals were diagnosed with cervical cancer, and about 350,000 succumbed to the disease worldwide in 2022, with the majority—more than 85%—of these incidents occurring in developing nations (11, 12). In China, according to the National Cancer Center's 2022 data, 150,700 incident cases of cervical cancer and 55,700 fatalities have been recorded, comprising 85,500 new cases and 30,200 deaths in urban areas and 65,200 new cases and 25,500 deaths in rural areas (13). A predictive study by Duan et al. (14) forecasts an increase in the number of HPV-associated cancer cases from 117,929 to 214,077 between 2016 and 2030, with age-standardized rates rising from 6.07 to 9.35 per 100,000 people. Cervical cancer is projected to account for 87.7% of all HPV-associated cancers. The burden of HPV-associated cancers in China is anticipated to continue rising over the next decade, imposing significant mental stress and disease on women.

Epidemiological research has shown that the majority of HPV infections are cleared spontaneously within 2 years due to autoimmune responses, although about 10–20% of women experience persistent infection (1, 4, 15). The course of HPV infection is affected by a combination of personal and environmental factors, which contributes to the substantial differences in clearance rates observed across regions (10, 16, 17). Vaccination with the HPV vaccine is an effective preventive measure against HPV infection (18–20). However, for individuals who are already infected, the clinical efficacy of anti-HPV drugs remains unclear, and further verification of the effectiveness of therapeutic HPV vaccines is needed (21–23). Additionally, the effect of physical therapy on HPV clearance varies significantly across individuals (24). Grasping of HPV infection outcomes within particular demographic groups is essential, enabling healthcare providers to effectively track and treat individuals with HPV. This knowledge also holds significant importance for the dynamic management of cervical lesions associated with HPV and for cervical cancer, underscoring the necessity for region-specific research and tailored intervention strategies.

While numerous studies have outlined the mechanisms of HPV infection, the processes of HPV clearance and the factors that influence this remain unclear. Particularly, debates persist regarding the outcomes of HPV coinfection, with significant variation observed across different countries, regions, economic statuses, and ethnic groups. To address these gaps, we propose a study on HPV outcomes in one of China's largest and youngest cities, aiming to examine the patterns of HPV outcomes in urban Chinese women.

This study used Shenzhen Longgang Central Hospital as the center, which was selected from 2018 to 2023 outpatient women with HPV infection for investigation and follow-up. To explore the outcome of female genital HPV infection, including the HPV infection negativity rate and negative time, the general demographic information, test results, and pathological results of patients were collected through the medical records system, and the related risk factors affecting the development of infection negativity were analyzed. These findings will contribute to a better understanding of the outcomes and related factors of HPV infection in women in Shenzhen, China. Moreover, these findings can provide a basis for the clinical diagnosis and treatment, disease management, and prognosis follow-up of HPV-infected women.

2 Objects and methods

2.1 Object

The selected patients were admitted to Shenzhen Longgang Central Hospital from 2018 to 2023, according to the expert consensus on HPV infection and HPV-associated diseases (25). For women diagnosed with HPV infection, patient information was obtained through an electronic medical record system. The inclusion criteria were as follows: (1) were women aged ≥ 18 years; (2) were married or unmarried patients with a sexual life history; (3) were in line with the Western diagnosis of human papillomavirus infection; (4) had complete clinical data; and (5) had two or more HPV-DNA tests. The exclusion criteria were as follows: (1) pregnant or lactating; (2) mental disorders or consciousness disorders; and (3) serious organ damage and related functional damage to the heart, liver or kidney. The study sample comprised 608 female participants who satisfied the inclusion criteria.

This study was conducted in accordance with relevant guidelines, regulations, and principles outlined in the Declaration of Helsinki. The Ethics Committee of Shenzhen Longgang Central Hospital reviewed and approved the study protocol (Approval Number: 2024ECPJ004). Written informed consent was obtained from all participants prior to their involvement in the study, ensuring compliance with ethical standards for human subject research.

To ensure participants' privacy and data security, this study strictly adheres to data protection regulations. All collected data is de-identified, and participants' personal and health information is kept confidential throughout the analysis process. Research data is restricted to authorized personnel and stored in an encrypted database. All analyses are conducted on anonymized data to prevent the disclosure of any personal identities. Furthermore, all research data will be securely destroyed upon completion in accordance with Shenzhen Longgang Central Hospital's data management policies. Any publicly available data will be fully de-identified and limited to aggregated results necessary for dissemination. Under no circumstances will the research data be shared with unrelated third parties, ensuring the full protection of participants' privacy rights.

2.2 Methods

Data collection: Age, ethnicity, occupation, ancestral place, home address, medical insurance type, marital status, birth history, abortion history, menopausal status, use of intrauterine devices, HPV infection status, cervical lesion degree, treatment method, and HPV outcome data were collected from the hospital medical record system. According to the correlation with the degree of cervical intraepithelial neoplasia (CIN), the 28 detected HPV types were classified as high-risk types (16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) or low-risk types (6, 11, 23, 40, 42, 43, 44, 54, 61, 81, 83). According to the correlation with CIN, the patients were divided into high-risk HPV (HR-HPV) infection (regardless of how many types of HPV they were infected, positive results included only HR-HPV), low-risk HPV (LR-HPV) infection (regardless of how many types of HPV they were infected, positive results included only LR-HPV), and mixed infection (regardless of how many types of HPV they were infected, positive results included both HR-HPV and LR-HPV). The participants in the study were categorized into 5 distinct groups based on the severity of their cervical lesions:

normal smear, CIN I, CIN II, CIN III and cervical cancer. The number of infected HPV strains can be divided into single infections and multiple infections, which can be divided into two infections, three infections or more. Follow-up: Follow-up was conducted via telephone, and patients returned to the hospital regularly for review. The follow-up time ranged from the first visit date to June 2024, and the follow-up interval was once every 6 months. The follow-up data concerned the outcomes of the HPV-infected women, and the outcomes were divided into negative and persistent infections. If the HPV-DNA test is negative at the time of review, the HPV infection of the woman is considered negative; persistent HPV infection is diagnosed if a woman's test results remain positive, indicating the need for further evaluation and management.

2.3 HPV-DNA testing

For women with normal menstruation, 10–18 days after menstruation is the best time for examination; vaginal irrigation should be avoided within 3 days before the examination; the use of contraceptive ointment and other vaginal drugs should be avoided; no sexual activity should occur within 24 h of the test; and the application of acetic acid or iodine solution should be contraindicated prior to the examination.

A special cervical exfoliation cell collector was used for sampling. The gynecologist first exposed the cervix with a voyeur or vaginal opener and then wiped the excessive secretions from the cervix with a cotton swab. The cervical brush was removed and placed in the cervical opening. The tip of the cervical brush was rotated 4–5 times in one direction to obtain sufficient epithelial cell samples. The tip of the cervical brush was then placed into the eluting tube, the handle was broken along the crease of the handle, the cover of the eluting tube was tightened, the sample was marked, and the eluting tube was kept upright. Once collected, the samples were submitted for examination as soon as possible: the samples were stored at room temperature for no more than 12 h, at 4°C for no more than 7 days, and at –20°C for no more than 3 months. Repeated freezing and thawing cycles were avoided. The foam box and ice bag were sealed, and the transit time did not exceed 48 h.

A human papillomavirus (HPV) genotyping test kit (Yaneng Biotechnology Co., Shenzhen, China) was utilized to identify 28 HPV types, comprising 17 HR-HPV types (HPV 16, 18, 31, 33, 35, 39, 45, 51, 52, 53, 56, 58, 59, 66, 68, 73, 82) and 11 LR-HPV types (HPV 6, 11, 23, 40, 42, 43, 44, 54, 61, 81, 83). Genotyping of HPV was conducted through PCR-based amplification followed by DNA reverse line blot hybridization. Specifically, designed primers targeted 23 HPV genotypes, amplifying specific fragments. The amplification products were then hybridized with 17 high-risk and 6 low-risk probes fixed on a membrane. The presence of hybridization signals indicated HPV genotype infection. The testing process consisted of HPV-DNA extraction, PCR amplification, hybridization, membrane washing, color rendering, and result analysis. Both positive and negative controls, provided in the kit, were used during testing according to the manufacturer's instructions.

2.4 Statistical methods

Data processing and statistical analysis were conducted using SPSS version 27.0. Normally distributed data are presented as $\bar{X} \pm S$ values, with group comparisons performed via independent samples *t*-tests or chi-square (X^2) tests. The non-normally distributed

measurement data are represented by $M (P25-P75)$, and the rank-sum test was used for comparisons between groups. Count data are represented as $N (%)$. Kaplan–Meier survival analysis, the log-rank test and the Cox proportional risk regression model were used for survival analysis and influencing factor analysis. In this study, $p < 0.05$ was considered statistically significant.

The HPV-negative conversion rate was calculated as the proportion of women who cleared their HPV infection during follow-up. The calculation was based on the following: denominator: the total count of women who had a positive HPV-DNA result during their initial visit (2018–2023) and subsequently underwent re-evaluation. Numerator: the number of women with first-time HPV-DNA detection (2018–2023) and who subsequently tested negative for HPV-DNA during follow-up as the numerator.

3 Results

3.1 General information

The study included a sample of 608 HPV-infected women who visited the clinic between 2018 and 2023. The median age at initial diagnosis was 40.00 years (quartile 33.00–47.00 years) and ranged from 18 to 73 years. Among the 608 women, 237 patients (38.98%) were negative, and 371 (61.02%) were still infected at the end of the follow-up period. The median duration of negative HPV results was 8.95 months (quartile range 4.20–16.175 months), with a range of 1.1–58.1 months. Among the 608 enrolled women, 169 (27.8%) had normal smears at initial diagnosis; 102 (16.8%) were diagnosed with CIN I. In total, 37 patients (6.1%) had CIN II, 292 patients (48%) had CIN III, and 8 patients (1.3%) had cervical cancer. The rank-sum test revealed statistically significant differences in age, ethnicity, occupation and treatment mode among groups with different cervical lesions ($p < 0.05$), as shown in Table 1. No substantial variations were observed in the infection types, incidence of multiple infections, or levels of infection risk across groups characterized by distinct cervical lesions ($p > 0.05$), as shown in Table 2.

In our study, the infection rates of HPV16 and HPV52 were the highest, at 27.632 and 27.467%, respectively, making them the most common types. HPV58 had an infection rate of 14.803%, which, while relatively high, was lower than that of HPV16 and HPV52. The infection rates of other HPV types gradually decreased, with HPV26 and HPV83 having the lowest rates at 0.164%. Overall, HPV16 and HPV52 were the predominant infection types. In terms of clearance rates, HPV6, HPV40, and HPV54 showed relatively high negative conversion rates of 62.5, 66.7, and 66.7%, respectively, indicating favorable clearance outcomes. In contrast, HPV35 had the lowest negative conversion rate at only 18.75%, suggesting a lower likelihood of clearance for this type. On the whole, the negative conversion rates of most HPV types ranged from 20 to 50%, indicating considerable variability in clearance probabilities, as shown in Figures 1, 2.

3.2 Prognostic analysis

3.2.1 Univariate survival analysis

Univariate analysis of 608 patients with HPV infection revealed that with increasing age, the rate of HPV infection tended to decrease,

TABLE 1 Baseline demographic, behavioral, and health characteristics of 608 HPV-infected women at Shenzhen Longgang Central Hospital from 2018 to 2023, divided by the degree of cervical lesions.

Variable		Grouping				<i>H</i>	<i>p</i> ^b
		Normal smear and CIN I ^a	CIN II ^a	CIN III ^a	Cervical cancer		
Age	<30	45	9	29	0	7.849	<0.05
	30–60	219	28	258	7		
	>60	7	0	5	1		
Gestational number	1–2	106	9	132	2	3.291	0.349
	>3	142	24	154	6		
	0	23	4	6	0		
Yield	1–2	186	26	221	5	6.211	0.102
	>3	51	6	57	3		
	0	34	5	14	0		
Ethnicity	Han nationality	267	35	272	7	10.893	<0.05
	Ethnic minorities	4	2	20	1		
Matrimony	Married	237	32	258	7	0.174	0.982
	Unmarried	34	5	34	1		
Occupation	Enterprise business service personnel	29	2	8	0	60.873	<0.01
	Workman	36	1	62	0		
	Farmer	40	13	100	2		
	Otherness	122	11	46	2		
	People in state organs, party organizations, public institutions	22	4	19	1		
	Out of work	22	6	57	3		
Ancestral place	Out of province	175	18	166	5	5.560	0.135
	Province	96	19	126	3		
Current address	City	175	21	192	2	6.548	0.088
	Rural area	96	16	100	6		
Medical insurance Category ^c	Shenzhen medical insurance	189	23	215	6	1.505	0.681
	Self-pay	66	11	44	1		
	Offsite ^d	16	3	33	1		
History of abortion	No	124	12	125	2	3.560	0.313
	Yes	147	25	167	6		
Menopause	No	209	31	243	5	5.173	0.160
	Yes	62	6	49	3		
Intrauterine devices	No	200	31	210	6	2.403	0.493
	Yes	71	6	82	2		
State of transition	1	117	15	104	1	5.779	0.123
	0	154	22	188	7		
Treatment mode	No	72	3	0	0	364.838	<0.01
	Pharmacotherapy	168	15	24	0		
	Surgical treatment	31	19	268	8		

^aCIN I denotes mild, CIN II indicates moderate, and CIN III signifies severe cervical cell abnormalities, with the associated cancer risk escalating in accordance with the severity of the grade.

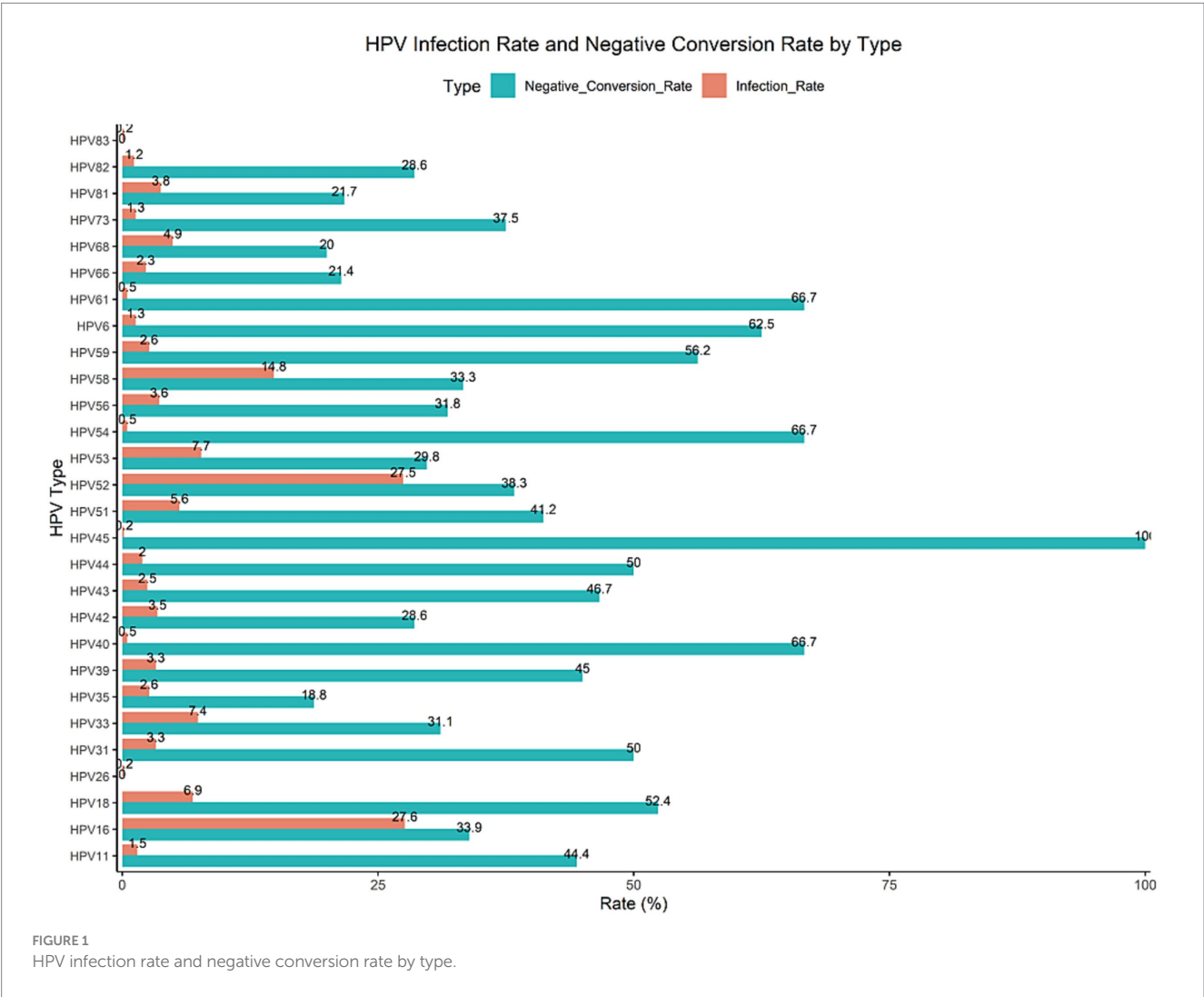
^b*p* values of infection differences between different cervical lesions.

^cShenzhen medical insurance is divided into three levels, which are applicable to different insured persons: I. First-level medical insurance is mainly for deep-household employees, who are jointly paid by the unit and individual. The outpatient and hospitalization reimbursement ratio is high. The insured can be in the city at any designated hospital for medical treatment without binding to a social health center. II. Second-tier health insurance: applicable to non-deep-household employees and other voluntary alternatives. Outpatient reimbursement is low, requiring binding to a social health center. The hospitalization reimbursement ratio is low, and out-of-city hospitalization requires referral procedures. III. Three-tier medical insurance: resident medical insurance, applicable to unemployed residents, students, etc. The payment is the lowest, and the reimbursement ratio is similar to that of the second grade. You need to bind to the social health center for outpatient reimbursement.

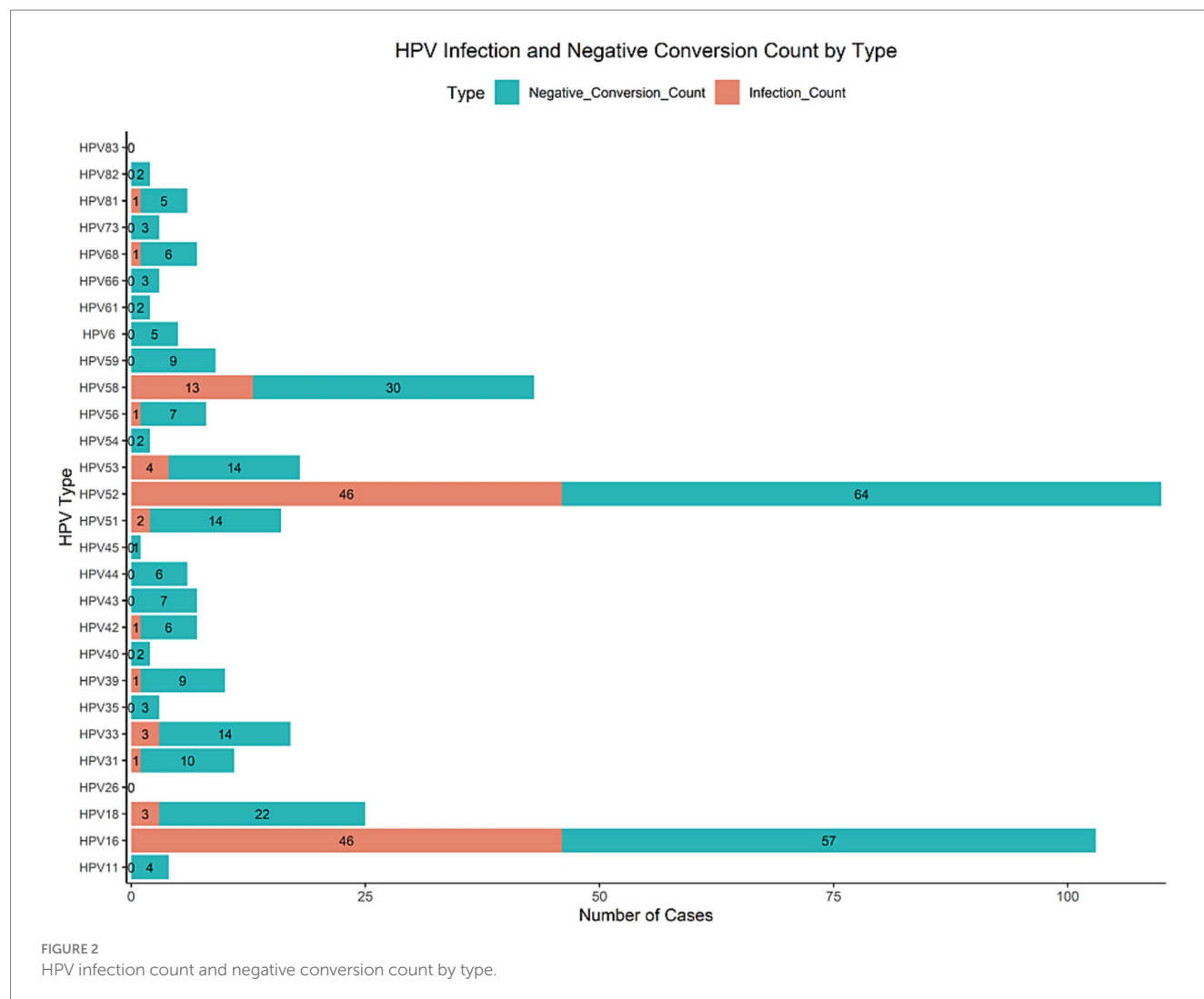
^dRemote medical insurance means that the insured can still enjoy medical insurance benefits when they seek medical treatment in places other than the insured place. It solves the problem of medical security for people from different regions, including direct settlement of medical treatment in other places and medical insurance services in long-term residence. The main forms of long-distance medical insurance are as follows: I. Long-distance medical settlement: the insured can directly settle the medical expenses through the designated hospitals in other places without the need to pay in full. I. Off-site filing: The insured shall put on record in the medical insurance center in advance to enjoy direct settlement in other places. II. Medical insurance for long-term living in other places: it is suitable for long-term residents or retirees who can enjoy medical insurance services in their place of residence after filing.

TABLE 2 HPV infection of 608 women infected with HPV in Shenzhen Longgang Central Hospital from 2018–2023, divided by the degree of cervical lesions.

Variable		Grouping				<i>H</i>	<i>p</i>
		Normal smear and CIN I	CIN II	CIN III	Cervical cancer		
Type of infection	Single infection	204	24	198	7	5.588	0.133
	Multiple infection	67	13	94	1		
Number of multiple infections	Single infection	196	24	197	7	3.535	0.316
	Double infection	59	11	67	1		
	Triple or above infection	16	2	28	0		
Infection type and risk level	HR-HPV infection	225	31	266	7	7.635	0.054
	LR-HPV infection	21	0	5	1		
	HR and LR HPV mixed infection	25	6	21	0		



and the <35 years age group had the highest rate of negative results, 46.60%. In the >50 years age group, the negative conversion rate was the lowest (28.41%). The rate of negative conversion in Han patients (39.59%) was higher than that in minority patients (25.93%). The negative conversion rate of premenopausal patients (41.39%) was significantly greater than that of postmenopausal patients (29.17%).



The infection status significantly influenced the negative conversion rate, with patients exhibiting a single infection showing a notably higher rate (43.19%) than those with multiple infections (28.57%). There were differences in the HPV conversion rate and conversion duration among patients with different medical insurance types. Patients with medical insurance in Shenzhen had the highest conversion rate (42.03%). Patients with remote medical insurance had the lowest rate of negative conversion, at only 13.21%.

A correlation was observed between the conversion rate of HPV infection negativity and the severity of cervical lesions, with the conversion rate decreasing as lesion severity increased. Analysis revealed that women with normal smears had a 44.38% HPV infection conversion rate, whereas cervical cancer patients had the lowest conversion rate of 12.50%. A comparison of treatment methods revealed that patients receiving drug therapy achieved the highest negative conversion rate (50.24%), whereas those receiving surgery had the lowest rate (33.44%).

There are also differences in HPV conversion rates among women in different occupations. Among them, enterprises and commercial and service workers had the highest negative conversion rates (43.59%). Unemployed patients had the lowest rate of negative conversion (28.41%).

The log-rank test revealed that eight of the 17 collected variables, specifically age, ethnicity, menopausal status, infection status, medical

insurance type, lesion degree, treatment mode, and occupation, demonstrated statistically significant associations with the negative conversion rate of infected patients ($p < 0.05$). The details are presented in Table 3 and illustrated in Figure 3.

3.2.2 Cox multivariate analysis

A multivariate Cox proportional hazards model was constructed, incorporating factors identified as statistically significant in the univariate analysis. Multivariate Cox regression analysis revealed that age (HR = 1.71, 95% CI 1.28–2.28), infection status (single infection vs. multiple infection; HR = 0.68, 95% CI 0.49–0.93), and medical insurance type (HR = 0.74, 95% CI 0.56–0.98) were independently associated with the outcome. In contrast, ethnicity, occupation, menopausal status, degree of cervical lesions, and treatment approach did not demonstrate significant associations in this multivariate analysis, as shown in Table 4.

4 Discussion

Variations in HPV incidence, clearance, and persistence have been observed across different regions and countries (26–28). Domestic studies (3, 17) have shown that about 50% of HR-HPV-positive women with normal baseline cytology clear the infection through

TABLE 3 Single-factor analysis outcomes in 608 women with HPV infection.

Influencing factors	Total number of cases	Negative/case	Negative conversion rate (n %)	Median survival	X ²	p
Age					6.21	<0.05
<35	206	96	46.60	14.60		
35–50	314	116	36.94	19.10		
>50	88	25	28.41	19.60		
Ethnicity					4.051	<0.05
Han nationality	581	230	39.59	17.5		
Ethnic minorities	27	7	25.93	9.9		
Matrimony					0.300	0.584
Married	534	211	39.51	17.4		
Unmarried	74	26	35.14	15.6		
Gestational number	608	237	38.98	17.3	0.16	0.21
Yield	608	237	38.98	15.55	0.43	0.356
Ancestral place					2.821	0.093
Fluidness	364	137	37.64	19.6		
Permanent residence	244	100	40.98	15.6		
Current address					0.087	0.768
City	390	157	40.26	17.8		
Rural area	218	80	36.70	15.6		
History of abortion					0.177	0.674
No	263	109	41.44	16.3		
Yes	345	128	37.10	19.1		
Menopause					4.929	<0.05
No	488	202	41.39	16.5		
Yes	120	35	29.17	19.6		
Intrauterine devices					3.544	0.060
No	447	190	42.51	16.3		
Yes	161	47	29.19	23.9		
Infection status					4.156	<0.05
Single infection	433	187	43.19	16.6		
Multiple infection	175	50	28.57	26.7		
Medical insurance Category					6.227	<0.05
Shenzhen medical insurance	433	182	42.03	16.20		
Self-pay	122	48	39.34	19.60		
Offsite	53	7	13.21	28.60		
Degree of lesion					10.87	<0.01
Normal smear	169	75	44.38	19.60		
CINI	102	42	41.18	23.90		
CINII	37	15	40.54	17.30		
CINIII	292	104	35.62	12.20		
Cervical cancer	8	1	12.50	13.60		
Treatment mode					10.06	<0.01
Naught	75	24	32.00	23.20		

(Continued)

TABLE 3 (Continued)

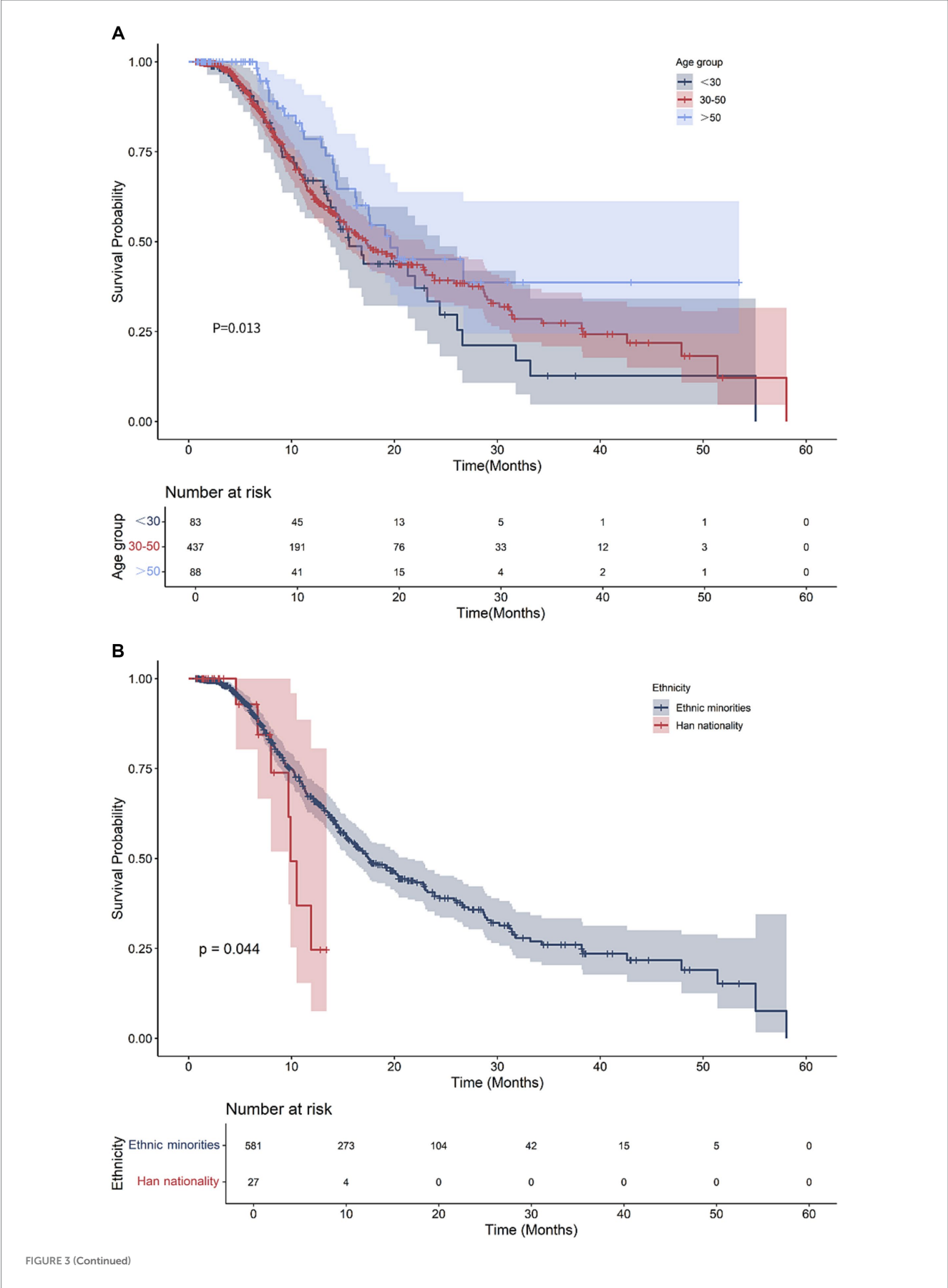
Influencing factors	Total number of cases	Negative/case	Negative conversion rate (n %)	Median survival	χ^2	p
Pharmacotherapy	207	104	50.24	20.40		
Surgical treatment	326	109	33.44	13.10		
Number of multiple infections					2.563	0.11
Single infection	424	180	42.45	16.20		
Double infection	138	45	32.61	21.30		
Triple infection	38	11	28.95	26.70		
Quadruple and above	8	1	12.50	INF		
Type of infection					1.182	0.277
LR-HPV infection	27	11	40.74	23.90		
HR-HPV infection	529	206	38.94	16.30		
HR and LR HPV mixed infection	52	20	38.46	23.20		
Occupation					4.196	<0.05
Employees in the corporate, business, and service sectors	39	17	43.59	25.80		
Industrial workers	99	34	34.34	14.80		
Farmers	155	59	38.06	14.70		
Others	181	89	49.17	17.60		
Personnel in government agencies, party organizations, and public institutions	46	13	28.26	29.40		
Unemployed individuals	88	25	28.41	15.80		

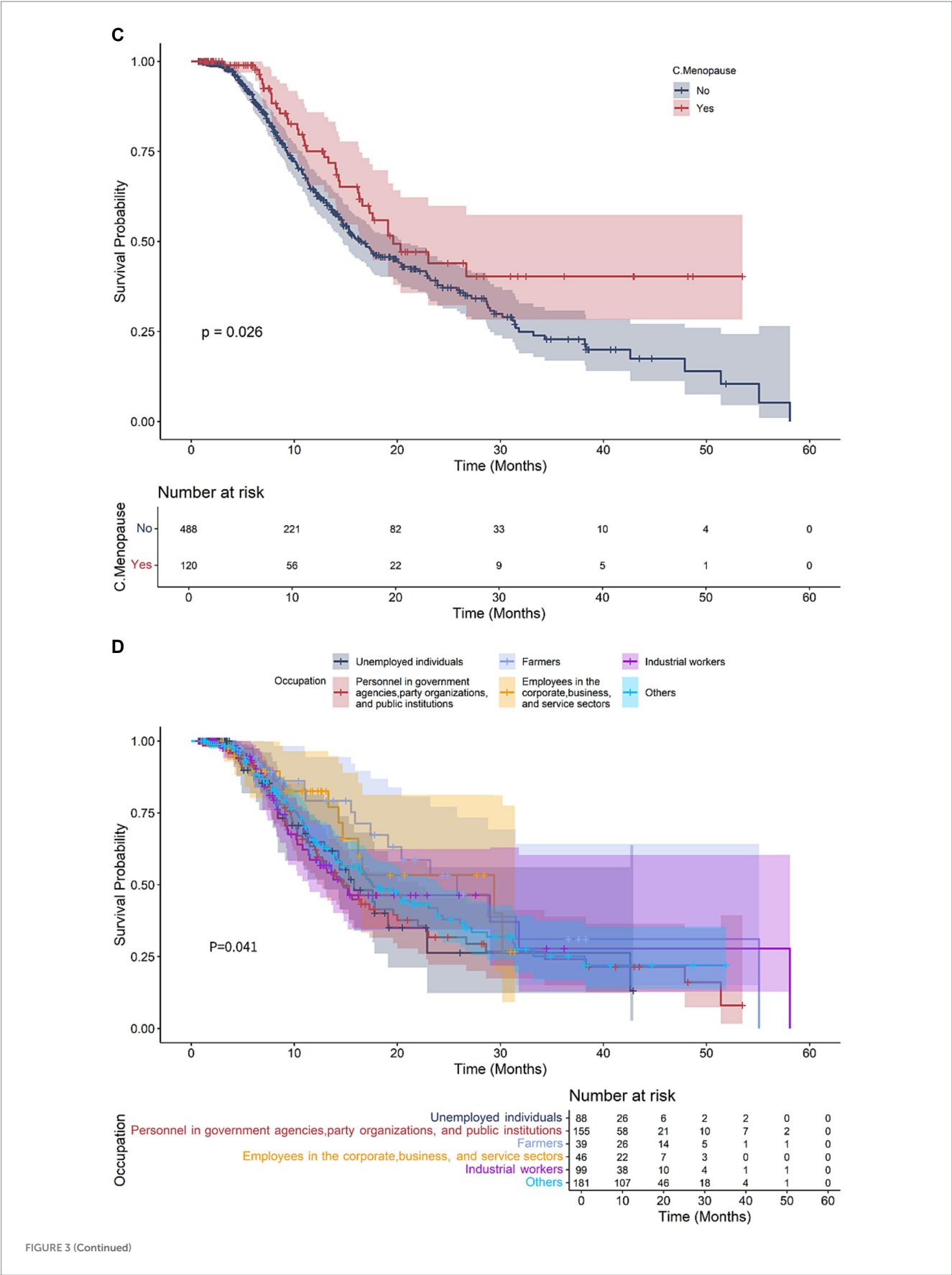
autoimmunity within 2–3 years without progressing to persistent infection.

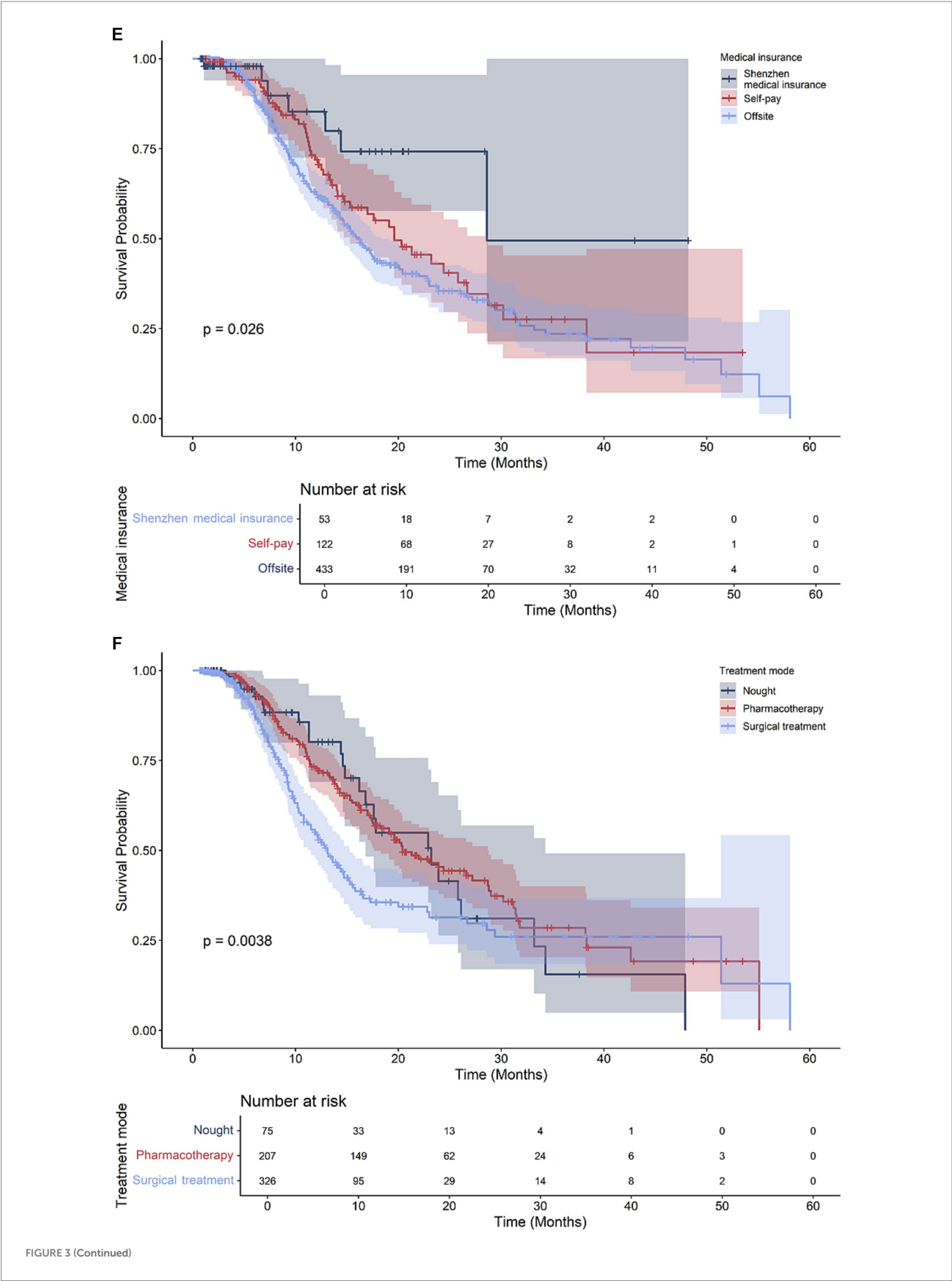
This study's follow-up data further highlight the complexity of HPV clearance. Among 608 HPV-infected women, the HPV clearance rate was 38.98%, with a median clearance time of 8.95 months, which is slightly longer than that reported in similar studies, potentially due to differences in study subject characteristics and follow-up methods. Previous studies have reported varying clearance rates and times. For example, Song et al. (29) reported a 68.9% clearance rate within 6 months among 173 cervical cancer patients in Beijing, China. Abudurexiti et al. (10) reported a median clearance time of 10.4 months among 97 individuals diagnosed with high-grade squamous intraepithelial lesions (HSIL) as well as 437 individuals with early-stage invasion (CC, Stage IA-IIA) in Xinjiang. Although the median clearance time of patients with HSIL was longer ($p < 0.05$), no significant differences were found between the HSIL grades, CC stages or age groups ($p > 0.05$). Bogani et al. (30), in a multi-center study of 3,212 women, reported that CIN3 patients had a significantly higher persistent HPV infection rate than CIN2 patients did (OR: 3.80, 95% CI 2.01–7.21). A systematic review and meta-analysis (31) revealed an increased risk of CIN1 progression due to persistent HR-HPV infection. However, Arthur et al. (32) reported no significant difference in the duration of HPV infection across different degrees of

cervical lesions. Similarly, while the univariate analysis in this study revealed an association between cervical lesion degree and HPV clearance, the multivariate analysis did not confirm this association, potentially because of differences in population selection, study design, and detection methods. The relatively low HPV clearance rate observed in this study may be attributed to natural differences in clearance rates among study populations, low patient follow-up rates, and small sample sizes. Future studies should continue to follow this cohort and collect additional data to draw more comprehensive conclusions.

Previous research has consistently identified age as a crucial factor influencing both the acquisition and outcome of HPV infection. This study's findings support this view, demonstrating that HPV-infected women aged younger than 35 years presented the highest rate of negative conversion (46.6%), whereas those over 50 years of age presented the lowest rate (28.41%). Similar findings have been reported by Li et al. (33), who revealed that advanced age was associated with a greater likelihood of persistent HPV infection (OR = 2.73, 95% CI = 1.07–6.93) in a one-year follow-up study of 152 baseline HPV-positive women in Daqing city. Feng et al. (34) research also revealed that age is an independent variable that influences the natural resolution of high-risk HPV (HR-HPV) infections among women with normal or low-grade cytology, with clearance rates







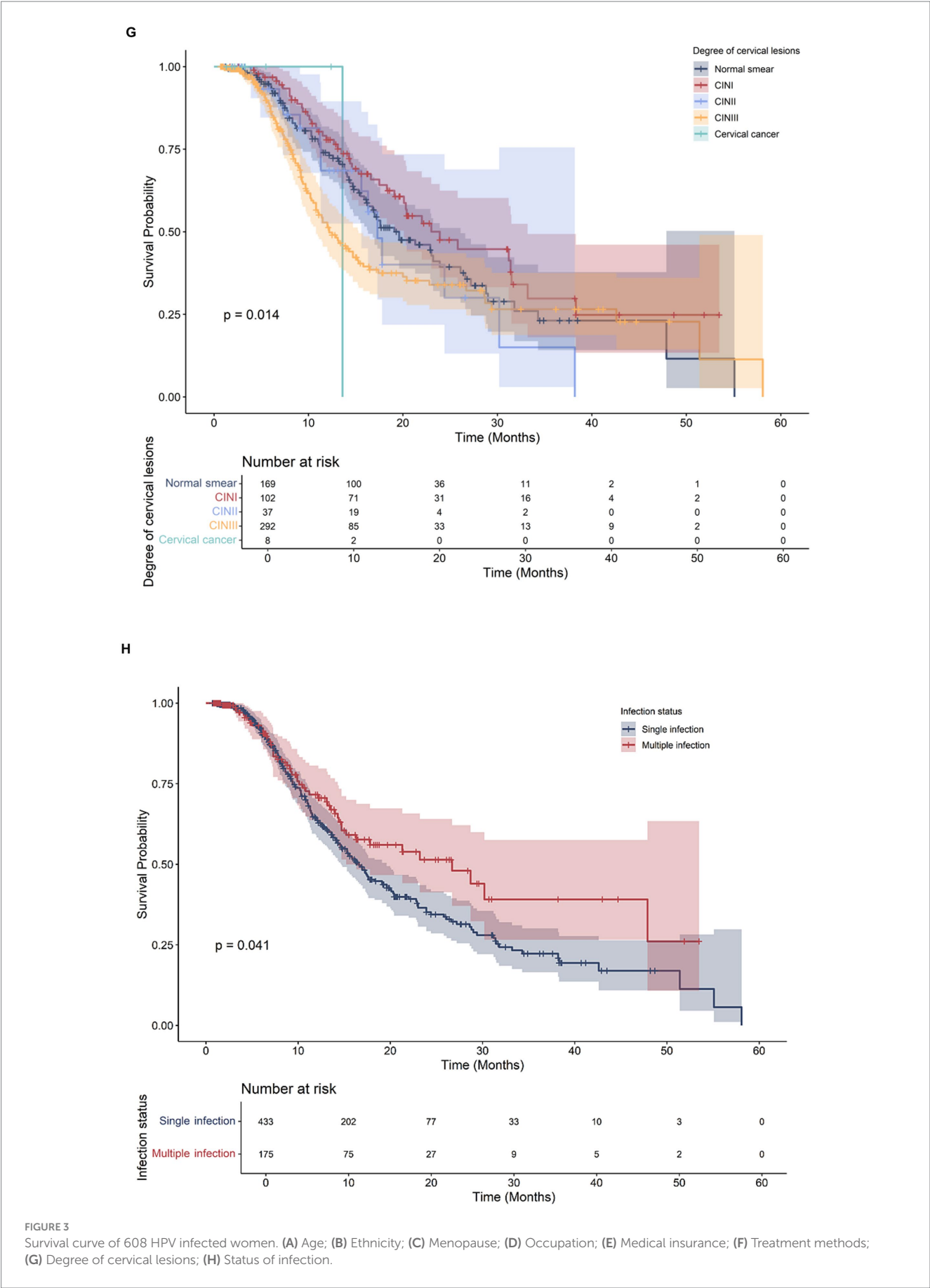


TABLE 4 608 cases of HPV infection female outcome of COX multivariate analysis.

Variable	HR	95% CI	p
Age			<0.01
35–50		Reference	
<35	1.71	1.28 ~ 2.28	
>50	1.34	0.69 ~ 2.60	0.3914
Ethnic group	1.42	0.65 ~ 3.13	0.3786
Occupation	0.99	0.92 ~ 1.06	0.6898
Type of medical insurance	0.74	0.56 ~ 0.98	<0.05
Menopause	0.86	0.48 ~ 1.54	0.6145
Infection status (single/multiple)	0.68	0.49 ~ 0.93	<0.05
Degree of cervical lesion	1.09	0.94 ~ 1.26	0.2653
Treatment mode	1.25	0.93 ~ 1.69	0.1389

generally being lower in older individuals. However, conflicting results have also been reported. Pan et al. (35) found that age was an independent risk factor for persistent HPV infection; however, paradoxically, HPV conversion rates were lower in women over 45 years of age than in other age groups. These discrepancies may be attributed to variations in study design, follow-up duration, age distribution, sample size, individual immune levels, hormone levels, and other factors.

Our study included 17 variables, of which 8 factors showed statistical significance in univariate analysis. In the multivariate analysis, three variables (age, medical insurance type, and infection status) were found to be associated with HPV clearance, while five variables (ethnicity, occupation, menopausal status, cervical lesion severity, and treatment method) did not show a significant relationship with HPV negativity. However, previous studies have indicated that factors such as ethnicity, occupation, menopausal status, cervical lesion severity, and treatment methods may influence HPV clearance outcomes. It is worth noting that this study was conducted in an area predominantly inhabited by Han people. In contrast, ethnic differences may be more pronounced in regions with a larger presence of minority populations. The lack of a significant association between occupation and HPV clearance may be attributed to the diverse occupations of patients in the surrounding area. In Shenzhen, a region characterized by low aging, high education levels, and strong treatment adherence among postmenopausal women, these factors could contribute to the limited differences observed in our study. Additionally, most of the patients in this study were of adult and pre-menopausal age, which may explain the lack of variation in menopausal status, cervical lesion severity, and treatment outcomes. Nevertheless, differences in regional characteristics and research methodologies may explain some of the discrepancies between our findings and those of other studies.

The age distribution of patients with HPV infection is bimodal, as demonstrated by several studies. A multi-center cross-sectional study in China (36) revealed that HR-HPV infection rates exhibit two peaks, during adolescence and the perimenopausal period, with the highest prevalence observed among women aged 15–24 and 40–45. The secondary peak may be attributed to increased HPV exposure resulting from aging and alterations in the internal environment and immune system. Furthermore, HPV infection rates in urban and rural areas display distinct trends with age (37, 38). A three-year follow-up

study on HR-HPV infection and the risk of cervical lesions in women with normal baseline cytology revealed that the HR-HPV negative conversion rate was consistently higher in women aged younger than 45 years than in those aged older than 45 years (17). These findings indicate that age significantly influences the natural history of HPV infection. This pattern potentially correlates with the decline in immune function attributed to aging, which may decrease the efficacy of the body in eliminating recent HPV infections or suppressing latent HPV. However, the relationship between age and HPV conversion requires confirmation in larger samples. Given that middle-aged and older adult women are more likely to experience persistent HPV infection, strengthening clinical follow-up and encouraging regular return visits are recommended to increase monitoring and intervention effectiveness.

Currently, the application of more perfect cervical cancer screening strategies and the results of HPV tests with higher analytical sensitivity in cervical cancer screening have focused on the detection of multiple HPV infections. Studies have shown that the incidence of HPV multiple infections is higher than expected, accounting for about 20–50% of HPV-positive patients (39). At present, research on multiple HPV infections has focused on two main aspects: the interaction of different types of HPV in multiple infections and its influence on persistent infection, and the impact of multiple infections on the risk of future cervical diseases. The results of this study revealed that HPV infection was dominated by a single infection (71.22%), whereas Chen et al. (40) reported that the HPV infection rates and subtype distributions of 326,824 cervical cancer screening and health examination subjects in Hunan Province were essentially the same, with a single infection accounting for 73.18% of the total. Furthermore, the research indicated that women with a solitary infection presented a negative conversion rate of 43.19%, markedly surpassing the 28.57% reported in those with multiple infections. However, the study failed to find a significant association between multiple infections and the risk of cervical lesions.

Multiple studies have shown that, compared with a single HPV infection, multiple HPV infections significantly increase the risk of cervical lesions. A study in South Korea (41) revealed that women with multiple HPV infections were more likely to develop persistent HPV infection than women with a single HPV infection and were more strongly associated with high-grade cervical lesions. Brot et al.

(42) reported that multiple HR-HPV infections are associated with ongoing low-grade squamous intraepithelial disease (LSIL). In contrast, Bruno et al.'s research (43) indicated that the risk of single HPV infection with cervical squamous cell carcinoma (SCC) is greater than that of multiple infections, which are associated mainly with early cervical lesions (CIN1–CIN2). Luo et al. (44) reported that the associations between multiple HPV infections and high-grade squamous intraepithelial disease (HSIL) and LSIL were stronger in 20,059 women undergoing physical examination in Chongqing, China, and certain types of combinations may synergistically increase the risk of HSIL and LSIL. The findings of these studies may be attributed to the independent pathogenicity theory of HPV (15, 45), where multiple infections accumulate individual risks without interactions between HPV types. Conversely, synergies between HPV subtypes could link multiple infections to persistent infections, leading to more severe cervical lesions.

Although some studies suggest that multiple infections increase the risk of cervical lesions, others find no significant effect. Zhong et al. (46) analysis of 7,036 cases from 2018 to 2022 revealed no association between multiple HR-HPV infections and an increased risk of CIN2 development. Another study showed (47) that the combination of HPV16 with other genotypes reduced its pathogenicity, potentially because of competitive interference rather than synergistic or cumulative carcinogenesis at the phylogenetic level. Multiple infections may stimulate a rapid immune response, shortening the HPV clearance time and improving clearance rates (48, 49).

A Chinese study suggests potential interactions between HPV vaccine types and non-vaccine HPV types, with possible type replacement occurring following vaccination (15). Some studies propose that antagonistic interactions may exist between different HPV genotypes in cases of multiple infections (39, 45, 50). Conversely, other research indicates that multiple infections might exhibit synergistic effects. For instance, certain combinations of HPV genotypes accelerate the virus's transcription, while others inhibit it. As a result, the relationships between HPV types in multiple infections are complex and variable. We hypothesize that the genotypes present in multiple infections may involve: (1) a persistent genotype, (2) a new genotype that initially antagonizes the pre-existing one, or (3) a cross-infection between the previous and newly acquired genotypes (51).

In this research, the infection rates of HPV16 and HPV52 were the highest, at 27.632 and 27.467%, respectively, making them the most prevalent types. In terms of clearance rates, HPV6, HPV40, and HPV54 exhibited relatively high negative conversion rates of 62.5, 66.7, and 66.7%, respectively, indicating favorable clearance outcomes. In contrast, HPV35 had the lowest negative conversion rate, at only 18.75%, suggesting that the likelihood of clearance for this type is relatively low. Studies (52) have revealed that the clearance times of different HPV types vary, with high-risk (HR) HPV types, such as HPV16 and HPV18, generally having longer clearance times than low-risk (LR) types. This extended clearance time makes HR-HPVs more dangerous. In cases of multiple infections, the combination of different HPV types results in varying clearance times among individuals, compared to single infections. Currently, both preventive and therapeutic HPV vaccines target specific HPV types. While some HPV types may be cleared more quickly in individuals with multiple infections, other types remain active, potentially promoting further infections. Therefore, more research is needed to understand the

dynamics of multiple infection types and their relationship with HPV clearance time. Future studies should explore the activation and elimination mechanisms of different genotype combinations, the effects of various treatments on HPV clearance, and the identification of variant lineages through genetic sequencing.

China's rapid economic development and urbanization have prompted a surge in cross-city population mobility, particularly between major cities such as Beijing, Shanghai, Guangzhou, and Shenzhen and neighboring provinces (53–55). The hukou system, implemented in the 1950s, restricts geographical mobility and defines access to employment, housing, social welfare, and education. Internal migrants (IMs), individuals relocating from their domicile cities, often face difficulties using medical insurance across regions because of differences in coverage between their home cities and current residences (54, 56, 57). Although the government has issued relevant policies to simplify the process of using cross-regional medical insurance, IMs still face some problems in practical use, such as difficult reimbursement, cumbersome procedures and insufficient coverage of community medical services (58, 59). These restrictions often cause IMs to rely on cross-provincial medical insurance settlements or self-funded medical treatment, which increases the economic burden and further affects medical decision-making.

The multivariable analysis conducted in this study revealed a substantial correlation between the type of health insurance and HPV infection outcomes. A cross-sectional study conducted by Holt et al. (55) among women who were either migrants or non-migrants aged 21–65 from seven Chinese provinces revealed that employer-provided medical insurance enhances cervical cancer screening uptake among the migrant female population in China. Furthermore, the scope and type of medical insurance significantly influence diagnostic and treatment investments, as well as ultimate medical decisions (60). Research by Zhao et al. (61) in 10 districts in Shenzhen revealed that patients with low-level medical insurance tend to opt for community health service centers for graded diagnosis and treatment. A study at a community health service center in Shenzhen's Bao'an (62) district revealed that medically insured women were more likely to register with family doctors and actively participate in community-based cervical cancer screening and follow-up programs. These findings align with international research on migrant workers (63). Improving the medical insurance system, particularly for migrant workers, is crucial for enhancing their disease prevention and diagnosis participation. Such reforms are expected to alleviate the national economic burden while increasing the negative conversion rate of HPV infection and improving overall migrant population health.

Access to healthcare across different regions is influenced by a range of factors, including health, social, and economic conditions. Consequently, the conclusions drawn in this study regarding the relationship between medical insurance and HPV clearance time should be viewed with caution, as our research does not establish a clear causal link between the two. While the reasons for population movement differ between European countries and cities within China, migrants face similar challenges in accessing healthcare: disparities in access to primary care at various levels of the healthcare system. Even in countries with well-established healthcare systems, immigrants often encounter significant barriers to obtaining health services. Studies from Italy, Norway, and Denmark (64–66) have shown that immigrant participation in cervical screening is lower than that of

local populations. However, some research in China (55) suggests that, unlike local residents, the participation of migrant women in cervical cancer screening is not directly linked to their medical insurance status. These women's families often purchase private insurance for them, allowing them to afford preventive screenings and treatment. It is therefore speculated that the economic situation of migrant families, along with mutual support among family members, may mitigate the impact of limited access to medical insurance, potentially enhancing HPV clearance rates.

There is a notable gap in preventive healthcare access between immigrants and local populations. To encourage greater participation in disease management, it is crucial for health authorities to implement policies that reduce barriers to HPV treatment among migrant populations. Healthcare services should develop more tailored and inclusive programs, particularly for rural migrants living temporarily in cities. Long-term, evidence-based prevention programs that are regularly evaluated are essential for migrants. Furthermore, leveraging Shenzhen's institutional strengths, with hospitals serving as central hubs and community health services as a foundation, public health policymakers could prioritize HPV screening and vaccination programs for the most vulnerable groups in the area. This would help alleviate the healthcare burden on both hospitals and patients (67, 68). It is also recommended that government agencies and healthcare institutions intensify public awareness, education, and training for both the migrant population and healthcare providers, focusing on HPV treatment and cervical cancer prevention.

Studies have also shown (69) that the prevalence of HPV infection increases in women over the age of 50, as immune system effectiveness declines with age. This decline in immunity makes older women more vulnerable to persistent HPV infections or reactivation of latent viruses. HPV vaccination is believed to play a positive role in promoting HPV clearance. While our study did not collect data on HPV vaccination status in women, research from various countries indicates that preventive vaccination is effective in facilitating HPV clearance. One study demonstrated that HPV vaccination significantly reduced persistent HPV infections and associated cervical intraepithelial neoplasia (CIN) in women aged 18 to 25 years (70). Other studies have also highlighted the benefits of vaccination for women aged 40–45 years and beyond, suggesting that vaccination can still protect against HPV infections and related conditions, such as cervical cancer, in older women. Although our study did not include young girls (14–18 years old), several studies suggest that completing the HPV vaccination program before puberty offers significant benefits. Vaccination is particularly important for young women to undergo before their first sexual activity (< 30 years of age). Furthermore, women over 50 should be regularly screened for cervical cancer (71–73).

Although our study does not explore lifestyle factors, existing research suggests that lifestyle may influence HPV clearance. Specifically, similar lifestyle patterns before and after HPV infection may provide common transmission routes and risk factors for persistent or cross infections. HPV is primarily transmitted through sexual contact. Due to traditional views, the number of men who proactively undergo HPV screening remains low, and those without symptoms are often reluctant to seek treatment. As a result, even if women are treated for HPV, continued sexual activity with untreated male partners increases the risk of persistent infection. Furthermore,

a study on the quality of life for women with pre- and postmenopausal HPV infections (74) found that postmenopausal women with HPV reported a lower quality of life, particularly those experiencing sexual disorders. These changes were linked to mental health issues, with the dual pressures of recovery and marital sexual demands contributing to psychological strain, which may hinder the process of HPV clearance.

With respect to contraceptive methods, condom use was reported by less than 20% of the 608 patients; thus, these patients were excluded from the analysis. The relationship between intrauterine device (IUD) use and persistent HPV infection remains debated (75). This study revealed a significantly lower HPV conversion rate among IUD users than among nonusers. However, multifactorial analysis revealed no significant correlation, precluding a definitive conclusion about the impact of the IUD on HPV infection outcomes. Further investigation is warranted to elucidate the potential link between the use of IUDs and the clearance of HPV infections.

5 Summary

This retrospective study analyzed 608 women with HPV infection from Shenzhen Longgang Central Hospital. The results revealed that a greater proportion of women aged 35–50 years at initial diagnosis had a single HPV infection and high-risk HPV infection. Multiple factor analyses revealed that age, type of medical insurance, and type of infection were independent factors influencing the conversion rates of HPV-negative patients. These findings underscore the importance of early screening, detection, and diagnosis of cervical cancer at the grassroots level. Increasing public awareness of cervical cancer screening, issuing timely health risk warnings to high-risk female groups, and ensuring thorough follow-up of HPV-infected patients are crucial measures. The coordinated development of medical and preventive measures is essential to mitigate the burden of HPV-associated cervical diseases in China. Effective implementation of these strategies will be critical in reducing the disease burden in the future.

This study is still inadequate. Owing to the limitations of a single-center retrospective study, the results can only represent the outcomes of HPV-infected women in Shenzhen but can provide new insights for the follow-up of HPV prognosis in other first-tier cities. Data on sexual behavior and HPV vaccination status were not gathered in this research, thereby limiting the ability to assess the impact of these significant factors on HPV outcomes. The degree of cervical lesions considered among the 17 variables in this study was time dependent, but the effect of baseline conditions on HPV infection outcome was analyzed only in this study, which may have skewed the results. In addition, there have been previous studies on menopausal status, lesion degree, and treatment. Although the single-factor analysis of this study revealed correlations, the multifactor analysis results revealed that multiple factors were not independent influencing factors, which was considered to be related to the uneven distribution of the population with relevant characteristics included in this study. In the future, high-quality, large-sample prospective studies should be carried out, the quality of life and anxiety and depression scale scores should be increased, and the diagnosis and follow-up plans for HPV-infected patients should be improved for clinical reference.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Research Ethics Committee of Longgang Central Hospital of Shenzhen. 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. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

ZheL: Conceptualization, Data curation, Formal analysis, Visualization, Writing – original draft, Writing – review & editing. XH: Writing – review & editing, Conceptualization, Methodology, Project administration, Resources, Supervision. ZhiL: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. YuY: Data curation, Formal analysis, Writing – original draft, Writing – review & editing. XZ: Data curation, Formal analysis, Writing – review & editing. CT: Data curation, Investigation, Writing – review & editing. YiY: Investigation, Writing – review & editing. YH: Investigation, Writing – review & editing. LY: Investigation, Writing – review & editing. HC: Investigation,

Writing – review & editing. YT: Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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Conflict of interest

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

Generative AI statement

The author(s) declare that no Gen AI was used in the creation of this manuscript.

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Human papillomavirus vaccination hesitancy among young girls in Ethiopia: factors and barriers to uptake

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Background: Human papillomavirus (HPV) vaccinations protect against HPV infections. The infection might lead to vaginal cancer, vulvar cancer, genital warts, cervical intraepithelial neoplasia lesions, and cervical cancer. This study assessed hesitancy of HPV vaccination, associated factors, and barriers to vaccination among youth girls in Ethiopia.

Methods: An institutional-based cross-sectional study was conducted among female undergraduate students at the University of Gondar, College of Medicine and Health Sciences, between July and August 2022. The data was collected using a self-administered questionnaire. A simple random sampling method was used to recruit participants. The data were entered and analyzed with SPSS version 26. Descriptive statistics were used to describe the participants' demographic characteristics. Logistic regression was performed to identify the significant factors associated with acceptance of the HPV vaccine. A p -value <0.05 was considered statistically significant.

Results: The study included 423 participants with a mean age of 22.5 ± 6.7 years. Only more than one-third (35.2, 95% CI: 27.2–44.1) received the HPV vaccine. Currently, more than one-fourth (27.9, 95% CI: 21.4–33.8) of participants are hesitant to receive the HPV vaccine. Higher monthly income (AOR = 1.52, 95% CI: 1.08–6.34), good knowledge of the HPV vaccine (AOR = 2.12, 95% CI: 1.12–4.87), and a positive attitude towards the vaccine (AOR = 3.03, 95% CI: 1.63–9.56) were significantly associated with acceptance of HPV vaccination. Safety concerns (63.1%), misinformation (42.8%), and parental concerns (42.3%) about the HPV vaccine were among the top perceived reported barriers to receiving the HPV vaccine.

Conclusion: This result showed that more than a quarter number of youth girls are still hesitant to receive HPV vaccinations. To increase vaccination acceptance, interventions should focus on awareness-raising programs about HPV infection and vaccines and addressing safety and parental concerns.

KEYWORDS

Human papillomavirus, HPV vaccine, vaccine hesitancy, vaccination acceptance, knowledge, attitude, young girls, Ethiopia

Introduction

Human papillomavirus (HPV) is a common sexually transmitted infection (STI) that can cause a variety of health problems, including cervical cancer, genital warts, and oropharyngeal cancer (1). The World Health Organization (WHO) estimates that approximately 630,000 women worldwide develop cervical cancer each year, resulting in 317,000 deaths. Most of these deaths occur in low- and middle-income countries, including Ethiopia. More than 85% of cases are diagnosed in developing countries (2). In Ethiopia, cervical cancer is the second most common cause of cancer death in women, with an age-specific incidence and mortality rate of 21.5 and 16 deaths per 100,000 females, respectively (3, 4).

The development of HPV vaccines has been a breakthrough in preventing HPV-related diseases (5–7). These vaccines are highly effective in preventing HPV infections and the subsequent development of cancer. The WHO recommends that all girls and young women aged 9–14 years receive two doses of the HPV vaccine as part of routine immunization programs (8). It is also recommended for girls and women aged 13 through 26 who have not yet been vaccinated or completed the vaccine series (9). Older people could be vaccinated, although this strategy is thought to be less effective given that this age group is more likely to have previously been exposed to HPV (10).

However, despite the availability and effectiveness of HPV vaccines, global vaccination rates remain suboptimal, and vaccination rates vary widely across different regions and countries (11). In some countries, vaccination rates are high, while in others they are low. Several factors contribute to vaccination hesitancy, including a lack of awareness about HPV and its consequences, concerns about vaccine safety, and cultural and religious barriers (12–15).

In Ethiopia, the HPV vaccination program is a relatively recent introduction and was introduced in December 2019 as part of the national immunization schedule for all 14-year-old girls through a school-based approach and in health centers (16). However, the uptake of the vaccine has been slow, with many girls and young women remaining hesitant to receive it (17–22). This is due to a variety of factors, including limited knowledge about HPV and its consequences (20), and concerns about vaccine safety (19), and cultural and religious beliefs that may discourage vaccination (13, 18).

Despite evidence showing barriers to HPV vaccination acceptance of youth girls in Ethiopia (17, 18, 20, 22, 23), most of the studies focused on knowledge and attitude-related factors and failed to comprehensively address other important potential barriers that influence vaccination acceptance in youth girls such as safety concerns and misconceptions about the HPV vaccine,

parental and peer-related concerns, and cultural and religious factors. This study aims to assess hesitancy and associated factors and potential perceived barriers to HPV vaccination. By identifying the barriers to HPV vaccination in Ethiopia, policymakers and healthcare providers can develop targeted interventions to address these challenges and increase HPV vaccination acceptance.

Methods

Study design, setting, and samples

An institutional-based cross-sectional study was employed among female medical and health science students at the University of Gondar (UoG) from 17 July 2022 to 30 August 2022. The UoG has five campuses, such as Atse Tewodros, Atse Fasil, the College of Medicine and Health Sciences (CMHS), Maraki, and Teda campuses. At present, with nine academic offices, it offers about 87 undergraduate and 167 postgraduate programs in regular, distance, extension, and summer programs.

All selected regular undergraduate female students in the College of Medicine and Health Sciences were used as a study population. Participants who were interested in completing the self-administered questionnaire were included in the study and those unable to take part in data collection because of illness or other problems were excluded.

Sample size determination and sampling technique

The sample size was calculated using the single population proportion formula: $n = (Z \alpha/2)^2 \cdot P \cdot (1-P) / D^2$. This is where 1.96 was used for $Z \alpha/2$, 50% for P , and 5% for D because there is no previous study in the study area. Where: D is the margin of error; P is the proportion of an outcome variable; and $Z \alpha/2$ is the 95% confidence interval.

After considering a 10% non-response rate, the final sample size was 424. Study participants were included in the study using a simple random sampling technique.

Study variables

Dependent variable

HPV vaccine hesitancy is the main outcome variable in this study, which is categorized as hesitant and not hesitant.

Independent variables

Independent variables include sociodemographic variables (age, religion, residence, marital status, sex experience, department of current study, year of study, academic performance, economic status, mother's education status, father's education status, family income monthly, family can afford the vaccine, family history of the HPV vaccine), knowledge, and attitudes of HPV infection and vaccinations.

Abbreviations: CDC, Communicable Disease Control; HPV, Human papillomavirus; WHO, World Health Organization.

Data collection instruments and procedure

Data were collected using a self-administered structured questionnaire adopted after reviewing previous literature on the topic (18, 22–25). Two data collectors with previous experience in data collection were recruited. The questionnaire consisted of sociodemographic characteristics and questions that were used to assess participants' hesitancy of HPV vaccinations; knowledge; attitudes toward cervical cancer, HPV infection, and HPV vaccination, and potential perceived barriers to HPV vaccination (Supplementary file 1).

Hesitancy to receive the HPV vaccine

Vaccine hesitancy is a delay or refusal to accept vaccines, even when they are readily available. The hesitancy of the HPV vaccination was determined directly from the respondents' responses, which asked for their willingness to accept the HPV vaccine (18). This item consisted of three questions related to previous vaccination status, current acceptance behavior, and their recommendation for families to receive HPV vaccines with a response of yes and no. The item's internal reliability was assessed using a Kuder–Richardson Formula 20 (KR-20) and found to be in an acceptable range of 0.74.

Knowledge of HPV and HPV vaccine

The knowledge of participants was assessed using a total of 15 questions of knowledge-related questions, specifically related to cervical cancer, HPV infection, and HPV vaccine. Participants are expected to respond Yes (score = 1) and No (score = 0) with a maximum possible score of 15. There were five questions for assessing knowledge of cervical cancer, five questions for knowledge of HPV infection, and five questions for the knowledge of HPV vaccine. The total score was calculated and those with mean and below mean scores were considered as having poor overall knowledge, while those with the above mean score were displayed as having good overall knowledge (23, 24). A KR-20 score of the items was found to be 0.71 and showed good internal reliability of the constructed items.

Attitude of HPV and HPV vaccine

Attitude towards HPV vaccination was measured using seven items that assessed attitudes of participants related to cervical cancer, HPV infection, and HPV vaccines on a five-point Likert scale: one for strongly disagreeing to five for strongly agree. Attitude scores for each participant then were calculated and attitudes towards HPV ranged from 1 to 7. Participants' attitude was categorized as follows: negative if they obtained a score of less than the mean score and positive for those who obtained more than the mean score of the total attitude score (23). The internal reliability of the constructed items found an acceptable score of a Cronbach alpha value of 0.81.

Data quality control

The quality of the data collection instrument was maintained. The instrument was assessed for content and face validity with expertise. It was also pretested using a 5% sample to ensure the accuracy of the responses, the clarity of the questionnaire, and applicability. The data collectors received one-day training about the purposes of the study and the ethical aspects of data collection. The collected data was checked for clarity, consistency, and cleanliness throughout the collection period.

Data entry and statistical analysis

The data were coded and entered using EpiData version 4.6 and analyzed with SPSS version 26 statistical packages. Shapiro-Willick test ($p > 0.05$), histogram, and Q-Q plots were used to assess the normality of continuous variables. Descriptive data statistics were used to describe the demographic characteristics of the participants, and the results were presented through text, tables, figures, and graphs. Multivariable logistic regression was performed to identify associations between other sociodemographic variables and knowledge and HPV vaccination acceptance among study participants.

Results

Sociodemographic characteristics of study participants

A total of 423 participants were included in the current study with a 99.9% response. The mean age of the participants was 22.5 ± 6.7 years. Most students reported to have academic performance (60.8%) and economic status (60.3%). Approximately three-fourths (74%) of the participants reported a personal history of sexual intercourse. Around one-third (30%) of the participants' families were vaccinated with the HPV vaccine (Table 1).

Participants' level of hesitancy of the HPV vaccine

Of the 423 participants, only 149 (35.2, 95% CI: 27.2–44.1) received the HPV vaccine before the survey. Currently, more than one-fourth (27.9, 95% CI: 21.4–33.8) of participants were hesitant to receive the HPV vaccine. About two-fifths (38.5, 95% CI: 31.4–44.9) of respondents were not confidently informing and recommending their family members and relatives to get the vaccines (Table 2) and (Figure 1).

Associated factors of HPV vaccination acceptance

Multivariable logistic regression analysis showed that vaccine acceptability was associated with the participants' monthly income, attitude, and knowledge of the HPV vaccine. HPV vaccine acceptance was significantly associated with higher monthly income (AOR = 1.52, 95% CI: 1.08–6.34), good knowledge of participants about cervical cancer, HPV infection, and HPV vaccine (AOR = 2.12, 95% CI: 1.12–4.87), and a positive attitude towards HPV vaccine (AOR = 3.03, 95% CI: 1.63–9.56) (Table 3).

Perceived barriers to the HPV vaccination

Less than two-thirds of the participants (63.1%) revealed that safety concern was the main barrier to receiving the HPV vaccine, followed by misconceptions about the HPV vaccine (42.8%) and parental concerns about the HPV vaccine (42.3%) (Table 4).

TABLE 1 Sociodemographic characteristics of study participants at the University of Gondar, 2022 (N = 423).

Variable		Frequency	Percent (%)
Age (average age = 22.5 ± 6.7 years)	<20	8	1.9
	20–24	343	81.1
	25–30	72	17
Religion	Orthodox	290	68.6
	Muslim	77	18.2
	Protestant	40	9.5
	Catholics	16	3.8
Residence	Urban	345	81.6
	Rural	78	18.4
Marital status	Marriage	71	16.8
	Single	352	83.2
Sex experience	Yes	110	26
	No	313	74
Department	Medicine	175	41.4
	Physiotherapy	14	3.3
	Anesthesia	11	2.6
	Pharmacy	35	8.3
	Public health	27	6.4
	Environmental and occupational health	26	6.1
	Optometry	20	4.7
	Medical laboratory	9	2.1
	Midwifery	38	9
	Nursing	58	13.7
	Health informatics	10	2.4
Year of study	2nd year	76	18
	3rd year	137	32.4
	4th year	130	30.7
	5th year	58	13.7
	6th year	22	5.2
Academic performance	Excellent	48	11.3
	Good	257	60.8
	Fair	97	22.9
	Below	21	5
Economic status	Good	255	60.3
	Acceptable	101	23.9
	Poor	67	15.8
Mother's education status	No education	103	24.3
	Primary education	138	32.6
	Secondary education	101	23.9
	More than secondary	81	19.1
Father's education status	No education	75	17.7
	Primary education	94	22.2
	Secondary education	118	27.9
	More than secondary	136	32.2

(Continued)

TABLE 1 (Continued)

Variable		Frequency	Percent (%)
Family income monthly	<8,000 birr	149	35.2
	>8,000 birr	274	64.8
Family can afford the vaccine	Yes	287	67.8
	No	136	32.2
Family history of the HPV vaccine	Vaccinated	127	30
	Not vaccinated	296	70

TABLE 2 Participants' level of acceptance of the HPV vaccine (N = 423).

Variable		Frequency	Percent (%)
Received HPV vaccine before this survey.	Yes	149	35.2
	No	274	64.8
Are you currently willing to receive the HPV vaccine if you get the chance of having an HPV vaccine for free?	Yes	305	72.1
	No	118	27.9
Encourage your family members, and relatives, to get a vaccine	Yes	260	61.5
	No	173	38.5

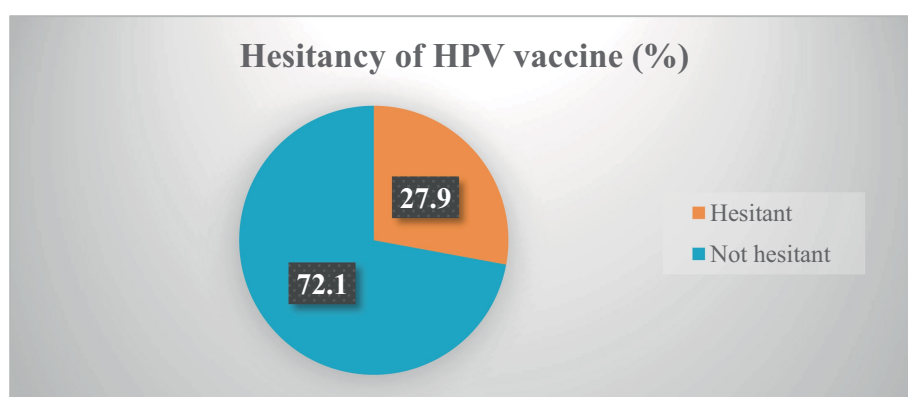
FIGURE 1
Participants' level of hesitancy of the HPV vaccine.

TABLE 3 Factors associated with acceptance of the HPV vaccine.

Variable		HPV vaccine acceptance		95% CI		P-value
		Yes	No	COR	AOR	
Residency	Urban	249	96	1.02 (0.08–3.56)	0.78 (0.05–4.16)	0.357
	Rural	56	22	Reference	-	
Family's monthly income	≥ 8,000	208	66	1.70 (0.14–4.59)	1.52 (1.08–6.34)	0.04*
	< 8,000	97	52	Reference	1	
Family history of the HPV vaccine	Vaccinated	92	35	1.02 (0.05–4.06)	0.67 (0.07–7.45)	0.381
	Not vaccinated	213	83	Reference	-	
Knowledge of cervical cancer, HPV infection, and vaccine	Good	182	70	1.55 (1.52–4.56)	2.12 (1.56–4.87)	< 0.001*
	Poor	123	48	Reference	1	
Attitude on HPV infections and vaccine	Positive	166	64	1.55 (1.52–4.56)	3.03 (1.63–9.56)	< 0.001*
	Negative	139	54	Reference	-	

*indicates *p*-value < 0.05. Bold values: Statistically associated variables.

TABLE 4 Perceived potential barriers to the HPV vaccination (N = 423).

Variable	Categories	Percent (%)	Percent
Potential barriers to the HPV vaccination	Safety concerns	267	63.1
	Misconceptions about the HPV vaccine	181	42.8
	Parental concerns about the HPV vaccine	179	42.3
	Lack of enough information about the HPV vaccine	138	32.6
	Cultural related issues	109	25.8
	Religion-related barriers	86	20.3
	Partners/peers pressure	77	18.2
	Cost and accessibility issues	73	17.3

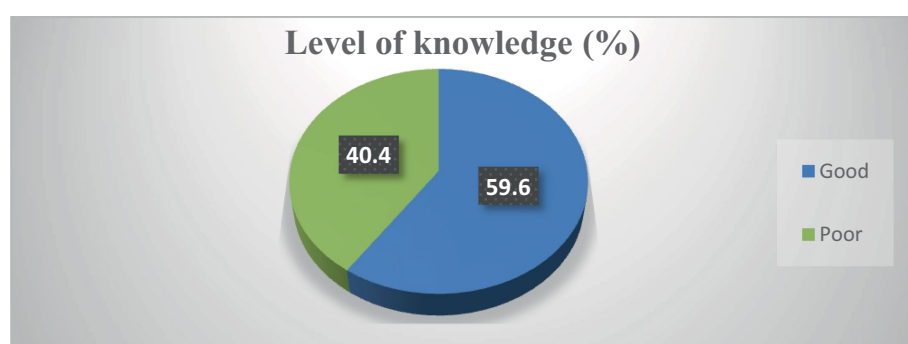


FIGURE 2
Participants' level of knowledge about cervical cancer, HPV infection, and vaccine (N = 423).

Participants' level of knowledge

Most participants heard about cervical cancer (92.9%), HPV infection (93.4%), and HPV vaccine (78%). However, most of them did not know about the risk factors of cervical cancer (87.9), risk factors of HPV infections (62.6%), and the method of prevention of HPV infection (83.9%) ([Supplementary Table S1](#)). Overall, 252 (59.6%) had good knowledge of cervical cancer, HPV infections, and vaccines ([Figure 2](#)).

Participants' level of attitudes

In the current study, more than one-third of the participants (33.5%) agreed or strongly agreed that cervical cancer is a deadly disease. About 97.2% also strongly agreed or agreed that vaccination helps to prevent HPV infection. Moreover, about 93.2% also agreed or strongly agreed that the vaccination was beginning to minimize cervical cancer ([Supplementary Table S2](#)). Overall, most (54.4%) respondents had a positive attitude towards the HPV vaccine ([Figure 3](#)).

Discussion

Vaccination hesitancy has continued to be a significant barrier to youths' HPV vaccination and this study also revealed that more than

one-quarter of youth girls were hesitant to receive HPV vaccines. HPV vaccination acceptance was significantly associated with the family's monthly income, knowledge, and attitude about cervical cancer, HPV infection, and HPV vaccine. This study also reported that safety concerns, misconceptions about the HPV vaccine, parental concerns about the HPV vaccine, lack of enough information about the HPV vaccine, and cultural and religious beliefs were among the top barriers to HPV vaccination of youth girls.

The findings of the current study showed that more than one-fourth of young girls are hesitant to receive the HPV vaccine currently. This figure is much lower than systematic reviews conducted on HPV vaccine uptake among adolescents in Ethiopia ([17, 19, 26](#)). A study conducted in South Africa ([27](#)), China ([28](#)), and other primary studies in Ethiopia ([18, 20–22](#)) also reported higher hesitancy levels than this study. This discrepancy could be due to differences in sociodemographic characteristics and health literacy. The study participants in the current study were health science students who were close to medical information, and they might be more aware of the importance of the HPV vaccine. The participants in China's study were also medical students, however, the methods of hesitancy scoring are different in that this study directly asked respondents about their willingness to vaccine acceptance, while the previous study calculated from composite scores of three components such as complacency, convenience, and confidence ([28](#)). However, the current finding is much higher as compared to other studies reported in China which ranged from 10.3 to 15.5% ([29, 30](#)). This could be because of

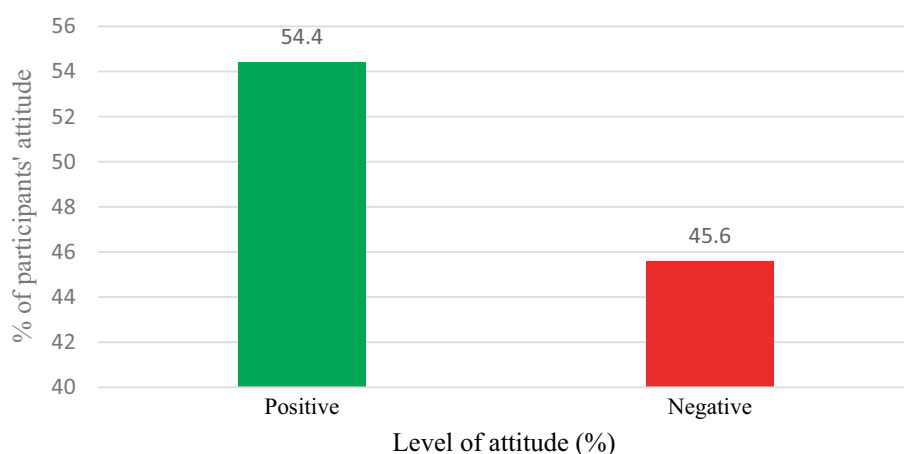


FIGURE 3
Participants' level of attitude towards the HPV and vaccination.

differences in socioeconomic status and access to healthcare and information, which can have an impact or contribute to vaccine acceptance or hesitancy. This finding suggests that vaccine hesitancy continues to be a public challenge and seeking tailored interventions considering contributing factors and potential barriers to vaccination uptake.

Consistent with earlier studies, parental monthly income was associated with acceptance of the HPV vaccine. Those participants with lower monthly incomes were less willing to vaccinate against the HPV vaccine compared to those with higher incomes (31). This justifies that the HPV vaccine can be expensive, especially when multiple doses are required. Lower-income families may struggle to afford this out-of-pocket expense, even if insurance partially covers it. However, in an Ethiopian context, this vaccination service is among the exempted healthcare services aiming to promote health service access. Therefore, public awareness creation could be important regarding the cost of the services.

This study also revealed that the knowledge and attitude of youth girls about cervical cancer, HPV infection, and HPV vaccines were determinant factors for HPV vaccine acceptance. These findings are consistent with other studies conducted in Ethiopia (17, 19, 20, 32) and Jordan (33). Participants with a positive attitude towards the HPV vaccine increased their chances of vaccine uptake by three times. This is consistent with a study conducted in Ethiopia (31) and Thailand (34). This might be because individuals who have a positive attitude towards the vaccine may have a higher intention of receiving the vaccine. Participants who had good knowledge of the HPV vaccine increased their vaccination uptake by about two-fold. This is a consistent study conducted in Nigeria (35) and Uganda (36). The findings highlight the importance of public awareness creation to enhance HPV vaccine acceptance in hesitant youth girls.

In the current study, safety concerns, misconceptions, and lack of enough information about the HPV vaccine were reported as the main barriers to HPV vaccination. This finding is consistent with earlier evidence (14, 15, 37, 38). This could suggest that healthcare providers should address specific concerns about vaccine safety and provide reassurance based on scientific evidence. Moreover, awareness creation campaigns may help dispel myths and provide

evidence-based information about HPV, its risks, and the benefits of vaccination. For instance, awareness creation campaigns focusing on cancer prevention and HPV vaccine knowledge have resulted in reduced misinformation and increased vaccination uptake (39, 40). In addition, vaccination promotion campaigns implemented in schools and integrating HPV vaccination as school routine vaccination increased vaccination acceptance (39, 41). Furthermore, effective eHealth communication messages and delivery methods could have a positive impact on increasing the uptake of HPV vaccinations in adolescents (42).

Parental concerns about the HPV vaccine were among the major barriers to receiving HPV vaccines among youth girls. Pooled evidence meta-analysis study also revealed that parental willingness to vaccinate their children was lower (19, 43–45). This finding highlights the importance of engaging parents and caregivers in the decision-making process to build trust and address their concerns. Clear and effective communication with parents or caregivers can play a crucial role in supporting them as they decide about vaccinating their children (46).

Cultural and religious beliefs were also among the reported potential barriers to HPV vaccination of youth girls. This is in line with previous studies (13, 47, 48). The finding underscores the need for vaccination campaign interventions to address cultural and religious beliefs, emphasizing the safety and effectiveness of the vaccines (49). Furthermore, a detailed exploration of the impacts of cultural and religious beliefs could be conducted using qualitative data.

Strengths and limitations of the study

This study presents a comprehensive finding regarding current vaccination status and their willingness to receive HPV vaccination among university students using a relatively larger sample. However, it has some limitations. Firstly, the study was conducted among only health science students, which is difficult to conclude for all young girls in Ethiopia. Secondly, this study focused only on HPV cancers. Additionally, despite HPV being recommended for males globally the current study accounts only for young girls. Furthermore, the nature

of data collection, a self-administered survey, causes a social disability bias, and the outcome might be overestimated. Finally, the causal relationship might be difficult to estimate using a cross-sectional study. As a result, the authors recommend a prospective follow-up study that incorporates qualitative exploration to understand potential barriers to the vaccination of youths.

Implication of the study

This study has implication highlights for important parties in this area such as healthcare providers, policymakers, and researchers.

Healthcare providers

Healthcare providers should be equipped with accurate and up-to-date information about HPV, its risks, and the benefits of vaccination. They should be able to effectively communicate this information to young girls and their parents, addressing common concerns and misconceptions. Providers should offer personalized counseling to address individual concerns and provide tailored information based on the patient's needs and cultural background. Establishing a trusting relationship with patients is essential. Providers should be empathetic, respectful, and patient in addressing concerns and building trust. Healthcare providers can also play a crucial role in advocating for HPV vaccination policies and programs, both at the local and national levels.

Policymakers

Policymakers should allocate sufficient funding for HPV vaccination programs, including education campaigns, vaccine procurement, and healthcare provider training. While mandating vaccination may be controversial, it can be a powerful tool for increasing coverage. Policymakers should carefully consider the potential benefits and drawbacks of mandatory vaccination. Implementing school-based vaccination programs can make it easier for young girls to receive the vaccine and improve access in underserved communities. Policymakers should also ensure that HPV vaccination programs are equitable and accessible to all young girls, regardless of their socioeconomic status, race, or ethnicity.

Researchers

Further research is needed to better understand the factors contributing to HPV vaccination hesitancy, particularly in diverse populations. This research can inform the development of more effective interventions. Longitudinal studies can track changes in vaccine hesitancy over time and evaluate the effectiveness of different interventions. Qualitative research can provide valuable insights into the perspectives and experiences of young girls, parent concerns, and their decisions regarding HPV vaccination and the potential impacts of cultural and religious beliefs. Researchers should also ensure that their studies are culturally competent and consider the diverse cultural and religious beliefs of different populations. Since HPV also causes other cancers and genital warts, future studies should consider including questions about additional HPV-related diseases. Furthermore, as HPV vaccination is administered to males globally, it would be valuable to include

males in such studies to better inform and target future vaccination efforts.

Conclusion

The current study revealed that more than one-quarter of young girls are hesitant to receive the HPV vaccine, indicating that it remains a persistent challenge in today's context. Interventional programs aiming to increase HPV vaccination in youth girls should focus on promoting awareness among youths and the public about the impact of HPV infection and the safety and importance of the HPV vaccine. Tailored interventions addressing parental concerns, and religious and cultural beliefs are also crucial to enhancing HPV vaccination in youth girls.

Data availability statement

The data supporting the conclusions of this article will be made available by the correspondence author upon reasonable request, without undue reservation.

Ethics statement

The studies involving humans were approved by the ethical review committee of the School of Pharmacy at the University of Gondar with a reference number of SOP/257/2022. 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

AS: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. BBA: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. BAA: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. AT: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. KM: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. MA: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. YT: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. AD: Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing, Data curation. MM: Data curation, Formal analysis, Investigation, Methodology, Software, Validation, Visualization, Writing – review & editing. GB: Conceptualization, Data curation, Formal analysis, Funding acquisition, Investigation, Methodology, Project administration, Resources, Software,

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Conflict of interest

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

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Supplementary material

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Epidemiology and spatiotemporal analysis of acute hemorrhagic conjunctivitis in Zhejiang province, China (2004–2023)

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Background: Acute hemorrhagic conjunctivitis (AHC) has become a significant public health issue in Zhejiang province. However, the analysis of epidemiological characteristics and spatio-temporal patterns of AHC in Zhejiang province has not been studied yet.

Methods: Monthly cases of AHC from 2004 to 2023 reported at the county level in Zhejiang province were obtained from the China Information System for Disease Control and Prevention. Demographic features, yearly county incidence, global spatial autocorrelation, local spatial autocorrelation analyses, and temporal and space–time cluster analysis were performed to identify and visualize the spatiotemporal patterns of AHC cases in Zhejiang province. The epidemiological characteristics of AHC outbreaks in the same period in Zhejiang province were also compared.

Results: A total of 52,119 AHC cases were reported in Zhejiang province, yielding an average incidence rate of 5.37 per 100,000. No fatalities were reported. The average age of those affected was 25.44 ± 4.37 years, with the highest incidence (16.70%) among individuals aged between 10 and 19 years. Seasonal peaks occur from August to October each year. Students and farmers experienced the highest incidence rates of infection. Significant positive spatial correlations for AHC were observed in Zhejiang province in the years 2007 (Moran's $I = 0.095$, $p = 0.039$), 2009 (Moran's $I = 0.075$, $p = 0.031$), and 2011 (Moran's $I = 0.173$, $p = 0.034$), indicating spatial clustering. Spatiotemporal scanning identified two distinct clusters: Cluster 1 and Cluster 2. Cluster 1, characterized by a relative risk of 21.44 ($p < 0.001$), was located in northeastern Zhejiang province, comprising 30 counties, with an active period from 1st September 2010 to 30th September 2010. Compared to low-risk regions, high-risk counties exhibited a different demographic profile with a higher proportion of men, older people, and farmers. Among the affected students during outbreaks, the predominant symptoms were conjunctival congestion, increased eye secretions, eye swelling, eye pain, photophobia and tearing, while the incidence of fever was relatively low.

Conclusion: The results of this study demonstrate the spatiotemporal heterogeneity of AHC cases in Zhejiang province and underscore the necessity for targeted prevention and control measures in high-risk areas to mitigate transmission and occurrence.

KEYWORDS

acute hemorrhagic conjunctivitis, spatiotemporal analysis, epidemiology, clustering, outbreak

Introduction

The pathogens responsible for acute hemorrhagic conjunctivitis (AHC) are diverse, mainly microribonucleic acid viruses, with enterovirus type 70 (EV70) being the most prevalent, followed by Coxsackie virus A group 24 variant (CA24v) and variants of adenovirus types 3, 7, 8, 11, 19, and 37 (1, 2). Patients infected with AHC mainly exhibit symptoms such as eye redness, stinging, increased eye secretion, tearing, and watery discharge (3). AHC is a self-limited eye infectious disease of the eye, with an incubation period generally ranging from 12 h to 48 h and up to a maximum of 6 days (4).

Patients are the main source of infection, with the main mode of transmission being contact transmission through eye-to-hand, object, and water-to-eye contact. Additionally, the virus can be transmitted via the fecal-oral route through contaminated water (5). AHC is noted for its rapid onset and high infectivity. Children aged 0–9 years are most commonly affected (6), often leading to global pandemics and frequent outbreaks in schools (7). Relevant reports indicated that the inflammatory response of conjunctivitis can spread to the cornea and cause a certain degree of visual impairment that may last over months or even years (8).

Since the first occurrence of AHC in Ghana in 1969 (9), it has spread rapidly worldwide. In recent decades, more than 10 million AHC cases have been reported worldwide (10), including several large outbreaks in Asia and South America. The largest outbreak of AHC was reported in South Korea in 2002, with over 1 million cases (11). In 2014, Thailand had an outbreak of AHC, with 300,000 cases over 3 months (12). The first outbreak of AHC in China was reported in Hong Kong in 1971 (13), and then spread to all provinces and cities. In 2022, influenza, other infectious diarrhoeal, hand-foot-mouth disease, epidemic mumps, and acute hepatitis C will be listed as the top five incidence rates of Class C infectious diseases in China (14). The incidence of AHC varies greatly in different regions in mainland China. Guangxi, Guangdong, Anhui, and Hubei are provinces with a larger number of cases from 2013 to 2020 (6). Currently, AHC is still an important public health issue in China, and its epidemic feature is worthy of further study.

In recent years, geographic information systems and spatial statistics have been widely applied to study the distribution characteristics of infectious diseases (15, 16). However, there is limited research on the spatial epidemiological characteristics of AHC across a province. This study analyzed the spatiotemporal aggregation characteristics of AHC incidence in Zhejiang province in the past 20 years (from 2004 to 2023). The results of the epidemiologic and spatiotemporal analysis in this article help provide a basis for targeting public health measures and allocation of health resources in disease prevention and control.

Materials and methods

Study area

Zhejiang Province is located on the southeast coast of China. Zhejiang province has 11 prefecture-level cities, with 90 municipal or county-level cities (including one autonomous county) (17). The epidemiological and spatiotemporal analyses in this study were carried out on the basis of counties.

Data collection

The data on AHC cases and outbreaks in Zhejiang province from January 2004 to December 2023 were obtained from the China Information System for Disease Control and Prevention (CISDCP) and the Management Information System for Public Health Emergencies, respectively. The information included the age, gender, occupation, residential address, date of illness onset, and date of illness diagnosis for the AHC cases.

The cases' communication information, address, and other personal information were not involved in this study. The diagnosis of AHC refers to the health industry standards for the diagnosis of AHC issued by the National Health Commission of the People's Republic of China (WS 217–2008; WS 217–2021) (18). See [Supplementary material](#). The demographical data of Zhejiang province was downloaded from the Zhejiang Statistical Yearbook (<https://tjj.zj.gov.cn/col/col1525563/index.html>). Maps of China and Zhejiang province were obtained from the National Basic Geographic Information System.

Spatial autocorrelation analysis

Spatial autocorrelation analysis is a spatial method used to quantify the spatial autocorrelation association based on the location of cases in different study regions. This analysis is typically divided into “global” and “local” categories. Both global and local Moran's I indices were employed to determine the presence of significant spatial autocorrelation regions for AHC within the study area. Moran's I coefficients, which range from -1 to 1 , were calculated using a Z-test. Values approaching 1 indicate a more significant aggregation of cases, while those approaching -1 suggest a more dispersed distribution. A Moran's I value near 0 indicates a global random distribution. Z and p values were used to evaluate the significance of Moran's I coefficients (19).

Local spatial autocorrelation analysis

In this process, Moran's I for local indication of spatial autocorrelation (LISA) measures the similarity or difference between the incidence of the given county unit and those of surrounding counties. LISA was adopted to identify significant spots into four categories: hotspots (high-high), cold spots (low-low), and outliers (high-low, low-high) of AHC. The significance level of aggregations was determined by the Z score generated by comparison of the local Moran's I data for the average incidence in each county. A high positive Z score represented that the surroundings had spatial clusters (high-high: high-value spatial clusters or low-low: low-value spatial clusters), and a low negative Z score represented the presence of spatial outliers (high-low: high values surrounded with low values or low-high: low values surrounded with high values) (20, 21).

Spatiotemporal cluster analysis

Spatiotemporal aggregation analysis was used to detect the location of possible high-risk space–time clusters for monthly AHC cases from 2004 to 2023 at the county level.

The basic principle is that Kulldorff's space-time scan statistic (SaTScan software) scanned the clusters by the variable-sized circular scanning window around the centroid of each county group (22). The null hypothesis assumed that the incidence's relative risk (RR) was the same within the window compared to outside. The difference in the number of cases inside and outside the scanning window was tested by log-likelihood ratio (LLR). The scanning window with the largest statistically significant LLR value is defined as the primary cluster, and other windows with significant LLR values are considered secondary clusters (23). A statistical test was evaluated using the Monte Carlo hypothesis simulation (set to the default value of 999) to calculate the corresponding *p*-value. The larger the LLR value ($p < 0.05$), the greater the probability that the area contained in the dynamic

window is an aggregation area (24). In this study, according to the county-level AHC cases, demographic data, and geographical data, the maximum spatial cluster size was set to 50% (25) of the population at risk in the spatial window in the process to fit discrete Poisson models (16). The scanning time extended from 1 January 2004 to 31 December 2023, with the time interval set to 'Month' to identify the aggregation of spatiotemporal clusters throughout the study period.

Statistical software

In this study, the R software program (v.4.3.1, <https://www.R-project.org/>) was used in descriptive statistical analysis, including

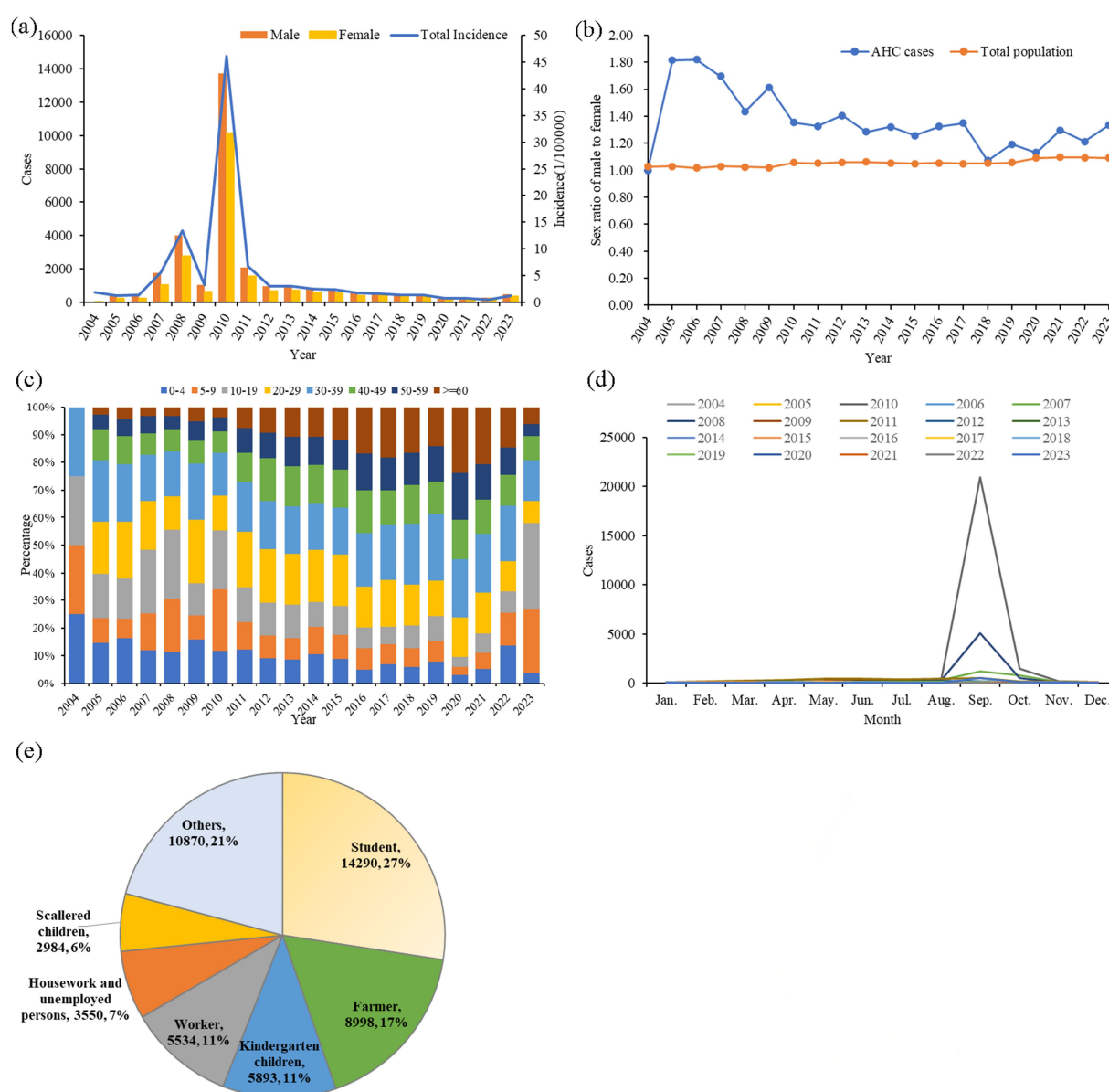
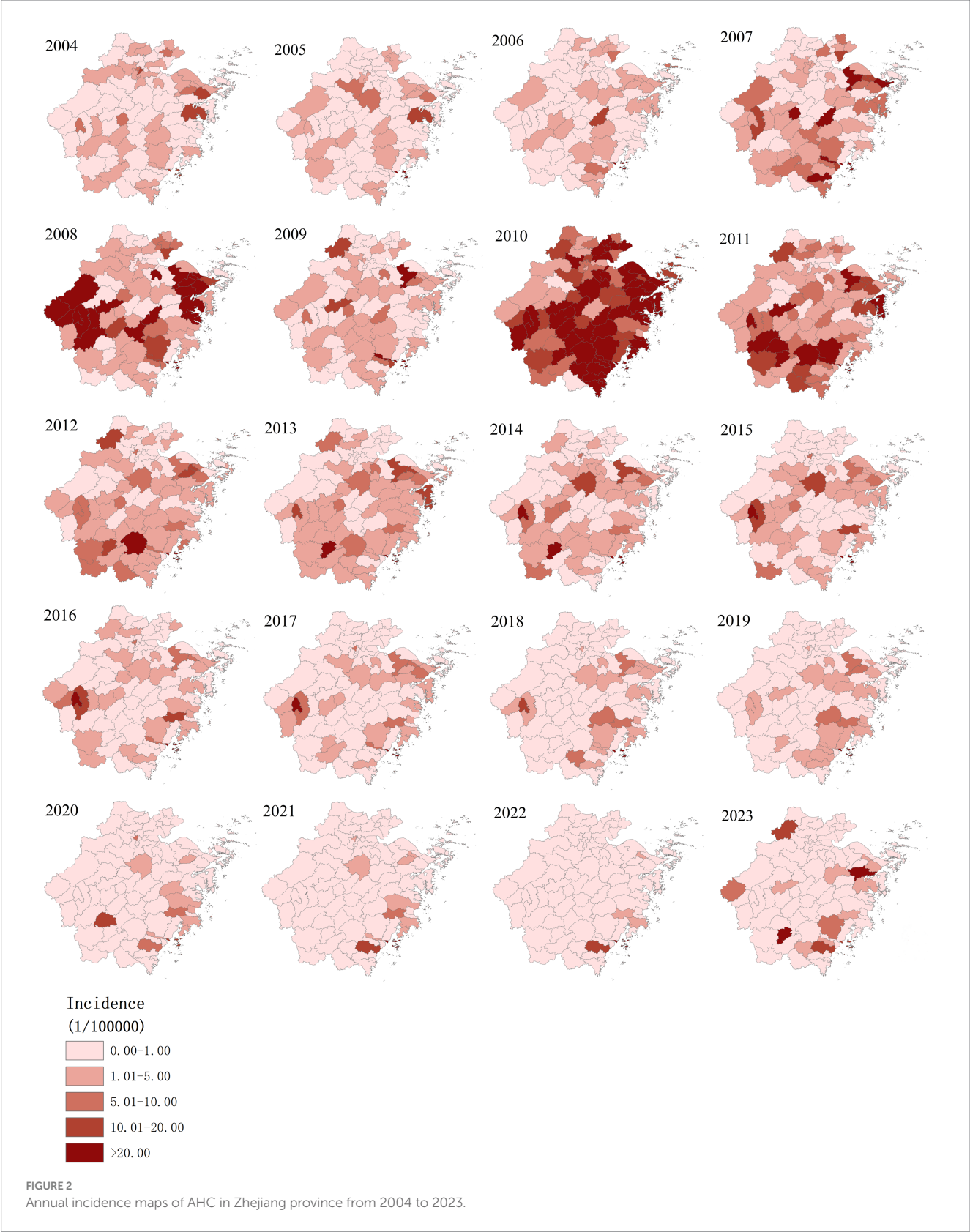


FIGURE 1

Epidemiological characteristics of AHC in Zhejiang province from 2004 to 2023. (A) Annual case numbers of AHC among men and women, along with total annual incidence; (B) annual male-to-female ratios in AHC cases and compared to the total population in Zhejiang province; (C) age distribution of annual AHC cases; (D) monthly distribution of AHC cases; and (E) occupational distribution among AHC cases.

investigating the population distribution, occupational composition, seasonal characteristics, diagnosis features, and outbreak investigation of AHC cases. GeoDa software (version 1.22, Spatial Analysis Laboratory, Urbana, IL, USA) was used for the global and local spatial

autocorrelation analysis. The spatiotemporal clusters were detected by SaTScan software (version 10.1.3 Martin Kulldorff, National Cancer Institute, Bethesda, MD, USA). ArcGIS software (version 10.3 ESRI, Redlands, CA, USA) was used for mapping and visualization analysis.



All results were considered statistically significant when the $p < 0.05$ for both sides.

Results

Epidemiological characteristics

From 2004 to 2023, a total of 52,119 AHC cases were reported in Zhejiang province, with an annual reported incidence of 5.37 per 100,000 (ranging from 0.77 per 100,000 in 2022 to 46.17 per 100,000 in 2010). The highest incidence was observed in 2010 (46.17 per 100,000), with the seasonal peak in September in almost all years (Figures 1A,D). During this study period, there were 30,212 male cases and 21,912 female cases. No fatalities were reported in 20 years. The average gender ratio of male to female is 1.38:1, which is higher than the gender ratio of common residents in Zhejiang Province each year (other than the year 2004) (Figure 1B).

The average age of all study cases was 25.44 ± 4.37 years. The largest age group was 10–19 years old, comprising 18.33% of cases, followed by those aged 30–39 years (16.78%) and 5–9 years (16.70%), as illustrated in Figure 1C. The most common occupations among AHC cases were students (14,290 cases, 27.42%), followed by individuals in other occupations (10,870 cases, 20.85%), farmers (8,998 cases, 17.26%), kindergarten children (5,893 cases, 11.31%), and workers (5,534 cases, 10.62%), as shown in Figure 1E. All cases in this study were clinically diagnosed.

Incidence map

The annualized incidence rate of AHC in Zhejiang province was mapped at the county level, ranging from 0.00 to 654.49 per 100,000 people. The high-incidence counties of AHC have changed over the years. The top 10 counties in terms of annual incidence were Dongtou (654.49 per 100,000), Pingyang (289.89 per 100,000), Yuyao (275.32 per 100,000), Longwan (156.02 per 100,000), Ninghai (142.37 per 100,000), Lucheng (132.64 per 100,000), Yongjia (117.89 per 100,000), Tongxiang (110.80 per 100,000), Wucheng (103.82 per 100,000) and Sanmen (101.95 per 100,000), a majority of the top 10 counties are located in the southeast of Zhejiang Province (Figure 2).

Spatial autocorrelation analyses

Except for 2007 (Moran's $I = 0.095$, $p = 0.039$), 2009 (Moran's $I = 0.075$, $p = 0.031$), and 2011 (Moran's $I = 0.173$, $p = 0.034$), the global spatial autocorrelation analysis of AHC incidence in other years did not show significant global correlation (Table 1). LISA analysis was performed to identify significant hotspots (High-High), cold spots (Low-Low), and outliers (High-Low and Low-High) of AHC cases in Zhejiang province from 2004 to 2023 (Figure 3). A total of 33 hotspots, 131 cold spots, and 82 outliers (63 low-high and 19 high-low clusters) were detected from 2004 to 2023. Hotspots were observed in southern counties such as Lucheng, Ou Hai, and Longwan counties, and the eastern counties

such as Zhenhai, Beilun, Fenghua, Xiangshan, and Ninghai counties. Years of 2005–2006 and 2013–2014 had detected no hotspot. Notably, Lucheng County was found to be a high-high cluster 12 times during the study period (Figure 3).

Temporal and space–time cluster analysis

Using Kulldorff's space–time scan statistic to detect spatiotemporal clusters across the study period, the result of the temporal scan showed that the most likely cluster was distributed in the southeast Zhejiang province and covered 30 counties ($RR = 21.44$, $LRR = 5373.20$, $p < 0.001$), with the time aggregation frame being 1st September 2010 to 30th September 2010 (Table 2). The secondary likely cluster located in the northwest regions of Zhejiang covered 35 counties ($RR = 7.23$, $LRR = 1369.73$, $p < 0.001$), which were recorded from 1st September 2010 to 30th September 2010 (Table 2; Figure 4).

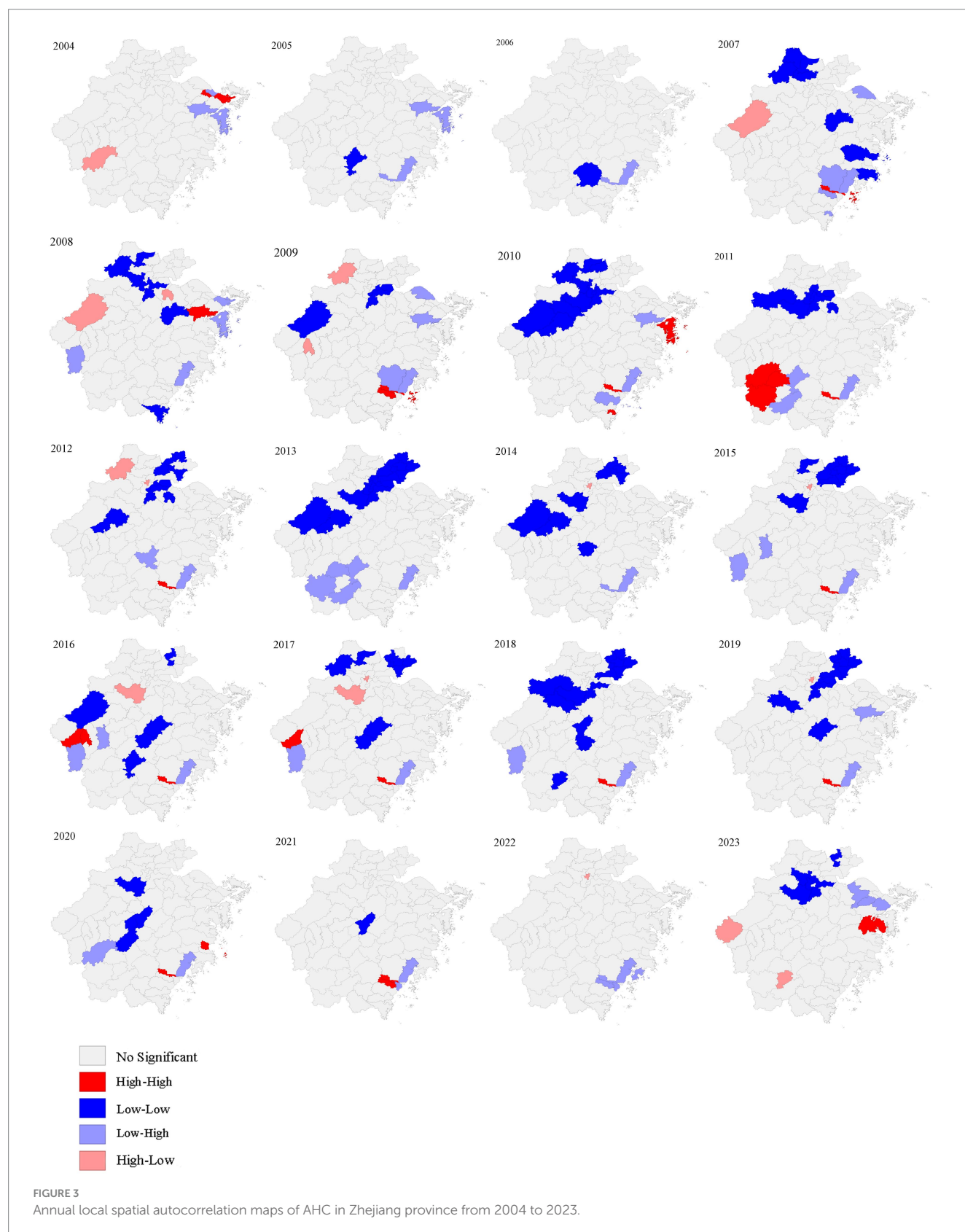
Comparative demographic characteristics within and outside temporal and space–time clusters

The proportion of male AHC in high-risk counties was lower than that in low-risk counties with a statistically significant

TABLE 1 The global spatial autocorrelation of AHC in Zhejiang province from 2004 to 2023.

Year	Moran's I	Z-Score	P-Value	Mean	SD
2004	−0.041	−0.343	0.407	−0.013	0.080
2005	−0.049	−0.749	0.179	−0.010	0.052
2006	−0.013	−0.123	0.451	−0.011	0.019
2007	0.095	2.255	0.039	−0.010	0.047
2008	0.049	0.901	0.161	−0.011	0.067
2009	0.075	2.898	0.031	−0.012	0.030
2010	0.122	1.818	0.052	−0.009	0.072
2011	0.173	2.388	0.034	−0.010	0.076
2012	0.038	0.915	0.178	−0.010	0.053
2013	0.003	0.126	0.261	−0.006	0.071
2014	−0.003	0.173	0.296	−0.009	0.033
2015	0.010	0.460	0.182	−0.010	0.044
2016	0.070	1.124	0.075	−0.009	0.071
2017	0.080	1.240	0.056	−0.010	0.072
2018	0.029	0.832	0.149	−0.010	0.046
2019	0.039	1.891	0.056	−0.011	0.027
2020	0.029	0.598	0.160	−0.013	0.070
2021	0.040	0.871	0.102	−0.010	0.058
2022	−0.012	−0.040	0.318	−0.010	0.032
2023	−0.034	−0.325	0.500	−0.011	0.071

SD, standard deviation.



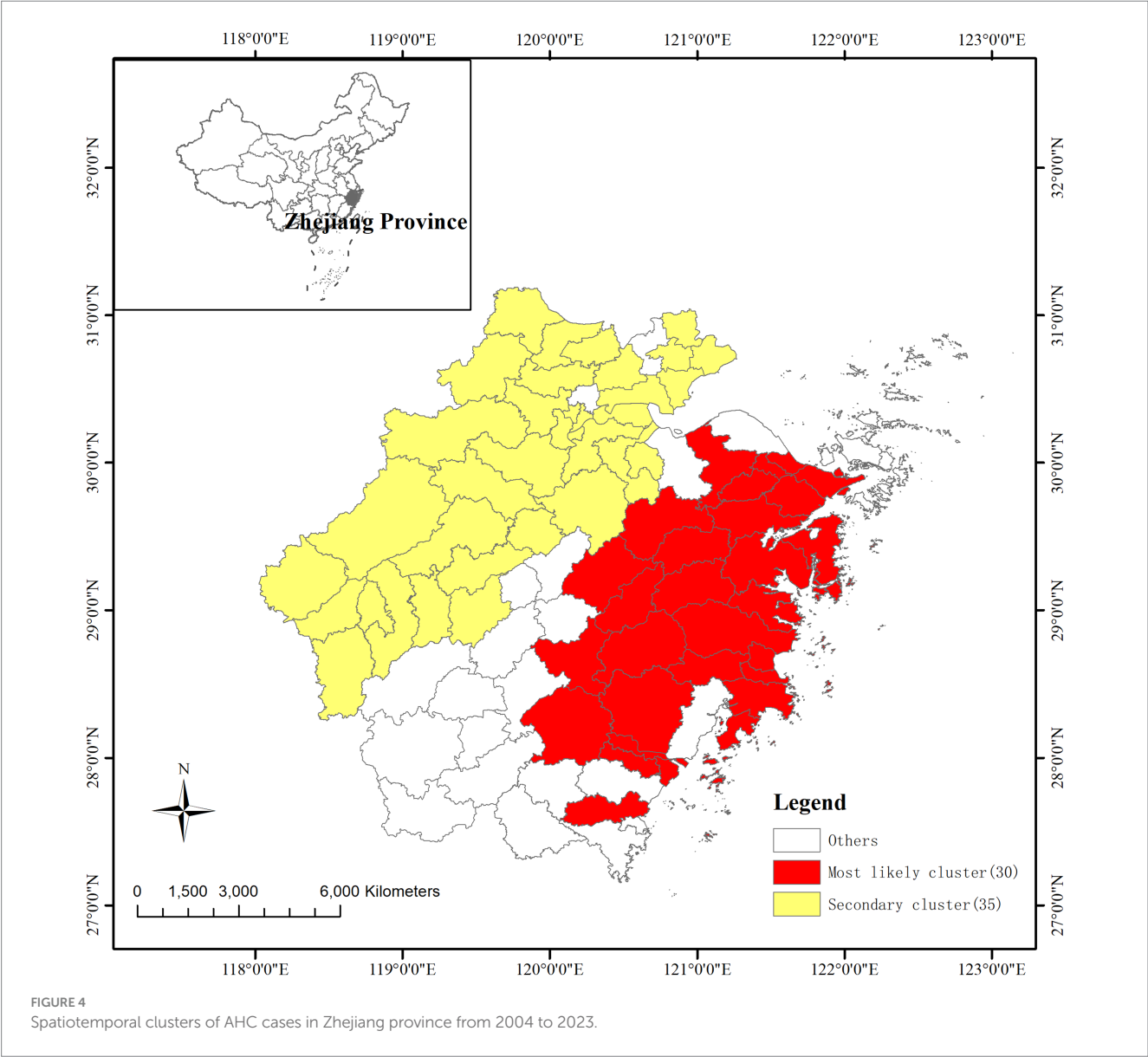
difference ($\chi^2 = 5.525$, $p = 0.019$). With regard to the difference in the age distribution of AHC, it was younger in cluster 1 than in the low-risk counties ($Z = -2.355$, $p = 0.018$), but it was older in cluster 2 ($Z = 3.509$, $p < 0.001$). The proportion of students in reported

AHC cases in cluster 1 was lower than that in low-risk areas ($\chi^2 = 169.241$, $p < 0.001$), but it was higher in cluster 2 ($\chi^2 = 99.703$, $p < 0.001$). Additionally, AHC cases from cluster 1 had fewer days from illness onset to diagnosis with a median of 1 day ($Z = -7.970$,

TABLE 2 The cluster results of space-time scan for AHC cases in Zhejiang province from 2004 to 2023.

Cluster type	Time frame	Counties (n)	Radius (km)	Observed cases	Expected cases	LLR	RR	p-value
Most likely	2010/9/1–2010/9/30	30	1.40	12,740	79.26	53739.20	212.44	<0.001
Secondary	2010/9/1–2010/9/30	35	128.08	4,535	86.65	13695.73	57.23	<0.001

RR, relative risk; LLR, log likelihood ratio.



$p < 0.001$), while cluster 2 had longer days than low-risk areas ($Z = 7.996$, $p < 0.001$) (Table 3).

Outbreak surveillance

There were three outbreaks caused by AHC, all reported in September 2023 in primary schools. A total of 104 clinically confirmed cases have been reported, and no fatalities have been reported. In each outbreak, gender ratios of male vs. female cases were larger than 1(7:3;

6:5, 43:29, respectively), with an outbreak duration of 8.33 ± 2.42 days (Table 4). The symptoms of the infected students were mainly conjunctival congestion, increased eye secretions, eye swelling, eye pain, photophobia, and tearing, and the proportion of fever was relatively low (Figure 5).

Discussion

This study described the epidemiological characteristics and spatiotemporal patterns of AHC cases in Zhejiang province using

TABLE 3 Comparison of characteristics of AHC cases between high-risk counties and low-risk counties identified by Kulldorff's spatiotemporal scan statistic in Zhejiang Province, 2004–2023.

Variables		High-risk counties		Low-risk counties
		Cluster 1	Cluster 2	
Gender	Male (%)	18,233 (57.83)*	7,770 (60.61)*	4,606 (59.29)
	Female (%)	13,298 (42.17)	5,050 (39.39)	3,162 (40.71)
Age	Median (Interquartile range)	23.00 (8–37)*	23.00 (10–39)*	23.00 (8–38)
Occupation	Students (%)	8,043 (25.51)*	4,122 (32.15)*	2,124 (27.34)
	Farmers (%)	4,615 (14.64)	2,841 (22.16)	1,542 (19.85)
	other (%)	18,873 (59.86)	5,857 (45.69)	4,102 (52.81)
Days from onset to diagnosis	Median (Interquartile range)	1.00 (0–2)*	1.00 (1–2)*	1.00 (1–2)

*Significant difference with lower value in high-risk counties compared with low-risk counties (χ^2 test or Z test with $p < 0.05$).

*Significant difference with higher value in high-risk counties compared with low-risk counties (χ^2 test or Z test with $p < 0.05$).

the systematic dataset of monthly AHC during the 20-year study period from 2004 to 2023, which provided a good basis for understanding the epidemiological characteristics of the infectious disease. Incidence data suggested that the total number of AHC male cases was higher than female cases in this study, and the sex ratio of male to female was higher than the general population of Zhejiang Province in the same period. This finding is in concordance with the results reported in Malaysia and other studies (4, 26).

AHC occurs year-round, with a peak incidence in September, potentially due to climatic factors. A study by Zhang et al. (27) found a moderate positive correlation between AHC and monthly mean temperature, relative humidity, and precipitation. Other enteroviruses (EVs) have also been found to have a similar relationship with temperature factors, such as hand-foot-mouth disease (HFMD) (28, 29). However, the exact mechanism behind the relationship between temperature and EVs remains unclear. We speculate that the survival of the virus in the environment, virus replication, and human behavior are possible factors. First, virological studies showed that human EV was sensitive to temperature, and 20°C was the most suitable temperature for its survival (30, 31). Second, children tend to participate in more outdoor activities when the temperature is appropriate, so it may increase the spread of AHC by increasing close contact between individuals and between individuals and contaminated surfaces (30, 32). In the meantime, school holidays were significantly associated with lower incidence (27). In September, students return to school after summer vacation. The gathering of students has exacerbated the spread or outbreak of AHC (33), which may also explain why September has become the peak month for AHC's popularity.

In summary, the epidemiological characteristics of AHC indicate that populations such as men, students, and farmers are more likely to be infected with AHC than other populations, which is consistent with the research results on the prevalence patterns of AHC in Chongqing (34) and Jinan (35). This phenomenon may be because (1) men tend to be hyperactive and engage in different types of occupational labor (36). (2) As a collective unit, the school has a crowding of individuals (37). Once there is a source of infection, students have more chances to be infected and even cause an outbreak. In addition, the evidence

of school holidays was significantly associated with lower incidence, with IRRs of 0.91 (an 8.57% reduction) compared to the non-school holiday (27), which has been proven that student gatherings during school contribute to the occurrence of AHC. (3) AHC is easy to spread in densely populated areas with poor medical and health conditions. Farmers lack sufficient health knowledge and good hygienic habits and live in places with insufficient medical and health facilities. The infection source was not effectively controlled and isolated, which is an important cause of infection (38).

This study found that it is difficult to collect laboratory diagnostic results of AHC-infected individuals, and the vast majority of these cases are clinical diagnosis cases defined by clinical doctors based on the patient's clinical symptoms, physical signs, and cytological examination. The lack of laboratory-confirmed results of cases may be because the symptoms of AHC infection include unique bulbar conjunctival bleeding follicular conjunctival reactions, and this infection has a short duration and self-limitation (39). Symptomatic treatment appears to be as effective as medical regimens to relieve symptoms. Therefore, doctors or patients are not motivated to retain samples for laboratory testing. To increase the proportion of pathogen detection in AHC cases, an active monitoring system should be established in the future to collect specimens from AHC patients and send them to the laboratory for testing instead of relying solely on existing passive monitoring systems.

Our study revealed that the incidence remained stable except for the years 2007, 2008, 2010, and 2011. Zhejiang had experienced the highest epidemic level so far in 2010. A similar situation of high AHC incidence also occurred in Guangxi, Guangdong, Chongqing, Shandong, and other regions in the year 2010 (34). In this study, we have found counties along the southeast coast of Zhejiang Province have higher incidence rates, which may be because coastal cities have more rainfall and migrant populations than inland cities (40). Results of multivariable analysis from other studies revealed that floods, city urbanization, and per capita play an important role in the occurrence of AHC. When a flood occurs, it pollutes water sources, worsens the living environment, and makes AHC easy to spread (41, 42). Additionally, urbanization and low per capita

TABLE 4 Basic information of three outbreaks reported in Zhejiang province from 2004 to 2023.

Name	Date of onset of the first case	Date of outbreak report	County	Place	Total number of students in the school	Number of clinically diagnosed cases	Gender ratio (male:female) of cases	Age range (years old)	Number of classes where cases occurred	Duration of outbreak (days)	Laboratory test results
Outbreak 1	2023/9/5	2023/9/11	Nanxun county	Primary school	2042	10	7:3	6–7	1 class	6	Positive universal nucleic acid test for enterovirus
Outbreak 2	2023/9/12	2023/9/15	Kaihua county	Primary school	2058	22	6:5	6–7	5 classes	4	None
Outbreak 3	2023/9/14	2023/9/20	Fenghua county	Primary school	889	72	43:29	6–12	17 classes	15	Positive universal nucleic acid test for enterovirus

usually mean poorer sanitary conditions and heavy traffic, making pathogens spread rapidly (7). Compared with the pre-pandemic of COVID-19 in the year 2019 and the post-pandemic of COVID-19 in the year 2023, 2020–2022 had the minimum incidence of AHC incidence, which may be affected by non-intervention measures during the pandemic of COVID-19 (43).

The global autocorrelation analysis revealed that AHC cases in Zhejiang province were not randomly distributed, indicating spatial clustering. Further LISA analysis results demonstrated that the high-high clusters varied over time. These areas have abundant precipitation, which is conducive to the occurrence of AHC. Temporal and space–time cluster analysis identified two high-risk clusters, which were generally consistent with local spatial autocorrelation results but also exhibited some differences. The two likely clusters include a total of 65 counties, accounting for 73% of the total number of districts in the whole of Zhejiang province. This indicates that large-scale outbreaks may exist from 1st September to 30th September 2010. Unfortunately, the monitoring network has not received any information on the outbreak during the same period. Only three outbreaks in schools were reported in September 2023 (which was probably due to the government strengthening the monitoring and reporting requirements for school epidemics due to the 19th Asian Games held in Hangzhou from 23rd September to 8th October 2023); clinical symptoms, including conjunctival congestion, increased eye secretions, eye swelling, eye irritation, fear of light and shedding tears, are the main clinical manifestations, which align with prior India and Thailand studies (12, 33, 44). Results of spatial clustering and outbreak characteristics suggest that the intervention of health administrative departments plays an important role in the improvement of AHC monitoring work. To further improve the level of disease prevention and control in areas with high incidence rates of AHC, recommendations for various levels of health administrative departments and public health practitioners are listed as follows: (1) Increasing the laboratory tests of patients with related symptoms in hospitals to make a clear diagnosis and implementing preferential measures for the test costs; (2) Strengthening the awareness of disease monitoring in schools and carry out daily morning and afternoon examinations for students in epidemic seasons; (3) Expanding the population scope of health education on disease prevention and control; and (4) The communication mechanism for the guidance of schools and other collective units on reporting and outbreaks disposal measures should be unblocked to control the number of infections in the outbreak as soon as possible.

Despite our research providing significant new insights into the epidemiological characteristics of AHC in Zhejiang province, it is important to consider two limitations when interpreting our findings. First, there is an inherent bias due to the use of passive surveillance data, which only includes cases that sought medical care in hospitals and were subsequently reported to the local disease control and prevention center for analysis. The quality of the data might also be affected by the availability of diagnostic techniques and the varying experience levels of doctors across different hospitals. Second, we failed to obtain the etiological classification of AHC cases; all cases were clinically diagnosed.

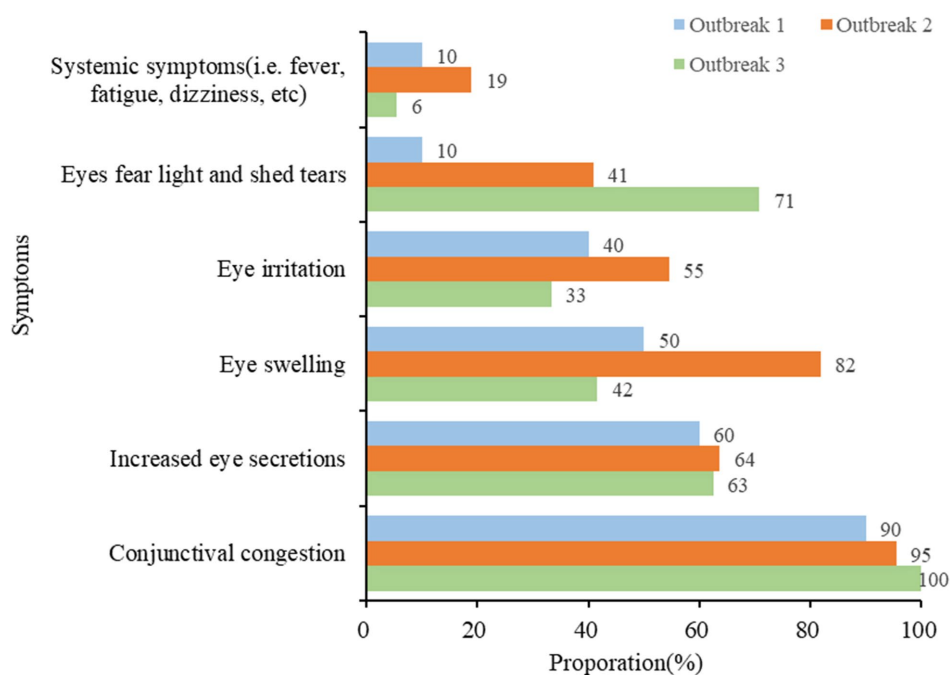


FIGURE 5
Distribution of clinical symptoms in three AHC outbreaks.

Conclusion

In summary, this study represents the first comprehensive and detailed analysis of the spatiotemporal dynamics of AHC in Zhejiang province from 2004 to 2023. Currently, no specific preventive measures are available for AHC. Transmission occurs mainly through eye-to-hand, object, and water. We recommend implementing public education for the population, improving their living environment, and enhancing accessibility to medical and health resources to prevent and control AHC outbreaks effectively. Our study demonstrates geographical distribution and seasonal variation in Zhejiang province, providing crucial evidence to health authorities and policymakers for optimizing the allocation of limited resources for disease prevention.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary material](#). Further inquiries can be directed to the corresponding author.

Ethics statement

This study was conducted in response to a public health emergency and received approval from the ethics committee of Zhejiang Provincial Centre for Disease Control and Prevention, with the ethics number 2016020. Informed consent was obtained from all participants or their legal guardians. The study was conducted in accordance with the Declaration of Helsinki.

Author contributions

WS: Conceptualization, Data curation, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. YC: Conceptualization, Investigation, Methodology, Software, Writing – original draft. SQ: Data curation, Investigation, Software, Supervision, Writing – review & editing. ZM: Conceptualization, Data curation, Funding acquisition, Investigation, Methodology, Project administration, Resources, 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.

Generative AI statement

The authors declare that no Generative AI was used in the creation of this manuscript.

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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.2025.1509495/full#supplementary-material>

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A population database analysis to describe the residual burden of varicella in Italy—a high vaccination coverage area—from 2004 to 2022

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Introduction: In Italy, universal varicella vaccination (VV) started in 2017 with a two-dose schedule administered in children aged 12–15 months and 5–6 years, achieving 90% coverage in 2019, though with regional variations. To address the limitations in surveillance databases for capturing varicella episodes, the study aimed to evaluate the burden of varicella disease in the pediatric population using a primary care database.

Methods: This cohort study used data from Pedianet, a comprehensive database of 193 family pediatricians in Italy. The incidence rate (IR) of varicella (International Classification of Diseases, Ninth Revision, Clinical Modification [ICD-9-CM] codes 052 and 052.0–52.9) was evaluated in children aged below 15 years, from January 2004 to April 2022, categorized by calendar year and region. Subjects were followed up from 2004 or their enrollment date until the end of assistance or the study period. Comorbidities and complications were identified.

Results: A total of 253,221 children aged below 15 years (resulting in a total follow-up of 1,430,249 person-years) were included in the study. A total of 35,614 varicella index cases were identified in 35,199 subjects (13.9%), with 1.2% experiencing two infections. Complications following varicella occurred in 467 children (1.3%), primarily affecting the skin and soft tissue (46.3%) and the respiratory tract (22.3%). The IR in regions that implemented the VV program before 2017 ranged from 38.3 per 1,000 person-years in 2007 to 0.8 per 1,000 person-years in 2022, while in those that implemented the VV in 2017, the IR decreased from 49.8 per 1,000 person-years in 2017 to 3.2 per 1,000 person-years in 2022. In the Veneto Region, following the implementation of VV in 2006, the IR significantly decreased by 20.5 annually (95% CI: –23.4, –17.5), ranging from 50.2 per 1,000 person-years in 2006 to 1.2 per 1,000 person-years in 2022.

Conclusion: The implementation of VV drastically reduced the IR of varicella, further confirming the importance of universal VV coverage.

KEYWORDS

varicella (chickenpox), children, Italy, epidemiology, disease complications

1 Introduction

Varicella zoster virus (VZV) is a highly contagious infection found worldwide, affecting between 2 and 16 individuals per 1,000 annually in the absence of preventive strategies or vaccination (1). VZV infection leads to two clinically distinct forms of the disease: varicella (chickenpox) and herpes zoster (shingles) (1).

Clinical manifestations of varicella generally develop after an incubation period of 10–21 days following exposure; they include a prodrome of fever, malaise, headache, anorexia, or pharyngitis, followed by a characteristic generalized pruritic vesicular rash. It is typically a mild-to-moderate disease in healthy children, in contrast to more severe cases in adults or immunocompromised patients of any age (1). Despite a benign, self-limited course in the majority of the episodes, severe complications can result after primary infection, leading to an increased risk of varicella-related hospitalization and mortality. The most frequent complications include skin and soft tissue infections, pneumonia, encephalitis, Reye's syndrome, and hepatitis (1, 2).

Since 1995, the implementation of routine childhood immunization has significantly altered its epidemiology, leading to decreased disease incidence, complications, hospital admissions, and deaths among children and in the general population, thereby indicating strong herd immunity. In the United States, following the introduction of the varicella vaccination (VV) program, the mortality rate for varicella decreased from 0.41 to 0.05 per million (2).

Nevertheless, only 12 European countries have implemented universal VV programs (3). In Italy, the vaccination for varicella was adopted and offered in a universal program at different times in different regions [i.e., Sicily (2003), Veneto (2006), Apulia (2006), Tuscany (2008), Basilicata (2010), Calabria (2010), Sardinia (2011), and Friuli Venezia Giulia (2013)] before being offered at national level starting from 2017 in all the regions (4). Since 2017, the schedule adopted is a two-dose schedule in children aged 12–15 months and 5–6 years (5, 6).

In 2010, for those regions that implemented the universal varicella vaccination, the coverage rates increased by 80% (i.e., Sicily, Veneto, and Apulia) (7). In 2019, the national first-dose coverage for varicella reached 90.5% for the 2017 cohort, with stable rates in subsequent years, though regional variations were observed (6).

Epidemiological data show a drastic reduction in varicella incidence, with almost half of the episodes from 2003 to 2013 among some Italian regions, but no up-to-date data are available at the national level (6).

Due to limitations in electronic health record databases in capturing varicella episodes in Italy, we aimed to assess the burden of varicella disease in the pediatric population using a primary care database. This study aims to update epidemiological and clinical data on varicella disease in the Italian pediatric population, focusing on the trends of incidence, clinical presentation, risk factors, and possible complications over the past two decades.

2 Materials and methods

This population-based birth cohort analysis complied with the European Network of Centres for Pharmacoepidemiology and

Pharmacovigilance methodological standards and did not require approval from the ethics committee.

2.1 Data source

The Pedianet database was used as the source of the study. Pedianet is a national population database that contains anonymous patient-level data of more than 500,000 children since 2004, corresponding to approximately 4% of the annual pediatric population, who received healthcare from 193 family pediatricians (FPs) from 1 January 2004 to 30 April 2022 in Italy who were part of the Pedianet network¹ using the same software package (Junior Bit®, Padova, Italy) in their professional practice. The data were extracted for this study on 23 October 2022.

According to the Italian National Health Service, each child is assigned to an FP, who is the primary referral for health-related matters. In Italy, there is a tax-funded public healthcare system that provides universal access, and patients do not incur any direct costs related to primary care visits. The Pedianet database captures various types of patient-level information, including the reason for accessing healthcare, health status, demographic data, diagnosis and clinical symptoms (in a free text or ICD-9-CM codes), drugs (Anatomical Therapeutic Chemical Classification codes), specialist appointments, diagnostic procedures, hospital or emergency room (ER) admissions, vaccinations, growth parameters, and clinical outcome data. Data are anonymized with a monthly update to a centralized database based in So.Se.Pe.—the legal owner of Pedianet, in Padova. Informed consent from children's parents is required to enter the data into the database. The data collected from the child's parents or tutors by pediatricians is entered into a dedicated cloud that is already encrypted and anonymized. Pedianet researchers are unaware of the anonymization process and cannot identify the owner of the data in any way.

2.2 Study design and study population

This is a retrospective population-based birth cohort analysis aiming at assessing the variation in the incidence of varicella over calendar years between 2004 and 2022 in children aged below 15 years. Any individual with missing data on age or sex was excluded because they were considered missing at random.

The varicella diagnoses were collected either with ICD-9 CM codes (052 and 052.0–52.9) or as free text in the medical notes (in Italian “varicella”) and manually validated by a clinical data manager to exclude possible false positives. Each visit, treatment and healthcare resource registered within 30 days from the varicella incident date were considered part of the same episode.

Regions were grouped in Veneto (where the universal VV program started in 2006), regions that introduced VV before 2017, named “Regions Pre2017” (Friuli Venezia Giulia, Sicilia, Puglia, Sardegna, and Toscana), and regions that offered the universal VV program starting from 2017, named “Regions 2017.”

¹ www.pedianet.it

Complications were associated with the varicella episode if a diagnosis of pneumonia, skin and soft tissue infection, neurological complications, and hepatitis were recorded as outpatient or inpatient visits within 30 days from the varicella episode index date. Every death event (not categorized as accidental death or suicide) was retrieved from the clinical diary and diagnosis field that were associated with the varicella episode if the event was recorded within 30 days from the varicella episode index date.

2.3 Statistical analysis

Descriptive analyses were summarized through tabulation and graphical representation of the means, medians, and standard deviations for continuous variables, as well as frequency distributions for categorical variables.

Each subject was followed from the 15th day of birth or the enrollment date, within 6 months of birth, until the end of assistance or the first varicella diagnosis or to the 30 April 2022, whichever came first. The incidence rate (IR) of varicella episodes was calculated by dividing the number of varicella episodes during the follow-up period by the total person-time computed as the number of years elapsed between the start and the end of the follow-up, expressed per 1,000 person-years. Furthermore, 95% confidence intervals were calculated by a group of regions. Significant trends over the years considered were assessed as average annual percent changes (AAPC) using the joint-point regression (8). The variation in the prescription rate was evaluated using the chi-square test. A *p*-value below 0.05 was accepted as statistically significant. Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS, IBM, Armonk, NY, USA) version 28.0 software program.

3 Results

During the 19-year-study period, 253,221 children aged below 15 years followed for a total of 1,430,249 years (mean follow-up of 5.6 years per person) were included in the analysis. A total of 35,614 varicella index cases were retrieved in 35,199 subjects (13.9%) (Figure 1). Overall, 51.7% were men, and Veneto was the region most represented accounting for 41.2% of total subjects. A total of 7,291 (2.9%) of children had at least a comorbidity; prematurity was the most frequent (1.3%, Supplementary Table 1s), followed by asthma (0.8% Supplementary Table 1s).

In 79.2% of children, the first varicella case was recorded after the age of 2 years (Table 1). A total of 415 children (1.2%) had two infections, and the majority of the cases (95.4%) occurred within 6 years of the index case. A total of 467 children (1.3%) had at least one varicella episode with at least a complication, the majority of which related to the skin and soft tissue (46.3%) and the respiratory tract (22.3%) (Table 1).

Overall, from 2004 to 2022, the incidence rate of varicella was 24.61 cases per 1,000 person-years, and no difference by sex was noted. The IR differed among the 13 regions, ranging from a minimum of 1.6 and 2.1 varicella episodes per 1,000 person-years in Sicily and Puglia regions, respectively, to a maximum of 61.7 varicella episodes per 1,000 person-years in the Marche region (Table 2).

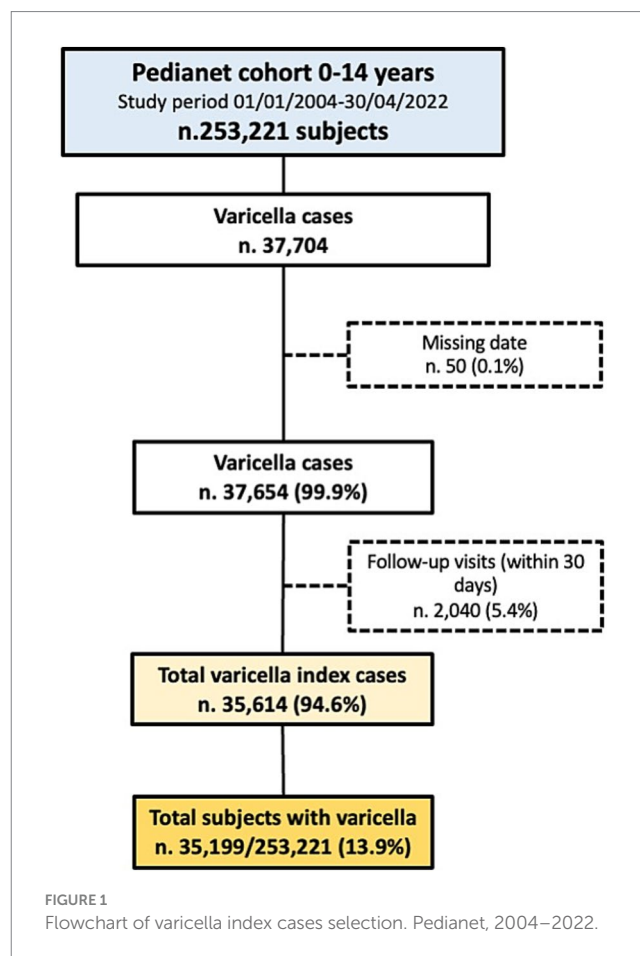


TABLE 1 Description of varicella cases by age class, number of varicella infections, and complications in children aged 0–14 years in Italy.

	Subjects with varicella (N = 35,199)
	N (%)
Age group at first varicella episode	
<1 year	3,151 (9.0)
1 year	4,172 (11.9)
2–5 years	22,681 (64.4)
>6 years	5,195 (14.8)
Number of varicella infections per child	
1	34,784 (98.8)
2	415 (1.2)
Complications	
No	34,732 (98.7)
Yes	467 (1.3)
Skin/mucosal/soft tissue	216 (46.3)
Respiratory	104 (22.3)
Neurological	11 (2.4)
Not specified	136 (29.1)

Pedianet, 2004–2022.

TABLE 2 Demographic characteristics, varicella episodes, and varicella incidence rates (with 95% CI) in children aged 0–14 years in Italy.

	Total subjects of the cohort N (%)	Total subjects with varicella N (%)	Follow-up (years)	Incidence × 1,000 person-years (95% CI)
Total	253,221 (100)	35,199 (13.9)	1,430,249	24.61 (24.36–24.86)
Sex				
Males	131,009 (51.7)	18,327 (14)	740,937	24.73 (24.38–25.09)
Females	122,212 (48.3)	16,872 (13.8)	689,312	24.48 (24.11–24.84)
Region				
Abruzzo	17,943 (7.1)	5,077 (28.3)	92,987	54.6 (53.14–56.06)
Campania	20,371 (8)	2,666 (13.1)	113,402	23.51 (22.63–24.39)
Friuli Venezia Giulia	5,869 (2.3)	1,631 (27.8)	30,111	54.17 (51.61–56.72)
Lazio	8,492 (3.4)	1,638 (19.3)	43,626	37.55 (35.76–39.33)
Liguria	1,429 (0.6)	321 (22.5)	6,788	47.29 (42.24–52.34)
Lombardia	17,455 (6.9)	3,995 (22.9)	73,733	54.18 (52.55–55.82)
Marche	22,306 (8.8)	6,712 (30.1)	108,857	61.66 (60.23–63.09)
Piemonte	18,783 (7.4)	4,924 (26.2)	91,426	53.86 (52.39–55.32)
Puglia	2,578 (1)	41 (1.6)	19,732	2.08 (1.44–2.71)
Sardegna	6,089 (2.4)	874 (14.4)	34,010	25.7 (24.02–27.38)
Sicilia	22,543 (8.9)	234 (1)	143,869	1.63 (1.42–1.83)
Toscana	4,973 (2)	444 (8.9)	30,076	14.76 (13.40–16.13)
Veneto	104,390 (41.2)	6,642 (6.4)	641,631	10.35 (10.10–10.60)

Pedinet, 2004–2022.

The IR varied from 52.6 episodes in 2007 to 1.8 per 1,000 person-years in 2022, with an annual percent change of -7.4 (95% CI: -13.6 , -0.7) from 2007 to 2017 and of -43.5 (95% CI: -52.3 ; -33.0) from 2017 to 2022. Specifically, in the Veneto Region following the implementation of VV in 2006 (9), the IR significantly decreased annually by 20.5 (95% CI: -23.4 ; -17.5), ranging from 50.2 in 2006 to 1.2 per 1,000 person-years in 2022 (Figure 2). In regions where universal VV programs started before 2017 (Regions Pre-2017), the IR significantly decreased annually by 40.5 (95% CI: -48.9 ; -30.8), ranging from 25.8 in 2012 to 0.3 per 1,000 person-years in 2020 (Figure 2). For the regions that implemented VV programs in 2017, a significantly decreasing trend after the vaccination introduction was observed; the IR decreased by 45.3 (95% CI: -54.7 , -33.8) (Figure 2).

4 Discussion

Continuous evaluation of the burden of vaccine-preventable disease, such as VZV, is necessary to promptly intervene in case of an increased trend in the incidence of the disease to inform further research to monitor the mutations in VZV genome, to discriminate vaccine virus from wild type virus, to study the phylogeny of VZV strains throughout the world, and to understand the evolution of the different clades of VZV. The implementation of VV drastically reduced varicella cases in all Italian regions, further confirming the importance of universal implementation of VV. In line with previous studies, the majority of the children were older than 2 years of age at first infection.

The incidence rates were very low in two Southern regions (Puglia and Sicilia), but the underreporting of cases was already noted (10). Children with comorbidities had lower incidence rates of varicella

compared to the overall population, possibly because those considered at risk were more prone to adopt non-pharmaceutical measures to reduce the risk of catching an infection.

Our results align with what was previously published on the population in Italy. In particular, an analysis using surveillance data from 2001 to 2010 in Italy revealed an average annual varicella IR of 9.49 cases per 1,000 inhabitants in children aged below 14 years (88.8% of the cases in the overall population). Notably, in this period, the universal vaccination for varicella had already been adopted by different regions: Sicily (in 2003), Veneto (in 2006), Apulia (in 2006), and Tuscany (in 2008), and vaccination coverage rates increased up to 80% in 2010 (5).

Consequently, in Sicily, the incidence of varicella decreased from 105.7 in 2003 to 9.2 per 100,000 inhabitants in 2010; in Veneto, the incidence decreased from 225.5 in 2007 to 55.7 per 100,000 inhabitants in 2010; in Apulia, the incidence decreased from 121.7 in 2006 to 13.1 per 100,000 inhabitants in 2010 (4), while in Tuscany, the incidence decreased from 350 in 1994 to 20 cases per 100,000 inhabitants in 2018 (11).

It should be considered that the sharp decline in the incidence rates from 2019 to 2022 might be attributed to the COVID-19 lockdown to some extent. Indeed, In Italy, voluntary and mandatory non-pharmaceutical interventions (NPIs) to reduce severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) circulation were first implemented in February 2020 and remained until the 18 May 2020 and included school (including nursery) and university closures, intrapersonal distancing of at least 1 m, ban of travel outside the city in which one person resides for reason other than work, public places and store closures, suspension of all public and private events including religious ceremonies (12). Notably, a surveillance analysis

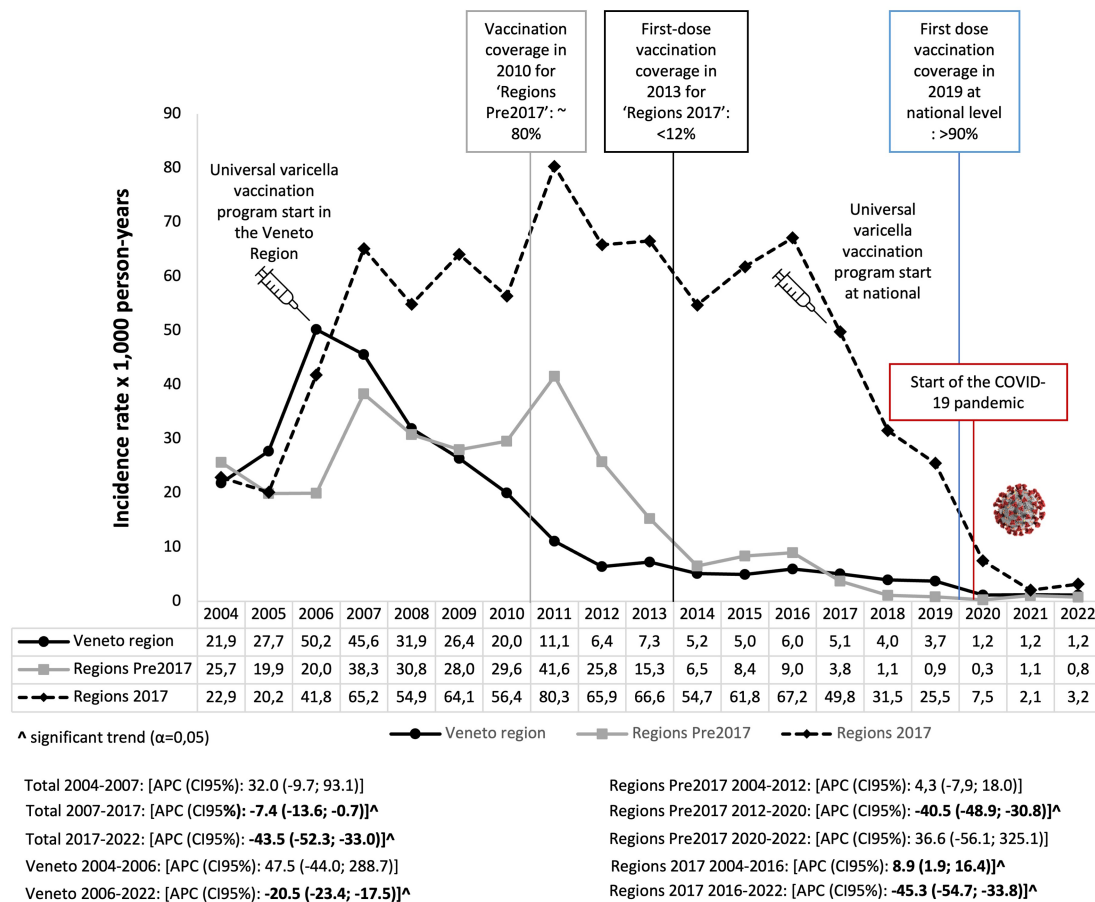


FIGURE 2
Annual trend of varicella expressed as the incidence rate of varicella episodes per 1,000 person-years in children aged 0–14 years in Italy. Peditanet, 2004–2022.

from France reported a reduction from 50 to 90% in varicella incidence from March through December 2020 (13). However, there was no active universal VV program in France during the study period (14).

The strength of this study is its size, generalizability, and representative coverage of pediatric patients. In Italy, enrolling in primary care is mandatory, and children are assigned to their FP based on their home proximity to the FP ambulatory.

A limitation of this study lies in its retrospective nature. The impossibility of confirming clinical assessment is a well-recognized limitation in working with real-world data because it may be subjective to the attending clinician. However, varicella is a well-defined disease typical of the pediatric age. Second, we could not assess the subjects' specific VV information for this study. Hence, variations that were noted in the disease burden in children with comorbidities might have resulted from a higher vaccination coverage in more fragile children. Third, the first available data regarding varicella vaccination coverage at the national level from the Ministry of Health are for children aged 5–6 years in 2013 (2006 birth cohort) (15). Hence it was not possible to fully assess the trend variation for the whole study period based on coverage rates and possible effect of regional catch-up programs. Fourth, limited representativeness in some regions was already

reported with this database; hence. The regional estimates should be evaluated with cautiousness.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: the data used in this study cannot be made publicly available due to Italian data protection laws. The anonymized datasets generated during and/or analyzed during the current study can be provided on reasonable request, from the corresponding author, after written approval by the Internal Scientific Committee (info@peditanet.it). Requests to access these datasets should be directed to Internal Scientific Committee (info@peditanet.it).

Ethics statement

Ethical approval was not required for the study involving humans in accordance with the local legislation and institutional requirements. Written informed consent to participate in this study was not required from the participants or the participants' legal guardians/next of kin

in accordance with the national legislation and the institutional requirements.

Author contributions

EB: Conceptualization, Methodology, Validation, Writing – original draft. SC: Conceptualization, Visualization, Writing – review & editing. PF: Formal analysis, Visualization, Writing – review & editing. AS: Data curation, Writing – review & editing. LC: Project administration, Software, Writing – review & editing. CG: Funding acquisition, Supervision, Writing – review & editing. VB: Supervision, Writing – review & editing.

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Conflict of interest

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Supplementary material

The Supplementary material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fpubh.2025.1412620/full#supplementary-material>

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The epidemiology and burden of respiratory syncytial virus and influenza infections in hospitalized children under 5 years old in Zhejiang, China (2018–2023)

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Objective: Estimate changes in hospital-based respiratory syncytial virus (RSV) disease burden before and after the COVID-19 pandemic and compare this burden with influenza among children younger than 5 years old in China's Zhejiang Province.

Methods: We enrolled hospitalized children under 5 years old from eight hospitals in Zhejiang Province. Multiple testing methods were used to compare epidemiological characteristics, and multivariate logistic regression analyses were used to estimate the odds ratio (OR) and 95% confidence interval (CI) between the two groups.

Results: In this study, of the 7,857 RSV and 2,571 influenza patients, the median age of the children was significantly lower for RSV infection than for influenza. Inpatients with RSV had longer hospitalization durations (mean: 5.66 days vs. 5.04 days; $p < 0.001$) and hospitalization costs (mean: 5,616.12 RMB vs. 5,352.99 RMB; $p = 0.023$) than those with influenza. RSV inpatients increased from 1,081 before the COVID-19 pandemic to 6,776 after the pandemic ($p < 0.001$), with 526.8% more hospitalizations than before the pandemic ($p < 0.001$). During 2020–2023, hospitalized children were older (16.86 months vs. 10.09 months; $p < 0.001$) and had a higher proportion of pneumonia (82% vs. 75% of hospitalized patients; $p < 0.001$) than during pre-pandemic seasons for children admitted due to RSV infection. However, the average RSV hospitalization cost was much lower after the pandemic (4,299.29 RMB vs. 5,697.51 RMB; $p < 0.001$). Compared with the prepandemic years (2018–2019), the influenza groups showed a similar

trend; the number of inpatients increased during the 2020–2023 season (1,949 vs. 622, $p < 0.001$), with older ages (33.13 months vs. 27.42 months, $p < 0.001$), a lower proportion of pneumonia (38% vs. 45%, $p < 0.001$), and lower costs (3,631.03 RMB vs. 3,742.59 RMB, $p < 0.001$). RSV infection was related to a higher risk of hospitalization in all age groups, and the greatest risk was observed in the 6–12 month age group (OR = 23.1; 95% CI, 18.0–29.6), followed by the 5 months and younger group (OR = 22.4; 95% CI, 17.3–28.9), compared with influenza infection.

Conclusion: RSV is a significant contributor to disease burden in hospitalized children under 5 years old, outweighing influenza. The COVID-19 pandemic impacted the epidemiological characteristics and disease burden of hospitalization for RSV and influenza infections. A more effective prevention strategy for both infections in young children, especially vaccinations against RSV and influenza, is urged.

KEYWORDS

respiratory syncytial virus, influenza virus, pneumonia, disease burden, children

1 Introduction

Respiratory syncytial virus (RSV) is an enveloped, single-stranded, negative RNA (Ribonucleic Acid) virus of the Pneumoviridae family of viruses (1). Globally, RSV is the most common pathogen responsible for lower respiratory tract infections (LRTIs) in children under 5 years old. It caused 33 million LRTI cases, 3.2–3.6 million hospitalizations, and more than 100,000 deaths annually before the coronavirus disease 2019 (COVID-19) pandemic (2). Notably, 99% of RSV deaths occur in low- and middle-income countries. China is one of the countries with a high prevalence of RSV, contributing nearly 50% of the global disease burden (2).

Treatment of RSV infection and measures to prevent infection should focus on children under 5 years because they are most vulnerable to RSV. In developed countries, either maternal RSV vaccination or infant immunization with RSV monoclonal antibodies prevents severe RSV disease in infants. In China, the long-acting monoclonal antibody Nirsevimab was officially approved by the National Medical Products Administration at the end of 2023 (3).

Globally, seasonal influenza circulates annually and causes substantial morbidity and mortality, with the highest burden among adults aged 65 years and older and children aged 5 years and younger (4). Every year, influenza causes an estimated 3–5 million cases of severe illness and 290,000–650,000 respiratory deaths. For children aged 5 years and younger, influenza is associated with 610,000–1,237,000 respiratory hospitalizations in the world annually (5). Influenza-associated infection (384 per 100,000 persons) or SARI (Severe Acute Respiratory Infection) (442–715 per 100,000 persons) hospitalization rate among children aged 5 years and younger is higher in China than in Singapore (186.8 per 100,000 persons) and Portugal (42.6 per 100,000 persons) (5). Although influenza vaccination can reduce the risk of illness caused by influenza virus infection by 40–60% among the general population, influenza vaccination is not included in China's National Immunization Program, and vaccination coverage in the Chinese population is only approximately 2% (6).

The COVID-19 pandemic has drastically perturbed the epidemiology of RSV and influenza infections (7). In the first year of the COVID-19 pandemic, when nonpharmacological interventions

(NPIs) were aggressively implemented, RSV and influenza cases immediately decreased worldwide (8). With NPI measures gradually relaxed, many countries, such as the USA and China, experienced an off-seasonal resurgence of RSV and influenza (9). Moreover, the testing model for respiratory infectious diseases in China was significantly changed from single to multipathogen by reverse transcription-polymerase chain reaction (RT-PCR) (10). All these public health measures may also have changed the disease characteristics and disease burden of other respiratory viruses, including RSV and influenza viruses.

China introduced a series of policies to control the influenza virus. First, the Chinese Center for Disease Control and Prevention recommends a yearly flu vaccine as the first and important action in reducing the risk of flu and its potentially serious outcomes. In China, the influenza vaccine was listed as the second-category vaccine that is self-paid and an optional inoculation; the coverage rate remained at a low level among children. Second, antiviral treatment may be considered in hospitals for symptomatic children with suspected or confirmed influenza disease, which has been covered by national medicare. For RSV, there were no RSV vaccines licensed in China, but the RSV antibody (Nirsevimab) was approved at the end of 2023 and has not been administrated largely to babies younger than 12 months of age. The etiology and epidemic characteristics of RSV were systematically monitored via a respiratory multipathogen monitoring system. Taking nonpharmaceutical interventions was most commonly used method for reducing the spread of influenza and RSV viruses in China.

To guide disease prevention strategies, it is critical to thoroughly characterize the evolving epidemiological patterns of RSV and influenza and the disease burden caused by the infection of the two viruses (11). Studies have explored RSV-related disease incidence, hospitalization rate, in-hospital mortality, and mortality rates (2, 9, 12, 13). Li Z-J (14) and Rha B (15) focused on analyzing the etiological composition of patients with respiratory tract infection, including acute respiratory infection (ARI) and acute lower respiratory tract infection, at different ages (including RSV and other respiratory viruses) and obtained the proportion of RSV in patients with respiratory tract infection. Studies by Shi T (16) and Sanz-Muñoz I (17) analyzed respiratory virus infection and the occurrence of

pneumonia before and after COVID-19 nondrug intervention measures. Other studies analyzed the risk factors of severe RSV patients (18).

In summary, large-scale intensive comparative research on RSV and influenza infections in epidemiological characteristics and disease burden is still lacking in mainland China. In this present study, we collected data on over 7,000 RSV- and 2,000 influenza-infected hospitalized children aged 5 years and younger from multiple health care centers in China's Zhejiang Province. We estimated the disease burden of influenza- and RSV-associated hospitalizations in prepandemic years vs. pandemic years and stratified the data by age, gender, underlying disease, and clinical severity.

2 Methods

2.1 Hospital selection

This multicenter retrospective cohort study was conducted between January 1, 2018 and August 31, 2023, from eight hospitals in the northern, central, southern, and coastal areas of China's Zhejiang Province. This area covered 75% of the local population, including rural and urban communities as well as provincial-level tertiary, municipal, county-level, and general hospitals as well as specialized pediatric facilities (more details are available in [Supplementary Figure 1](#) and [Supplementary Table 1](#)).

2.2 Study population

The participants of this present study consisted of all hospitalized children aged 5 years and younger who tested positive for either influenza A, B, or RSV, which was determined by using a combined RT-PCR or rapid antigen test of nasopharyngeal swab samples or lower respiratory tract samples during a hospital presentation during the study period. No change was found in the diagnostic criteria during the study period. Patients with a positive report for both viruses and those who had tested positive and reported in the previous month were considered to be different cases.

2.3 Data collection and analysis

All children aged 5 years and younger admitted to the eight hospitals during the study period were identified from the hospital information system (HIS). All RSV-confirmed inpatients were screened from the HIS, and hospitalization information was extracted from anonymized electronic medical records from the eight hospitals. Demographic variables collected for each patient encounter during the study period included age and sex; clinical characteristics included onset date, confirmed date, hospital admission and discharge dates, and presence of complex chronic conditions; outcomes assessed included discharge diagnosis, clinical outcome (s), hospital stay, and hospitalization cost (RMB). Our study only included hospitalized children younger than 5 years old who were confirmed by the eight hospitals to have RSV or influenza, we excluded outpatients with mild symptoms during the study period. There were no changes in the

investigation criteria and data analyzed in different hospitals or different years.

Each patient had a unique medical record number used as an identifier to retrieve and link all variables from different databases. Records were excluded if they had data missing from the age, primary discharge, admission diagnosis, or admission date fields. Specific names, ID numbers, home addresses, and other private information were not included.

Based on 2019 national guidelines for the diagnosis and treatment of community-acquired pneumonia in children, pneumonia is defined by the presence of lung consolidation on chest imaging (radiology or computed tomography scan) interpreted by a radiologist (10). The clinical severity categories in our study included acute upper respiratory tract infections, acute bronchitis, acute bronchiolitis, and pneumonic. These four clinical diagnostic classifications are made by an attending physician during a patient's visit to a hospital according to the clinical manifestations, imaging examinations, laboratory tests, and specific criteria of China. We extracted corresponding information from a patient's case and conducted a statistical analysis; no modifications were made to clinical classifications.

The study population was described in terms of epidemiological characteristics and disease burden. Age groups were categorized as follows: ≤ 5 months, 6–12 months, 13–24 months, 25–36 months, 37–48 months, and 49–60 months. To compare the disease burden of RSV and influenza infections between the pre-pandemic and pandemic periods, the period from 1 January 2018 to 31 December 2019 was considered the pre-pandemic period, and the period from 1 January 2020 to 31 August 2023 was considered the post-pandemic period.

2.4 Statistical analyses

First, normality was tested for continuous variables. As the distribution of variables including age, length of hospital stay, and hospitalization cost were non normal, we calculated the median range. Categorical variables were expressed as frequencies and percentages. T-, chi-square, and Fisher's exact tests were applied to compare continuous and categorical variables as appropriate.

Second, a multivariate logistic regression was used to estimate the odds ratio (OR) and 95% confidence interval (CI) associated with gender, age, pneumonia, severe illness, mixed infection, underlying disease, length of hospital duration, and hospitalization cost of each inpatient infected with RSV and influenza. All statistical analyses were performed using the R software program (v. 4.3.1). Statistical significance was set at $p < 0.05$ (two tails).

3 Results

3.1 Overall hospitalization burden of RSV infection vs. influenza infection

Over the five seasons between 2018 and 2023, 10,428 hospitalized children aged ≤ 5 years (7,857 RSV infections and 2,571 influenza infections) were enrolled in this study. RSV was responsible for approximately three times as many hospitalizations as influenza in the ≤ 5 years age group (1,571 vs. 514 cases per

TABLE 1 Comparison of the disease burden of hospitalization attributable to influenza and RSV among children 0–5 years old during 2018–2023 in Zhejiang Province, China.

Variable	RSV	Influenza	Test value ^a	<i>p</i> value ^a
Overall No. (%)	7,857 (75.35)	2,571 (24.65)		
Stages No. (%)				
Prepandemic years	1,081 (13.75)	622 (24.19)	154.36	< 0.001
Pandemic years	6,776 (86.25)	1,949 (75.81)		
Gender No. (%)				
Male	4,746 (60.40)	1,520 (59.12)	1.331	0.249
Female	3,111 (39.60)	1,051 (40.88)		
Age (Months) (median, range)	12 (0–60)	36 (0–60)	–37.29	< 0.001
No. (%)				
0–5	2,374 (30.22)	336 (13.01)	1,798.47	< 0.001
6–12	2,806 (35.71)	440 (17.11)		
13–24	1,133 (14.42)	367 (14.27)		
25–36	1,049 (13.35)	530 (20.61)		
37–48	376 (4.79)	451 (17.54)		
49–60	119 (1.51)	447 (17.39)		
Underling diseases No. (%)				
Yes	1,417 (18.03)	374 (14.55)	16.57	< 0.001
No	6,440 (81.97)	2,197 (85.45)		
Mixed infections No. (%)				
Yes	1,601 (20.38)	367 (14.27)	47.08	< 0.001
No	6,257 (79.62)	2,204 (85.73)		
Clinical severity No. (%)				
Pneumonia	6,360 (80.95)	1,032 (40.14)	3,421.03	< 0.001
Acute bronchitis	754 (9.60)	532 (20.69)		
Acute bronchiolitis	652 (8.29)	19 (0.74)		
Acute upper respiratory tract infection	91 (1.16)	988 (38.43)		
Clinical outcome	All cases were discharged	All cases were discharged		
Hospitalization duration(days) (median, range) (ratio)	5.00 (1–64)	4.00 (1–62)	8.30	< 0.001
Underling diseases(yes/no)	1.20 (6.00/5.00)	1.25 (5.00/4.00)	/	/
Mixed infections(yes/no)	1.00 (5.00/5.00)	1.25 (5.00/4.00)	/	/
Pneumonia(yes/no)	1.00 (5.00/5.00)	1.25 (5.00/4.00)	/	/
Hospitalization expenses (RMB) (median, IQR; Range) (ratio)	Median: 4460.73 (IQR: 3,364.98–6207.55; Range: 684.29–285,655.90)	Median: 3664.00 (IQR: 2,732.41–5,016.66; Range: 340.00–352,008.68)	6.11	0.023
Underling diseases (yes/no)	1.49 (6244.62/4,197.73)	1.53 (5,302.28/3,456.85)	/	/
Mixed infections (yes/no)	1.07 (4,704.31/4,395.61)	1.49 (5226.10/3,497.51)	/	/
Pneumonia (yes/no)	1.13 (4,558.51/4,047.55)	1.40 (4,524.24/3,227.82)	/	/

RSV = respiratory syncytial virus; RMB = Renminbi; IQR = interquartile range.

^a*p*-value is used to assess the difference between the RSV group and influenza group in the corresponding classification. The test value of count data is the chi-square test, and the test value of quantitative data is the T-test.

year) (Table 1). The hospitalization rate of RSV cases was higher than that of influenza cases in the same year (Supplementary Table 2).

For population distribution, there was no difference between males and females in the two groups ($p = 0.249$). However, the mean age was lower for RSV than for influenza (15.92 vs. 31.75 months;

$p < 0.001$); the 0–2 years group accounted for approximately 80% of RSV infections, and the 3–5 years group accounted for 55.54% of influenza virus infections (Table 1).

Regarding comorbidities, 18.03% of RSV inpatients and 14.55% of influenza inpatients had at least one underlying medical condition, respectively ($p < 0.001$) (Table 1). The categories of comorbidities of RSV and influenza inpatients were similar; the top five most common comorbidities in the RSV groups were atrial septal defect (21.32%), anemia (14.33%), asthma (13.20%), allergic rhinitis (9.88%), and adenoidal hypertrophy (6.28%); for influenza inpatients, the most common comorbidities were anemia (18.99%), allergic rhinitis (15.24%), asthma (13.11%), atrial septal defect (12.57%), and adenoidal hypertrophy (12.04%) (Figure 1; Supplementary Table 3).

For coinfections, the percentage in the RSV groups was greatly higher than that in the influenza groups (20.38% vs. 14.27%; $p < 0.001$). The categories of pathogens resulting in mixed infections in RSV and influenza hospitalized patients were similar. In terms of viral coinfection, the top three most common viruses were human rhinovirus, cytomegalovirus, and human adenovirus in the RSV groups, and human rhinovirus, rotavirus, and human adenovirus in the influenza groups (Supplementary Figure 2A). The top three most common bacteria for both the RSV and influenza groups were mycoplasma pneumonia, streptococcus pneumonia, and haemophilus influenza (Supplementary Figure 2B).

For disease burden, infections in the RSV groups, with a higher percentage of pneumonia, were more severe than those in the influenza groups (80.95% vs. 40.14%; $p < 0.001$). Inpatients with RSV had longer hospitalization duration (median: 5.00 vs. 4.00 days; $p < 0.001$) and hospitalization cost (median: 4,460.73 vs. 3,664.00 RMB; $p = 0.023$) than those with influenza (Table 1). The distributions

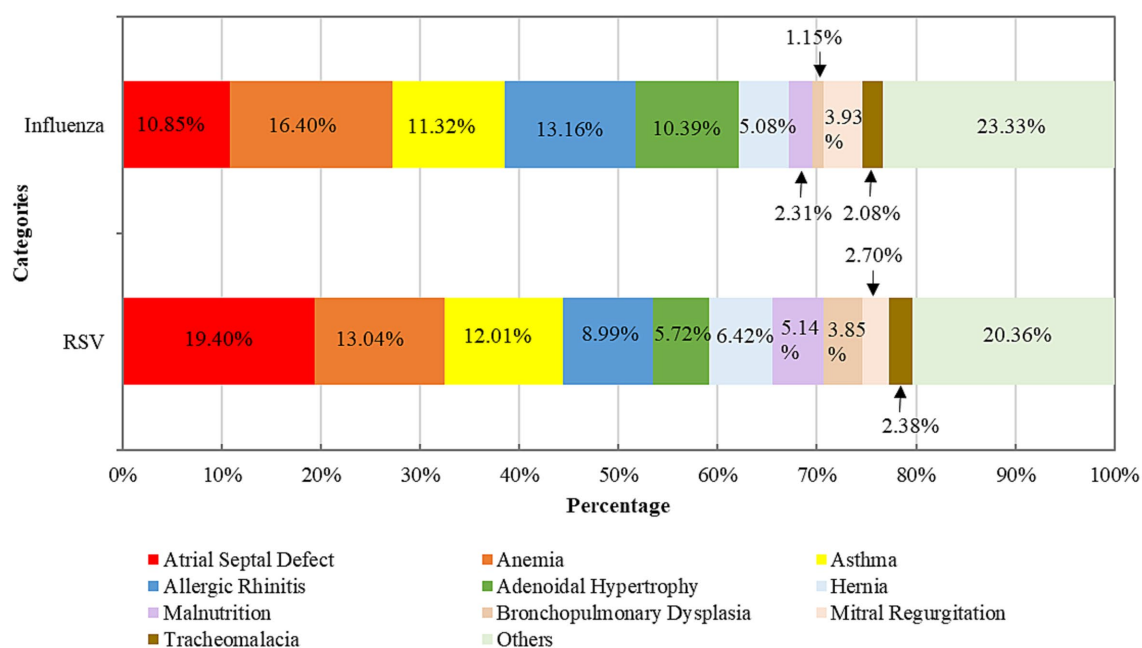
of hospital stays and hospitalization costs are shown in Supplementary Figure 3.

3.2 Epidemiological changes during the pre- and post-pandemic periods

The number of hospitalizations due to RSV and influenza was higher in the post-pandemic period than in the prepandemic period (6,776 vs. 1,081 cases for RSV; 1,949 vs. 622 cases for influenza, respectively; $p < 0.001$) (Table 1).

In 2018–2019, RSV and influenza epidemics typically followed seasonal patterns, peaking in December or January, but the COVID-19 pandemic disrupted RSV seasonality in 2020–2023 (Figure 2). In 2020–21, the typical winter RSV and influenza epidemics did not occur. RSV circulation began earlier and continued longer in 2021–2022 than during the prepandemic years. The 2022–2023 season started later than the 2021 season but earlier than the prepandemic seasons. The peak of the RSV season was delayed to the spring–summer of 2023 (Figure 2A). In contrast, influenza showed an off-season peak in July 2022 and March 2023 (Figure 2B).

A shift toward older children with RSV symptomatic infections was observed in the post-pandemic period. For the RSV groups, the median age was larger for pandemic years than for pre-pandemic years (16.86 vs. 10.09 months; $\chi^2 = 217.62$, $p < 0.001$). This increase was primarily observed among children aged 13–24 months, representing 9% of all RSV hospitalizations from 2018 to 2019 to 15% in 2020–2023. In contrast, compared with pre-pandemic seasons, the proportion of the ≤ 5 months group (45% vs. 28%) and the 6–12 month group (38% vs. 35%) decreased in the pandemic seasons for RSV



Note: The numbers in the figure are proportion of each comorbidity.

FIGURE 1

Comparison of the underlying diseases of hospitalization due to respiratory syncytial virus and influenza infections among children ≤ 5 years old in 2018–2023 in Zhejiang Province, China.

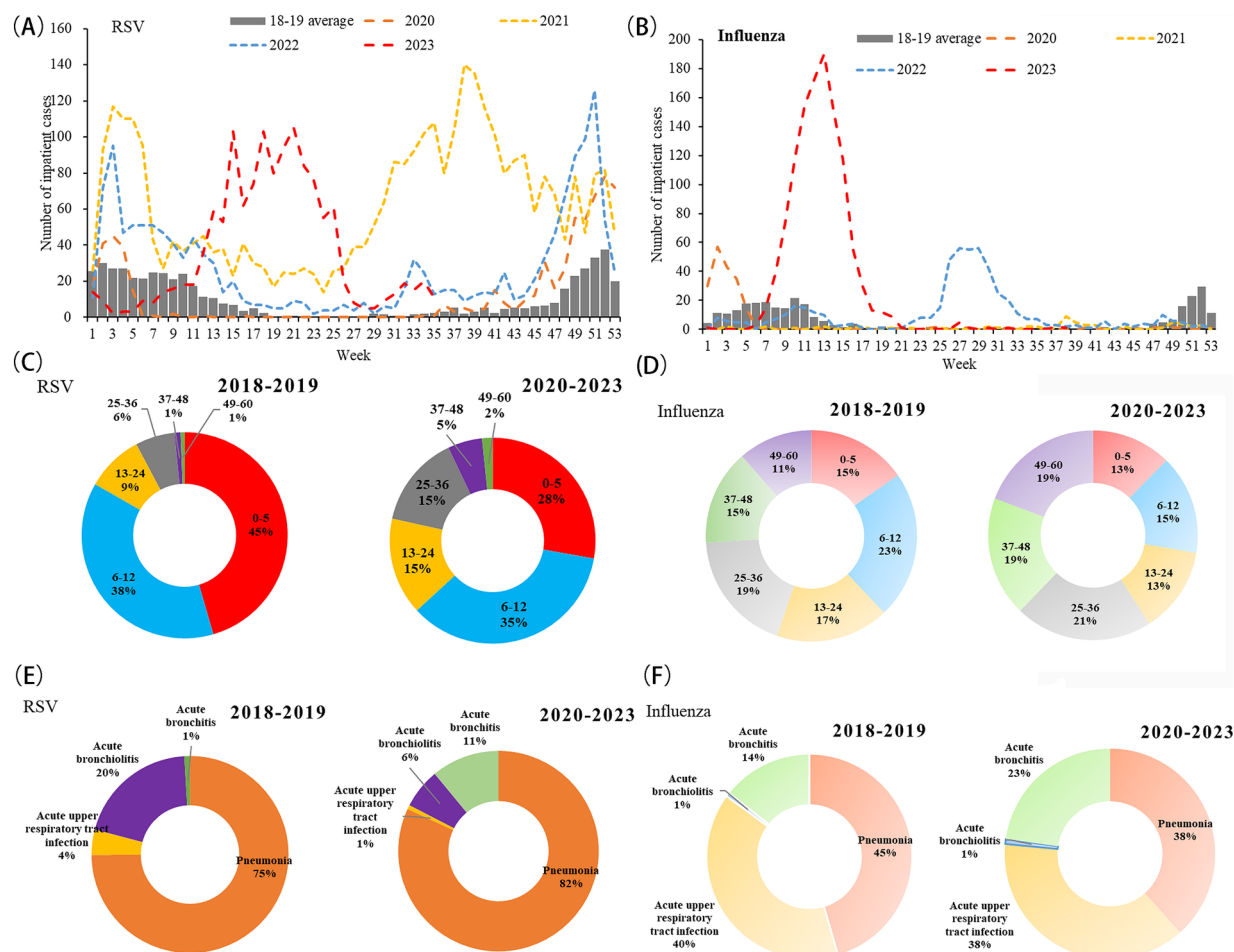


FIGURE 2

Hospitalization trends, age distribution, and disease spectrum in hospitalized children younger than 5 years with respiratory syncytial virus and influenza infections before and after the COVID-19 pandemic. (A) Temporal distribution of RSV (number of cases: 1,081 in 2018–2019 and 6,776 in 2020–2023); (B) temporal distribution for influenza (number of cases: 622 in 2018–2019 and 1,949 in 2020–2023); (C) age distribution for RSV; (D) age distribution for influenza; (E) disease spectrum for RSV; (F) disease spectrum for influenza.

infection cases (Figure 2C). Influenza groups showed a similar trend; the median age was 27.42 months in 2018–2019 to 33.13 months in 2020–2023 ($\chi^2 = 45.66$, $p < 0.001$) (Figure 2D). The most noticeable increase was observed in the 37–48 month group (15% vs. 19%) and the 49–60 month group (11% vs. 19%), respectively.

Disease severity parameters showed an increase in RSV groups during pre-pandemic seasons vs. pandemic seasons (percentage of acute bronchiolitis: 1% vs. 11%) (Figure 2E). For influenza infection, this percentage of influenza increased from 14 to 23% in 2020–2023 compared with 2018–2019 (Figure 2F).

3.3 Disease burden during the pre- and post-pandemic periods

The average hospitalization cost (4299.29 vs. 5697.51 RMB; $p < 0.001$) and duration (5 vs. 6 days; $p < 0.001$) for RSV inpatients in 2020–2023 were significantly lower than those in 2018–2019. Analyses stratified by age showed that disease burden significantly decreased among the groups aged younger than 6 months and 6–12 months separately (all $p < 0.05$) (Figures 3A,B).

The hospitalization cost (3,631.03 vs. 3,742.585 RMB) and duration (4 vs. 5 days) for influenza inpatients in 2020–2023 were also significantly lower than those in 2018–2019 ($p < 0.001$). Analyses stratified by age showed a downward trend for disease burden among the groups aged younger than 2 years (all $p < 0.05$) (Figures 3C,D).

3.4 Risk factors for hospitalization burden

A multivariate logistic regression analysis revealed that the odds of all variables were significantly higher among patients hospitalized with RSV compared with those hospitalized with influenza (Figure 4). This analysis confirmed that, compared with influenza, RSV was related to an increased burden in all groups; the greatest burden was seen in the 6–12 months group (OR = 23.1; 95% CI, 18.0–29.6), followed by the ≤ 5 months group (OR = 22.4; 95% CI, 17.3–28.9), 13–24 months group (OR = 8.8; 95% CI, 6.80–11.4), 25–36 months group (OR = 6.0; 95% CI, 4.7–7.7) and 37–48 months group (OR = 2.7; 95% CI, 2.0–3.5) (Figure 4). Compared with influenza patients, RSV patients had a higher risk of pneumonia (OR = 5.8; 95%

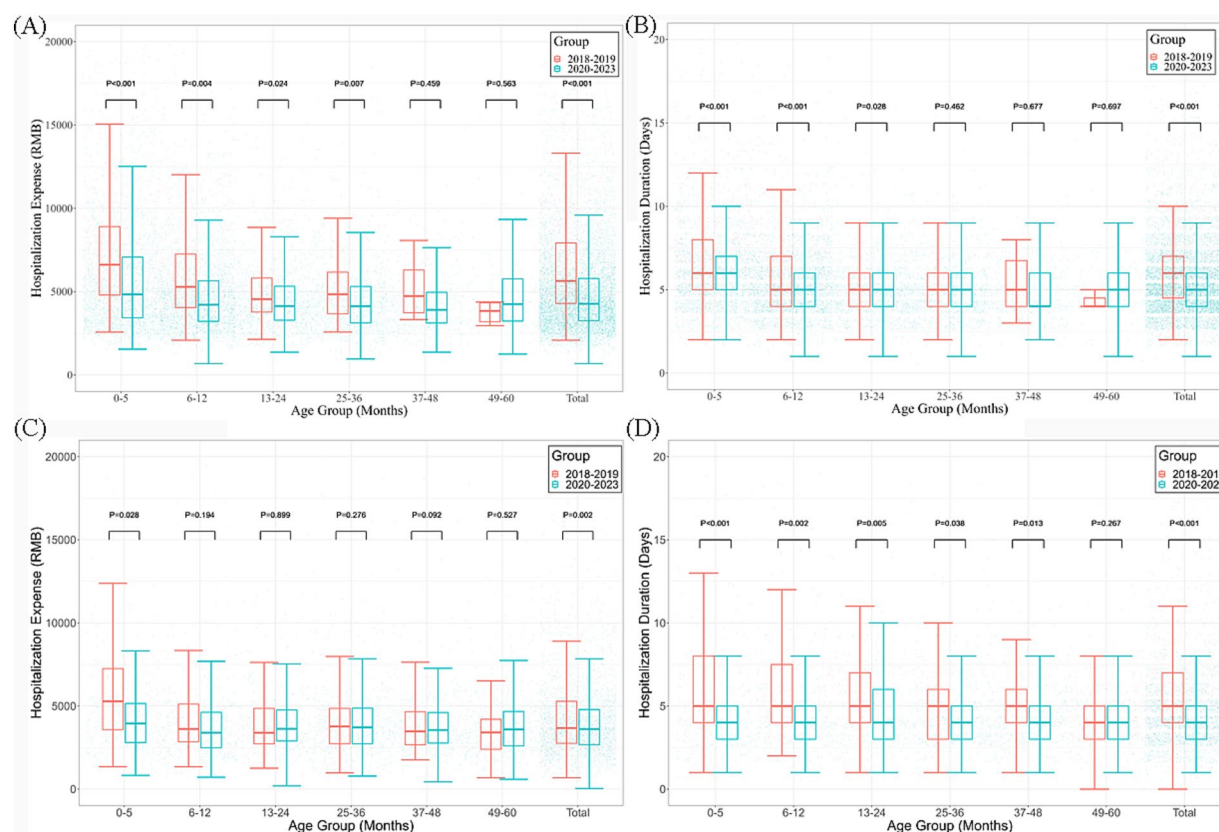


FIGURE 3

Median hospital stay and median hospitalization cost of inpatients due to influenza and RSV infections among children ≤ 5 years old before and after the COVID-19 pandemic in Zhejiang Province. (A,B) Hospitalization costs and hospital stays for RSV infection, respectively; (C,D) Hospitalization costs and hospital stays for influenza infections, respectively.

CI, 5.1–6.5), severe pneumonia (OR = 1.4; 95% CI, 1.15–1.6), and mixed infection (OR = 1.1; 95% CI, 1.1–1.3) (Figure 4).

Compared to the influenza groups, the RSV group had a higher risk for the average length of hospital stay [4–6 days for (OR = 2.1; 95% CI, 1.9–2.5); 7–15 days (OR = 1.6; 95% CI, 1.2–2.0)]. However, the risk of having to be hospitalized for 15 days or more was lower in the RSV groups (OR = 0.5; 95% CI, 0.3–0.8). On the same, RSV patients had higher hospitalization costs [$>9,000$ RMB, OR = 1.80; 95% CI, 1.5–2.2], and lower costs in the group [3,001–6,000 RMB, OR = 0.4; 95% CI, 0.3–0.5] compared with influenza patients (Figure 4).

4 Discussion

In this retrospective observational study from multiple centers in Zhejiang Province, 7,857 RSV cases and 2,571 influenza cases were analyzed for nearly five epidemic seasons of RSV and influenza between 2018 and 2023. This study found that RSV was associated with a higher risk of disease burden (younger age, higher percentage of comorbidities, coinfections, pneumonia, hospitalization costs, prolonged hospital stays) compared with influenza in children aged 5 years and younger. NPIs during the COVID-19 pandemic had varied impacts on both RSV and influenza infections. Compared with the pre-pandemic period, the

number of cases, ages of inpatients, and percentage of pneumonia significantly increased during the pandemic years, whereas hospitalization cost and hospital stay per admission decreased slightly.

Previous studies showed that children under 5 years old were the high-risk group for RSV infection. Cui et al. found that children under 5 years old accounted for 87.13% of all RSV and 8.34% of influenza-infected patients in nine provinces of China (19). In our study, compared with influenza, RSV was associated with higher health care costs, leading to hospital overcrowding for all age groups, with the highest risk observed in children aged 12–24 months. These findings align with recent studies (20). For example, in the UK, RSV-attributable disease leads to 20,000–30,000 hospitalizations annually, which is significantly higher than influenza (up to 20 times higher for children under 6 months) and accounts for over 55,000 annual bed days (21).

These results may be explained by several reasons. First, RSV has a much higher proportion of pneumonia cases compared to influenza, whereas influenza has a higher proportion of acute upper respiratory tract infections. Therefore, pneumonia (OR = 5.8) and severe pneumonia (OR = 1.4) were more frequent in the RSV group than in the influenza group. Furthermore, RSV infections are more frequently associated with coinfections (22, 23). In Singapore, for RSV infection in children less than 6 months of age, the average cost per bronchiolitis hospitalization was US\$2,209, rising to US\$5,942 for hospitalization for pneumonia with complications (22).

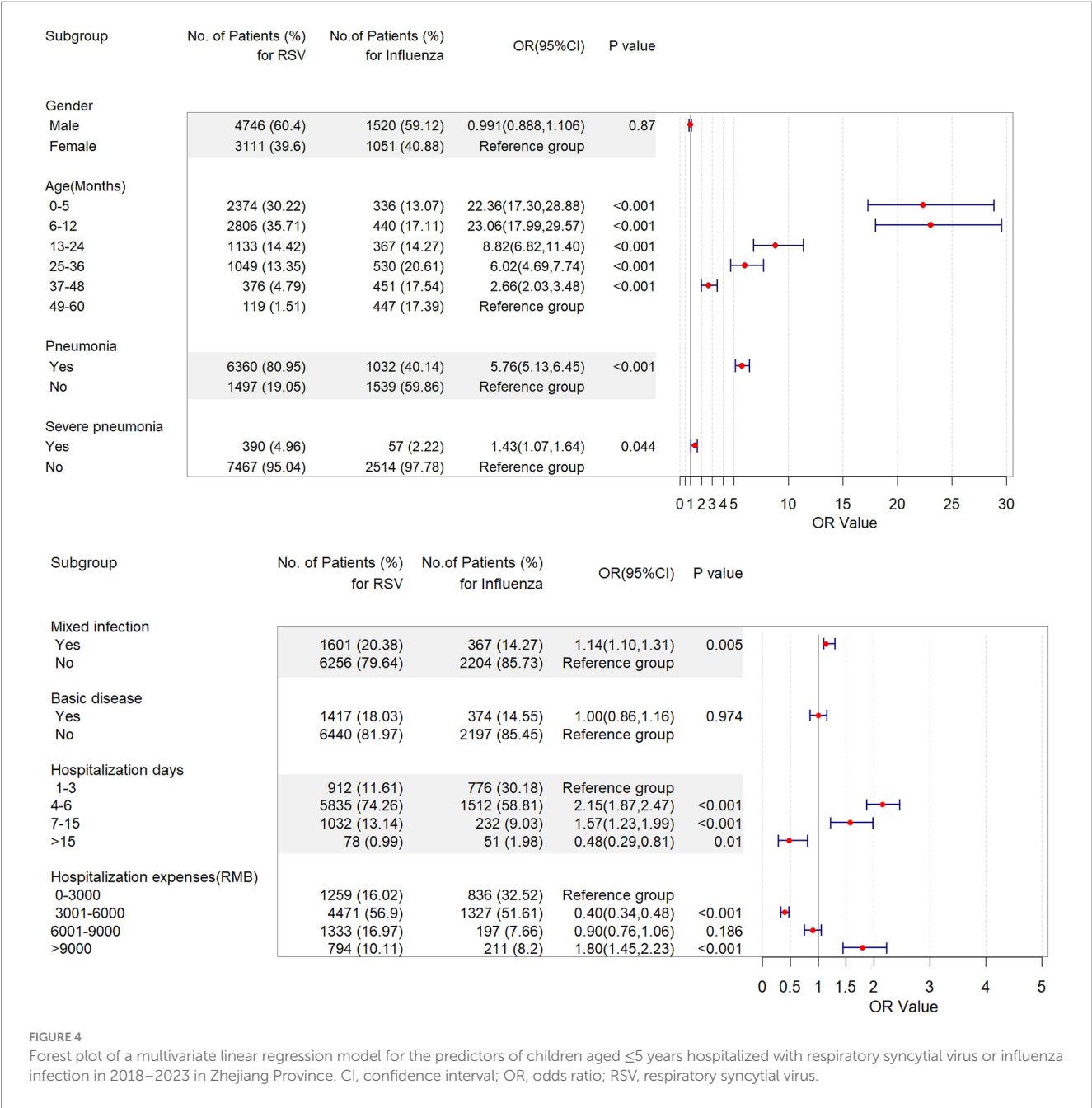


FIGURE 4 Forest plot of a multivariate linear regression model for the predictors of children aged ≤ 5 years hospitalized with respiratory syncytial virus or influenza infection in 2018–2023 in Zhejiang Province. CI, confidence interval; OR, odds ratio; RSV, respiratory syncytial virus.

Second, this study also confirmed that hospitalized patients with RSV were younger than those with influenza (16 vs. 32 months); the findings are consistent with two previous studies examining RSV age (24, 25). Younger patients presented with higher respiratory signs and symptoms (dyspnea, oxygen desaturation, and wheezing), characteristic of the pathophysiology of RSV infection (26). Third, Oseltamivir is a specific antiviral drug that is effective in reducing the disease burden of influenza (27).

Compared with other countries, the mean length of hospital stay in this current study was longer among children aged ≤ 5 years (5.66 days in RSV and 5.06 days in influenza) than RSV infections reported in other countries. For example, one study reported that across Europe, the average length of hospital stay for RSV infection was 2–4 days (28). In Brazil, the median length of hospital stay for

influenza was 3.0 days, while in Mexico, the median length of hospital stay was 5.0 days (29).

Conversely, the median direct medical cost for hospitalization of children with RSV (5,616.12 RMB) and influenza-related (5,352.99 RMB) illnesses was lower in China than in other countries. The average cost of RSV-associated hospitalization was estimated at US \$3,300 in Japan and at US\$4,500 in Australia (22). The cost of influenza hospitalization in the USA was \$3,366–\$19,444 (30). Compared with other domestic research results, the average cost of RSV-related hospitalization cases was lower than the research results of Ren et al. on the direct economic burden caused by RSV infection in hospitalized children aged 0–59 months in Henan Province (1055.3 US dollars (US\$) (95% CI: 998.2–1112.5 US\$) for each episode) (31). This difference might be due

to the different levels of economic development and medical care systems.

The COVID-19 pandemic had a notable impact on the circulation of seasonal respiratory viruses, including RSV and influenza virus (32). For RSV groups, the usual winter peak did not occur in the winter of 2020, which was like studies from the USA and UK, when most COVID-19 restriction measures stopped, RSV infections and hospital admissions rose sharply in the summer of 2021, autumn of 2022, and spring of 2023 (33). However, compared with 2018–2019, the total number of RSV hospitalization cases in 2020–2023 remained high in all age groups of patients ≤ 5 years, suggesting higher intensity of circulation (32). Similarly, in 2020–2021, the intensity of influenza activity continued to decrease significantly, while it increased in the summer of 2022 and the spring of 2023.

This result is consistent with the research results of Cui AL et al. on the epidemiological characteristics of RSV infection in 16 provinces of China (excluding Zhejiang Province) from January 2009 to September 2023 (34) and the study by Li MZ et al. on RSV epidemiology in Beijing during 2015–2023 (35). This altered activity patterns between the two viruses hinted that the public health measures against COVID-19 had a more effective impact on influenza than RSV (34). One plausible reason for this observation could be the ability of RSV to survive for a longer duration out of a host and to spread quickly by direct contact (36). Also, children, particularly those under 1 year, could not fully benefit from preventive measures during the pandemic of COVID-19, such as wearing masks.

In our study, compared with the clinical severity of RSV cases in 2017–2018, our results showed a significant increase in the percentage of acute bronchiolitis for RSV and influenza. We propose three hypotheses for this phenomenon. First, during the COVID-19 pandemic, PCR techniques were widely used in hospitals, resulting in the timely, accurate detection of specific or broad-spectrum pathogens (10). This initiated specific treatments and reduced the occurrence of complications and disease burden (37). Second, we found that during pandemic seasons, the age distribution of children infected with RSV or influenza decreased with the proportion of children aged 0–12 months decreasing and the average age increased. A similar older age structure of RSV and influenza patients was observed in the USA, France, and Australia; this situation could be attributed to ‘immunity debt’ resulting from the accumulation of immune-naïve children due to the strict implementation of NPIs in 2020–2023 (33). This older age population influenced a host’s immune response and thus increased its ability to fight against both viruses and reduce the disease burden. Third, more of the population appeared to be vaccinated against influenza during the pandemic years, demonstrating a reduction in hospitalization burden. Consistent with the above results, the disease burden (including expenses and duration) of each RSV or influenza-infected patient was lower during the pandemic period than during the pre-pandemic period.

In this retrospective cross-sectional study, we report a surge in RSV and influenza infections in older children from 2020–2023. Meanwhile, we found that the disease burden of children infected with RSV and influenza was severe. Increased cases and disease burdens may continue to pose considerable strains on healthcare systems. In response to these challenges, clinicians and program planners should take measures to increase China’s health care capacity and ICU (intensive care unit) capacity to meet greater demands for RSV and influenza admissions. Policymakers have introduced imminent new

RSV and influenza prevention strategies, including increased overall influenza vaccine coverage and approved RSV monoclonal antibodies in China, to minimize the burden of the two diseases in this age group.

The advantage of this study is the large sample, multicenter survey, and comparative study of the epidemiology characteristics and disease burden of RSV and influenza infections. However, this study has several limitations due to differences in the criteria for admission, treatment plans, and laboratory testing capabilities in several hospitals. Additionally, the lack of relevant data on viral genotyping, family economic status, and indirect economic burden may influence whether parents decide to hospitalize their children, which is also useful in evaluating epidemiological features and disease burdens.

5 Conclusion

This study, conducted at eight hospitals for nearly five consecutive epidemic seasons of RSV and influenza, included the largest cohort of RSV- and influenza-infected hospitalized children under 5 years of age. This study indicates that the disease burden of RSV in all groups aged ≤ 5 years, especially those under 1 year, was significantly higher than that of influenza; however, the burden of RSV and influenza infections per admission was reduced during the pandemic compared with the prepandemic period. The seasonal pattern of the two viruses was significantly disrupted by the easing of COVID-19 restriction measures, but RSV infections remained at a higher level than influenza infections during the pandemic (38). It is uncertain whether this change will continue in upcoming seasons.

Future studies should focus on specific mechanisms via the seasonality and epidemiologic characteristics of RSV and influenza. Second, it is critical to conduct a comprehensive study on RSV and influenza immunity at the population level and to inform the prioritization of immunization platforms. Third, the safety of RSV and influenza vaccines as well as monoclonal antibody immunogenicity should be thoroughly assessed.

Data availability statement

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

Author contributions

WS: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing. QD: Data curation, Writing – original draft. LG: Data curation, Formal analysis, Investigation, Writing – original draft. AZ: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. AT: Conceptualization, Data curation, Investigation, Methodology, Writing – original draft. ZM: Data curation, Formal analysis, Investigation, Supervision, Writing – original draft. YZ: Data curation, Writing – review & editing. FY: Investigation, Methodology, Software, Supervision, Writing – original draft. XF: Data curation, Investigation, Methodology, Writing – original draft. SS: Data curation, Investigation, Methodology,

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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.2025.1470770/full#supplementary-material>

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Feasibility and acceptability of human papillomavirus self-sampling compared with clinician sampling in urban areas of western China: a cross-sectional survey

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Introduction: Cervical cancer, driven by persistent high-risk human papillomavirus (hrHPV) infection, remains a global health challenge, especially in low- and middle-income areas such as western China. Despite the critical role of HPV testing in early detection, coverage in China remains low due to cultural, psychological, and other barriers. Self-collected urine and vaginal samples offer alternative methods for sample collection. This study aimed to evaluate the feasibility and acceptability of detecting hrHPV and cervical intraepithelial neoplasia grade 2 or worse (CIN2+) via urine and vaginal self-sampling compared with clinician sampling in urban areas of western China.

Methods: A cross-sectional survey was conducted from November 2022 to March 2023 in urban areas of western China. The participants provided self-collected urine and vaginal samples for hrHPV testing and completed questionnaires on acceptability of self-sampling. The HPV positivity, agreement, and kappa value were calculated to assess concordance between self- and clinician sampling. The sensitivity, specificity, agreement, predictive values, and likelihood ratios were used to evaluate the clinical performance of both methods for detecting CIN2+.

Results: A total of 2,228 female subjects aged 21–71 years were recruited, and self-collected urine samples, vaginal samples, and clinician-collected cervical samples were obtained. The sensitivity of clinician sampling, urine self-sampling and vaginal self-sampling were 80.00% (95% CI: 44.22–96.46), 70.00% (95% CI: 35.37–91.91) and 90.00% (95% CI: 54.12–99.48) for CIN2+; the specificity for <CIN2 were 98.33% (95% CI: 97.68–98.81), 98.23% (95% CI: 97.56–98.72) and 98.50% (95% CI: 97.87–98.95%); and the agreements for CIN2+ were 98.25% (95% CI: 97.59–98.74), 98.83 (95% CI: 98.26–99.22) and 98.82 (95% CI: 98.25–99.21). All methods yielded high negative predictive values, high positive likelihood ratios, and low negative likelihood ratios. Additionally, participants reported high

acceptability of self-sampling, citing less discomfort and embarrassment than clinician sampling.

Conclusion: Self-collected urine and vaginal samples for the detection of hrHPV and CIN2+ demonstrate high diagnostic accuracy and acceptability, making them viable alternatives to clinician-collected samples. Self-sampling methods may improve screening accessibility and compliance, especially in resource-limited settings, thereby supporting the prevention and early detection of CIN2+.

KEYWORDS

vaginal self-sampling, urine self-sampling, human papillomavirus testing, cervical intraepithelial neoplasia, cervical cancer screening, acceptability

1 Introduction

Cervical cancer, which is strongly associated with persistent high-risk human papillomavirus (hrHPV) infection, ranks as the fourth most common cancer in both incidence and mortality among women, with approximately 660,000 new cases and 350,000 deaths globally in 2022 (1). Notably, 80% of the decline in cases occurred in lower-middle-income countries (LMICs) (2). Unfortunately, China remains among the developing countries with a high burden of cervical cancer, making it the most prevalent gynecological cancer in terms of both incidence and mortality (3). Cervical cancer could be the first cancer eliminated worldwide through strategies such as the 90–70–90 targets proposed by the World Health Organization (WHO) in 2020. These targets aim for 90% of girls to be fully vaccinated with the HPV vaccine by age 15, 70% of women to be screened with a high-performance test by age 35 and again by age 45, and 90% of women diagnosed with precancer or invasive cancer to receive appropriate treatment (4).

In addition to vaccination, HPV testing is a crucial method for cervical cancer screening, offering greater effectiveness in reducing cervical cancer incidence compared to cervical cytology, due to its higher sensitivity and negative predictive value (5–8). As of 2021, 48 countries have recommended primary HPV-focused screening, either independently or in combination with other screening methods such as cytology (9). Low rates of HPV vaccination and cervical cancer screening coverage remain significant challenges in China, where the cumulative full vaccination rate among women aged 9–45 was only 6.21% (10), and 70.5% of women aged 20–64 years have never been screened (11). This situation is attributed to organizational barriers such as limited medical access and prolonged wait times; interpersonal challenges such as stigma and a lack of family support; and personal factors such as low risk perception, discomfort, and fear (12, 13).

HPV self-sampling techniques are recommended by the WHO to be incorporated into cervical cancer screening protocols, aiming to

facilitate the achievement of the 2030 targets (4, 14). As of 2021, 17 countries have integrated self-sampling into national programs or guidelines, targeting underscreened populations or as a primary method. Several studies have shown the high feasibility and acceptability of the HPV-DNA test using self-collected urine/vaginal samples and clinician-collected cervical samples (13, 15–18). Advantages include being less invasive, offering greater accessibility for self-collection, and reducing the need for infrastructure such as examination beds and costly collection kits. Self-sampling methods have also been shown to be cost-effective (19–21), making them a promising way to increase women's participation in cervical cancer prevention, especially in LMICs.

Despite evidence supporting the feasibility and acceptability of self-sampling methods, studies in China on these techniques remain limited (22, 23). Therefore, our study aimed to evaluate the feasibility and acceptability of detecting high-risk HPV (hrHPV) and cervical intraepithelial neoplasia grade 2 or worse (CIN2+) using urine and vaginal self-sampling compared with clinician sampling in urban areas of western China.

2 Methods

2.1 Study design and participants

A cross-sectional study was conducted from November 2022 to March 2023 in urban areas of western China. To be eligible for the study, women had to be between 21 and 71 years of age at the time of invitation, must have refrained from sexual intercourse, vaginal medication, vaginal contraceptives, and the use of vaginal cleansers for at least 48 h prior to the examination, and must have signed the informed consent. Patients were excluded from the study if they had a history of uterine or cervical surgery (such as hysterectomy), were pregnant or within 8 weeks postpartum, or were experiencing menstrual bleeding at the time of the examination.

Eligible patients were recruited at our partner hospital in Shuangliu District, Chengdu, where they registered, signed informed consent, underwent cervical and self-sampling, and completed a questionnaire. The physician-collected hrHPV-DNA test was performed at the partner hospital, while the self-sampling hrHPV-DNA test was conducted at Hangzhou central laboratory. Cytology and immunohistochemistry were handled by Guangzhou central laboratory and sent to our hospital's pathology department for slide review. Colposcopy and sampling were performed at our partner hospital, with slides prepared and reviewed by our hospital's pathology department.

Abbreviations: ASC-US, atypical squamous cells of undetermined significance; CI, confidence interval; CIN2+, cervical intraepithelial neoplasia grade 2 or worse; HPV, human papillomavirus; hrHPV, high-risk human papillomavirus; ICC, immunocytochemistry; IQR, interquartile range; LCT, liquid-based cytology testing; LMICs, lower-middle-income countries; NLR, negative likelihood ratio; NPA, negative percent agreement; NPV, negative predictive value; OPA, overall percent agreement; PLR, positive likelihood ratio; PPA, positive percent agreement; PPV, positive predictive value; PUMCH, Peking Union Medical College Hospital; WCSUH, West China Second University Hospital; WHO, World Health Organization.

2.2 Basic information collection, sample collection and acceptability assessment

After registration and informed consent, the participants completed a questionnaire covered basic participant information (such as age, education, marriage, family income), histories of cervical cancer prevention, previous female reproductive system diseases, menstrual cycles, and contraceptive use.

Before sample collection, video guidance based on the manufacturer's instructions for self-sampling was given to all participants. The participants were instructed to collect at least 25 ccs of anterior first void in a sterile urine container. After urine collection, a self-sampling swab for vaginal sampling was obtained. Researchers remained outside the bathroom to offer further assistance and collect the samples. Finally, trained gynecologists conducted gynecological examinations and collected cervical samples, completing a corresponding examination questionnaire.

After sampling, the participants completed a questionnaire assessing the acceptability of the HPV screening protocol. This survey contained 20 questions evaluating their experiences, comfort, and safety during the procedure, as well as their method preferences and reasons for those choices. All paper questionnaires were transcribed into a Microsoft Access 2016 database by two data stewards for data cleaning and coding, with a third steward ensuring consistency of the double-entered data.

2.3 hrHPV-DNA detection, cytology, immunocytochemistry, colposcopy, and histopathology

Cervical samples were evaluated for hrHPV-DNA with HBRT-H14 (HybriBio Biotechnology Ltd. Corp., Guangdong, China) (24), whereas urine and vaginal self-samples were evaluated with CareHPV systems (QIAGEN, Hilden, Germany).

Liquid-based cytology testing (LCT) and P16/Ki-67 immunocytochemistry (ICC) were conducted on clinician-collected cervical samples if urine, vaginal, or cervical samples tested negative for HPV16/18 but positive for the other 12 types of hrHPV.

According to the screening results, women meeting any of the following criteria are required to undergo colposcopy within 1 month: (1) positive for HPV16/18; (2) positive for the other 12 types of hrHPV, along with a positive result for LCT [atypical squamous cells of undetermined significance (ASC-US) or higher] or P16/Ki-67 ICC. Tissue biopsies were performed by an experienced physician on women who tested positive during colposcopy. If abnormalities were detected, a direct biopsy was taken at the abnormal site. If a high-grade lesion was indicated by cytology but no abnormalities were found during colposcopy, endocervical curettage (ECC) and random biopsies (at positions 2, 4, 8, and 10 on the cervical squamocolumnar junction) were conducted.

All LCT, ICC, and histopathology samples were blinded and independently evaluated by two experienced cytopathologists or histopathologists. In cases of diagnostic discrepancies, the specimens were re-evaluated to reach a consensus. Cytological diagnoses were made according to the Bethesda System (3rd Ed.), and the histological results were based on the highest level of diagnosis.

2.4 Statistical analysis

Statistical analyses were conducted via SPSS version 23.0 (IBM Corp., Armonk, NY, United States) and VassarStats (online). The HPV positivity rate, agreement rate, and kappa value were calculated to assess the concordance between self-sampling and clinician sampling. The sensitivity, specificity, agreement rate, predictive values, and likelihood ratios were used to evaluate the clinical performance of self-sampling and clinician sampling for detecting CIN2+. HPV positivity rates between self-sampling and physician sampling were compared using McNemar's test. Participants' feelings about self-sampling were compared using Chi-Square test, and Bonferroni's correction was used for multiple comparisons. The distributions of difficulty levels between self-sampling and physician sampling were compared using the Mann-Whitney U test. All tests were two-sided, and p values < 0.05 were considered statistically significant.

2.5 Ethics approval and consent to participate

This study was approved by the ethics committees of both the Peking Union Medical College Hospital (PUMCH) (approval code ZS-3293) and West China Second University Hospital (WCSUH). Written informed consent from all the study participants was obtained, and all materials complied with the Declaration of Helsinki and ethical standards.

3 Results

Figure 1 presents a flow chart of the study. Among the 2,235 eligible participants, 2,228 provided self-collected urine and vaginal samples, and 2,212 completed the questionnaire.

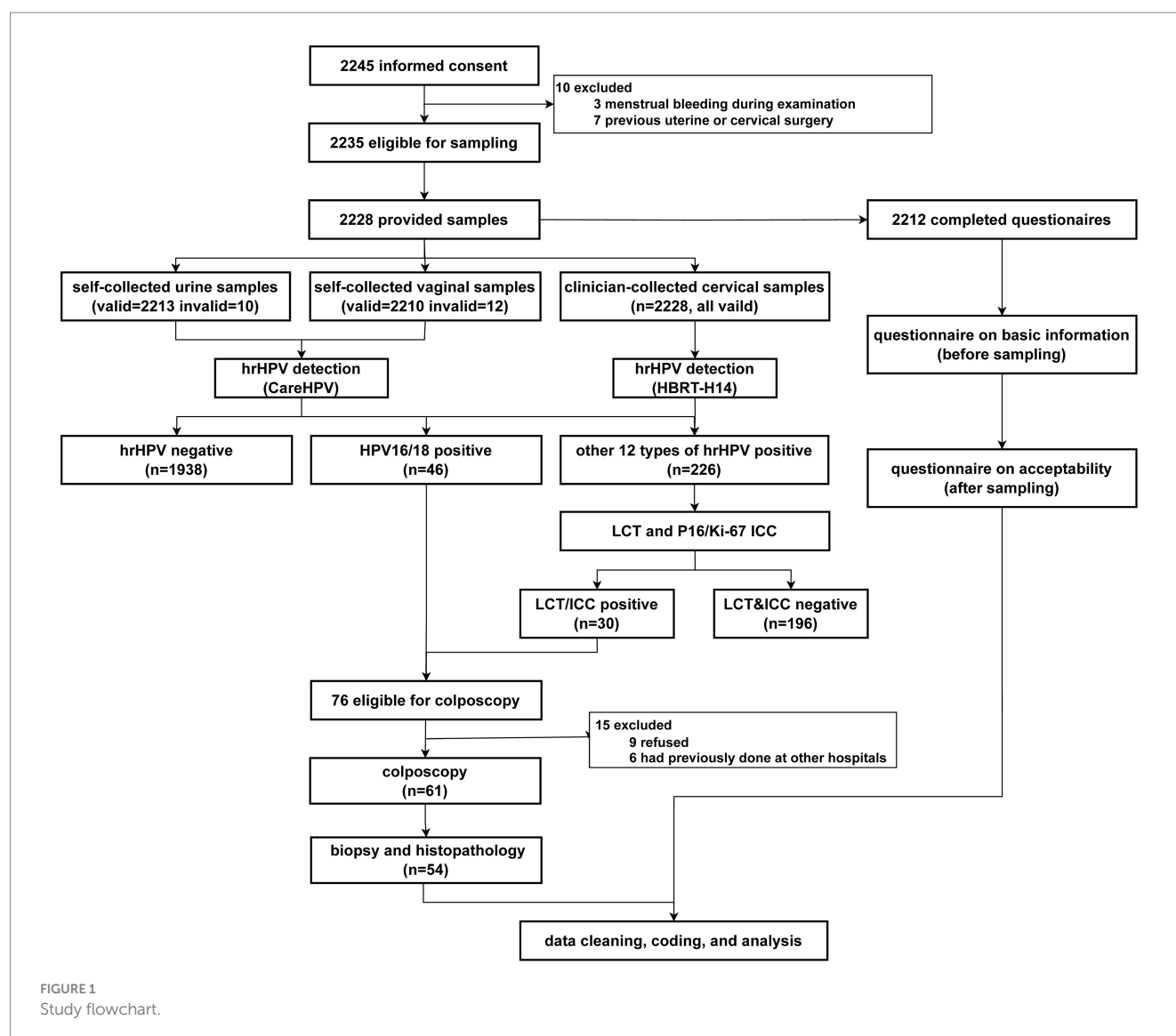
3.1 Basic characteristics

The median age of the 2,228 participants was 52 years (IQR: 40–64 years). Most participants were married (2,071/2,228, 93.0%), and approximately 98.8% (2,202/2,228) had completed primary or higher education. Additionally, 91.4% (2,037/2,228) had not received the HPV vaccine, and 65.5% (1,459/2,228) had never undergone cervical screening (Table 1).

3.2 Accordance of HPV testing between self-sampling and clinician sampling

The comparison among the tests is shown in Table 2 and Supplementary Table 1. The hrHPV positivity rate was similar between urine and clinician samples (9.9% vs. 9.9%, $p > 0.05$) and between vaginal and clinician samples (9.4% vs. 9.9%, $p > 0.05$).

Urine self-sampling had an overall percent agreement (OPA) of 95.66%, a positive percent agreement (PPA) of 78.54%, and a negative percent agreement (NPA) of 97.54%, with a Cohen's kappa of 0.758. Vaginal self-sampling had an OPA of 96.61%, a PPA of 80.37%, and a NPA of 98.39%, with a Cohen's kappa of 0.806. The combined



self-sampling methods yielded an OPA of 96.05%, a PPA of 84.93%, and a NPA of 97.27%, with a Cohen's kappa of 0.788.

3.3 Cytological and immunocytochemical findings of the population

Among those with cytological alterations (25/226, 10.8%), ASC-US was the most common (20/226, 8.6%). ICC was positive for both P16 and Ki-67 in 17 samples (7.5%). Overall, 30 samples (13.3%) were positive for either cytology (ASC-US or higher) or ICC [P16 (+), Ki-67 (+)] ([Supplementary Table 2](#)).

3.4 Histological findings of the population

Of the 76 participants eligible for colposcopy, 61 (80.2%) completed the procedure, with 54 cervical samples (71.1%) collected. Histological findings showed 24 women (44.4%) with less than cervical intraepithelial neoplasia grade 1 (< CIN1), 20 (37.0%) with

CIN1, and 10 (18.5%) with grade 2 or worse ($\geq$ CIN2) ([Supplementary Table 3](#)).

The accuracy rates for detecting CIN2+ lesions are shown in [Table 3](#). Clinician-collected cervical samples had a sensitivity of 80.00% and a specificity of 98.33%, and an agreement of 98.25%. Urine self-sampling had a lower sensitivity of 70.00% and a specificity of 98.23%, and an agreement of 98.10%. Vaginal self-sampling had a sensitivity of 90.00% and a specificity of 98.50%, and an agreement of 98.46%. The combined approach of urine and vaginal self-sampling maintained a sensitivity of 90.00% and a specificity of 98.17%, with an agreement of 98.14%. All methods yielded high negative predictive values (NPVs), high positive likelihood ratios (PLRs), and low negative likelihood ratios (NLRs).

3.5 Awareness, feelings, and acceptability of self-sampling

The participants' awareness of cervical cancer, HPV, and self-sampling is presented in [Table 4](#). Awareness of cervical cancer was

TABLE 1 Characteristics of the participants ($N = 2,228$).

Characteristics	No. of women n , %
Age [median (IQR)]	52 (40–64)
Education	
College graduate or more	599 (26.9)
High school graduate	377 (16.9)
Middle school graduate	925 (41.5)
Primary graduate	301 (13.5)
Uneducated	26 (1.2)
Marriage	
Married/living with partner	2,071 (93.0)
Divorced/separated/widowed	131 (5.9)
Single	26 (1.2)
Contraception (within 3 years)	
Yes	680 (30.5)
No	1,548 (69.5)
HPV vaccination	
Yes	191 (8.6)
No	2,037 (91.4)
Previous cervical screening	
Yes	769 (34.5)
No	1,459 (65.5)
Cervical screening time	
Within 3 years	620 (80.6)
Beyond 3 years	131 (17.0)
Not sure	18 (2.3)
Cervical screening method	
Pap smear or LCT	516 (67.1)
HPV testing	405 (52.7)
VIA/VILI	11 (1.4)
Other	6 (0.8)
Not sure	121 (15.7)

The results are presented as n (%) if not otherwise stated.

IQR, interquartile range; HPV, human papillomavirus; LCT, liquid-based cytology test; VIA/VILI, visual inspection with acetic acid and Lugol's iodine.

high, with 86.1% (1,905/2,212) of participants aware of the disease, and 71.6% (1,583/2,212) believing it could be prevented. In contrast, only 35% (775/2,212) were aware of HPV, and 88.9% (245/2,212) had never heard of HPV self-sampling. Among those aware of HPV self-sampling, medical professionals were the primary source of information (51.4%, 126/245).

The participants' feelings about self-sampling are presented in Table 5. Most were confident in performing self-sampling (urine vs. vaginal: 97.7% vs. 86.5%, $p < 0.0001$). Embarrassment was reported less frequently during urine self-sampling (3.6%, 80/2,212) than during vaginal (12.5%, 277/2,212, $p < 0.0001$) or clinician-collected sampling (11.8%, 262/2,212, $p < 0.0001$). Discomfort or pain was reported less frequently during urine self-sampling (2.2%, 48/2,212) than during vaginal (18.6%, 412/2,212, $p < 0.0001$) or clinician-collected sampling (48.4%, 1,071/2,212, $p < 0.0001$), and less

frequently during vaginal self-sampling than during clinician-collected sampling (18.6% vs. 48.4%, $p < 0.0001$).

The participants' preferences for self-sampling are presented in Table 6. Most (75.9%, $n = 1,679$) favored clinician sampling, mainly because of trust in physicians (94.2%, $n = 1,582$) and concerns about incorrect sampling (34.1%, $n = 573$). In contrast, 22.0% ($n = 487$) preferred self-sampling, citing the inconvenience of gynecological exams (39.8%, $n = 194$), better privacy (37.2%, $n = 181$), and less pain and fear (33.5%, $n = 163$). Among those preferring clinician sampling, 41.6% ($n = 699$) would choose self-sampling if clinician sampling was unavailable. When comparing urine to vaginal self-sampling, 35.5% ($n = 785$) preferred urine for its convenience (96.3%, $n = 756$), 11.0% ($n = 243$) preferred vaginal self-sampling for its reliability (94.2%, $n = 229$), 52.8% ($n = 1,167$) had no strong preference.

The participants' acceptance of self-sampling is presented in Table 7. Urine self-sampling was perceived as easier than vaginal self-sampling ($Z = -34.0$, $p < 0.001$). Urine sampling was rated as "very easy" by 72.7% ($n = 1,608$) and "relatively easy" by 25.9% ($n = 572$), while for vaginal sampling, the rating were 30.4% ($n = 672$) and 46.4% ($n = 1,026$). Regarding preferred locations for self-sampling, 61.7% ($n = 1,365$) preferred guidance by medical staff in hospitals. Additionally, 86.2% ($n = 1,907$) were willing to send self-collected samples to a hospital or testing institution, and 80.9% ($n = 1,790$) were willing to recommend self-sampling to others.

4 Discussion

This study aimed to evaluate the feasibility and acceptability of detecting hrHPV and CIN2+ via urine and vaginal self-sampling compared with clinician sampling in urban areas of western China.

Our study evaluated the feasibility and acceptability of self-sampling in a population with low vaccination and screening rates. The HPV vaccination rate among the study population was 8.6% (191/2,228), which is higher than the national average in China (10) but lower than the global average of 15% (25). This may be because Shuangliu, the study location, was among the first regions in China to initiate cervical cancer vaccination programs. The overall cervical cancer screening rate was 34.5% (769/2,228), which was lower than the national average in China of 36.8% (95% CI: 35.1–38.4) (11).

Self-sampling could be a viable alternative in hrHPV screening, potentially improving accessibility and compliance. Arbyn's 2022 meta-analysis (16) of 4 studies conducted in screening populations reported a pooled OPA between vaginal self-sampling and clinician sampling of 88.1% (95% CI: 81.8–93.3), a kappa statistic of 0.65 (95% CI: 0.51–0.79), a PPA of 74.8% (95% CI: 60.0–87.1), and a NPA of 92.0% (95% CI: 86.9–94.8). Bober's 2021 meta-analysis (26) reported the diagnostic accuracy of first-void urine sampling versus clinician-collected sampling. For hrHPV detection, the sensitivity of urine self-sampling was 78% (95% CI: 70–84), and the specificity was 89% (95% CI: 81–94). For HPV16/18 detection, the sensitivity of urine self-sampling was 87% (95% CI: 74–94), and the specificity was 91% (95% CI: 83–96). Our study demonstrated strong concordance between urine and vaginal self-sampling and clinician-collected samples for hrHPV detection, with kappa values of 0.758 for urine, 0.806 for vaginal, and 0.788 for the combined methods. All three self-sampling methods (urine, vaginal and combined) resulted in high OPA, PPA, and NPA.

TABLE 2 Results of hrHPV testing—self-collected urine and vaginal samples compared with clinician-collected cervical samples.

Method	TP	TN	FP	FN	McNemar's χ^2	<i>p</i> value	OPA, % (95% CI)	PPA, % (95% CI)	NPA, % (95% CI)	Cohen's Kappa (95% CI)
hrHPV										
Urine self-sampling	172	1,945	49	47	0.04	> 0.05	95.66 (94.71 ~ 96.46)	78.54 (72.39 ~ 83.66)	97.54 (96.74 ~ 98.16)	0.758 (0.711 ~ 0.804)
Vaginal self-sampling	176	1,959	32	43	1.61	> 0.05	96.61 (95.74 ~ 97.30)	80.37 (74.35 ~ 85.29)	98.39 (97.71 ~ 98.88)	0.806 (0.763 ~ 0.848)
Combined self-sampling ^a	186	1,927	54	33	5.07	< 0.05	96.05 (95.12 ~ 96.80)	84.93 (79.34 ~ 89.26)	97.27 (96.43 ~ 97.93)	0.788 (0.746 ~ 0.831)
HPV 16/18^b										
Urine self-sampling	30	2,170	10	3	2.77	> 0.05	99.41 (98.97 ~ 99.67)	90.91 (74.53 ~ 97.62)	99.54 (99.13 ~ 99.77)	0.819 (0.722 ~ 0.916)
Vaginal self-sampling	29	2,170	7	4	0.36	> 0.05	99.50 (99.08 ~ 99.74)	87.88 (70.86 ~ 96.04)	99.68 (99.31 ~ 99.86)	0.838 (0.744 ~ 0.932)
Combined self-sampling ^a	31	2,154	13	2	6.67	< 0.05	99.32 (98.85 ~ 99.60)	93.94 (78.38 ~ 98.94)	99.40 (98.95 ~ 99.67)	0.802 (0.704 ~ 0.901)
Other 12 types of hrHPV										
Urine self-sampling	146	1,974	46	47	0.01	> 0.05	95.80 (94.85 ~ 96.58)	75.65 (68.86 ~ 81.40)	97.72 (96.95 ~ 98.31)	0.735 (0.684 ~ 0.787)
Vaginal self-sampling	152	1,990	27	41	2.88	> 0.05	96.92 (96.09 ~ 97.59)	78.76 (72.18 ~ 84.17)	98.66 (98.03 ~ 99.10)	0.800 (0.754 ~ 0.846)
Combined self-sampling ^a	159	1,958	49	34	2.71	> 0.05	96.23 (95.32 ~ 96.97)	82.38 (76.11 ~ 87.33)	97.56 (96.76 ~ 98.17)	0.772 (0.725 ~ 0.820)

hrHPV, high-risk human papillomavirus; TP, true positive; TN, true negative; FP, false positive; FN, false negative; OPA, overall percent agreement; PPA, positive percent agreement; NPA, negative percent agreement; CI, confidence interval.

^aEither sample (urine or vaginal sample) tested HPV positive is considered as positive.

^bEither HPV 16 or HPV 18 positive is considered as positive.

Our findings concerning the detection of CIN2+ are consistent with those of other studies investigating the accuracy of HPV testing via self-collected vaginal or urine samples. Previous studies, including clinical trials and meta-analyses, have shown that HPV testing on self-collected vaginal or urine samples has a similar accuracy to that of clinician-collected cervical samples (15, 27–32). For example, a 2018 meta-analysis by Arbyn et al. (27) revealed that compared with clinician sampling, PCR-based hrHPV assays had similar diagnostic accuracy for CIN2+ or CIN3+ patients via vaginal self-sampling (pooled sensitivity ratio for CIN2+: 99, 95% CI: 0.97–1.02). Additionally, two randomized trials (28, 32) confirmed comparable accuracy between self-collected and clinician-collected samples for detecting CIN2+ or CIN3+ lesions. The 2021 meta-analysis by Cho et al. (33) demonstrated that HPV testing via a PCR-based urine detection method showed similar clinical accuracy to clinician-collected samples in detecting CIN2 or more severe lesions.

In terms of participants' awareness of cervical cancer, HPV and self-sampling, the majority were aware of cervical cancer and thought that it could be prevented. However, their knowledge of HPV is limited and may contribute to the low rates of HPV vaccination and cervical cancer screening in China. Most participants (88.9%, $n = 1,967$) were introduced to self-sampling techniques and underwent self-sampling for the first time throughout our project. Consequently, their experiences in this self-sampling initiative could directly influence their acceptance of the technique. Since self-sampling is not

yet widely adopted for cervical cancer screening in China, our study offers a genuine reflection of initial impressions and acceptance levels among the screening population. Consistent with previous studies (34, 35), most participants were confident in their ability to collect samples correctly (97.7%, $n = 2,162$) and reported less embarrassment (3.6%, $n = 80$) and pain (2.2%, $n = 48$) during the process.

Nelson's 2017 meta-analysis (36) evaluated patient acceptance and preferences for self-sampling versus clinician-collected sampling across 23 studies ($N = 12,610$), finding an average preference rate of 59% (95% CI: 0.48–0.69) for self-sampling. In our study, only 22.1% ($n = 487$) of the participants preferred self-sampling, whereas most (75.9%, $n = 1,679$) favored clinician sampling because of their trust in physicians (94.2%, $n = 1,582$), lack of confidence in self-sampling (34.1%, $n = 573$), and fear of self-inflicted injury (5.5%, $n = 93$). This may be due to low awareness of self-sampling and the fact that most participants were sampling themselves for the first time. However, if clinician assistance was unavailable, half (50.7%, $n = 852$) of those who preferred clinician sampling would opt for self-sampling. When asked about their preferred self-sampling method, most (50.7%, $n = 852$) reported that both urine and vaginal self-sampling were acceptable. Those favoring urine sampling cited convenience (96.3%, $n = 756$), whereas those preferring vaginal sampling considered it more reliable (94.2%, $n = 229$). Thus, in areas with limited medical resources, self-sampling could be a viable alternative to clinician-collected sampling. Urine self-sampling was considered easier

TABLE 3 Accuracy rates for the detection of CIN2+ in cervical, vaginal, and urine samples.

Method	TP	TN	FP	FN	Sensitivity, % (95% CI)	Specificity, % (95% CI)	Agreement, % (95% CI)	PPV, % (95% CI)	NPV, % (95% CI)	PLR (95% CI)	NLR (95% CI)
Clinician routine ^a	8	2,181	37	2	80.00 (44.22 ~ 96.46)	98.33 (97.68 ~ 98.81)	98.25 (97.59 ~ 98.74)	17.78 (8.51 ~ 32.58)	99.91 (99.63 ~ 99.98)	47.96 (30.73 ~ 74.84)	0.20 (0.06 ~ 0.70)
Urine routine ^b	7	2,164	39	3	70.00 (35.37 ~ 91.91)	98.23 (97.56 ~ 98.72)	98.10 (97.42 ~ 98.61)	15.22 (6.84 ~ 29.48)	99.86 (99.56 ~ 99.96)	39.54 (23.71 ~ 65.93)	0.31 (0.12 ~ 0.79)
Vaginal routine ^b	9	2,167	33	1	90.00 (54.12 ~ 99.48)	98.50 (97.87 ~ 98.95)	98.46 (97.83 ~ 98.92)	21.43 (10.84 ~ 37.24)	99.95 (99.70 ~ 100)	60.00 (40.35 ~ 89.21)	0.10 (0.02 ~ 0.65)
Combined routine ^c	9	2,150	40	1	90.00 (54.12 ~ 99.48)	98.17 (97.50 ~ 98.68)	98.14 (97.46 ~ 98.64)	18.37 (9.24 ~ 32.50)	99.95 (99.70 ~ 100)	49.28 (34.03 ~ 71.34)	0.10 (0.02 ~ 0.65)

HPV: Human papillomavirus; TP: true positive; TN: true negative; FP: false positive; FN: false negative; PPV: positive predictive value; NPV: negative predictive value; PLR: positive likelihood ratio; NLR: negative likelihood ratio; CI: confidence interval.

^aDefined as HPV16/18 positive in clinician-collected samples, or positive for other hrHPV types in clinician-collected samples with concurrent positive LCT/ICC.

^bDefined as HPV16/18 positive in self-collected urine/vaginal samples, or positive for other hrHPV types in self-collected urine/vaginal samples with concurrent positive LCT/ICC.

^cEither routine (urine or vaginal routine) positive is considered as positive.

TABLE 4 Participants' awareness of cervical cancer, HPV, and self-sampling (n = 2,212).

Awareness	No. of women n, %	
Are you aware of cervical cancer?	Yes—1,905 (86.1)	No—307 (13.9)
Do you think cervical cancer can be prevented?	Yes—1,583 (71.6)	No—64 (2.9)
	Not sure—565 (25.5)	
Are you aware of human papillomavirus or HPV?	Yes—775 (35.0)	No—1,437 (65.0)
Are you aware of HPV self-sampling?	Yes—245 (11.1)	No—1,967 (88.9)
How did you learn about HPV self-sampling?		
Participated in research projects.	33 (13.5)	
By hospital or medical professionals.	126 (51.4)	
Heard from relatives or friends.	45 (18.4)	
Seen online.	46 (18.8)	

The results are presented as n (%). HPV, human papillomavirus.

(Mann–Whitney U test, Z: −34.0, $p < 0.001$), suggesting that it may achieve greater acceptance in resource-limited settings.

Nelson’s 2017 meta-analysis (36) reported that 97% (95% CI: 0.95–0.98) of patients reported that self-sampling was generally acceptable (7 studies, N = 1,470), and 87% (95% CI: 0.73–0.95) expressed a willingness to repeat it (9 studies, N = 2,660). In our study, 86.2% ($n = 1,907$) of the participants were willing to send self-collected samples to a hospital or testing institution, and 80.9% ($n = 1,790$) were willing to introduce self-sampling to others, indicating a promising outlook for promoting self-sampling. Di Gennaro’s meta-analysis (13) revealed similar preferences for home sampling (66, 95% CI: 57–74%) and clinical settings (67, 95% CI: 62–71%; $p = 0.841$), whereas Nishimura’s review (37) of 8 studies revealed a preference for home collection. In contrast, our study, which provided video and professional instructions, revealed that most participants (61.7%, $n = 1,365$) preferred self-sampling under medical guidance. This preference may stem from a lack of confidence in self-sampling skills and concerns about accessing treatment when needed. These findings suggest that combining media with professional guidance could effectively promote self-sampling adoption.

Our study presents certain innovations. It focuses on western China, where low- to middle-income populations are more common and where medical resources, screening rates, and compliance are lower than those in the more developed eastern regions. Given the limited research in this area, our study on the feasibility and acceptability of self-sampling provides valuable insights and potential strategies to improve screening rates and compliance in this region.

However, our study has several limitations. As this study focused on primary screening, the low number of CIN2+ cases may have affected the feasibility assessment. Expanding the screening scale is necessary to ensure a more representative evaluation of diagnostic accuracy. Self-sampling techniques, especially urine self-sampling,

TABLE 5 Participants' feelings about self-sampling ($n = 2,212$).

Feelings		No. of women n , %			Pearson χ^2	p value ^a
		Urine self-sampling	Vaginal self-sampling	Clinician sampling		
I was able to collect samples correctly.	Yes	2,162 (97.7)	1,914 (86.5)	–	194.13	< 0.0001
	No	46 (2.1)	292 (13.2)	–		
	Refusal ^b	4 (0.2)	6 (0.3)	–		
I felt embarrassed during sampling.	Yes	80 (3.6)	277 (12.5)	262 (11.8)	U vs. C: 105.51	< 0.0001
	No	2,111 (95.4)	1,916 (86.6)	1,924 (87.0)	U vs. V: 118.15	< 0.0001
	Refusal ^b	21 (0.9)	19 (0.9)	26 (1.2)	V vs. C: 0.42	> 0.017
I felt discomfort or pain during sampling.	Yes	48 (2.2)	412 (18.6)	1,071 (48.4)	U vs. C: 1257.08	< 0.0001
	No	2,141 (96.8)	1,771 (80.1)	1,117 (50.5)	U vs. V: 323.02	< 0.0001
	Refusal ^b	23 (1.0)	29 (1.3)	24 (1.1)	V vs. C: 440.94	< 0.0001

The results are presented as n (%).

^aBonferroni's correction for multiple comparisons. p values < 0.017 were considered statistically significant.

^bThe participant refused to answer this question.

U: urine self-sampling; V: vaginal self-sampling; C: clinician-collected sampling.

TABLE 6 Participants' preferences for self-sampling ($n = 2,212$).

Preference	No. of women <i>n</i> , %	
Which do you prefer: self-sampling or clinician-collected sampling? Why?		
Clinician sampling	1,679 (75.9)	
Trust in physicians.	1,582 (94.2)	
Lack of confidence in self-sampling.	573 (34.1)	
Fear of injury during self-sampling.	93 (5.5)	
Other reasons.	31 (1.8)	
Self-sampling	487 (22.1)	
Gynecological examinations are inconvenient.	194 (39.8)	
Transportation to the hospital is inconvenient.	65 (13.3)	
Better protection of privacy.	181 (37.2)	
Less pain and fear.	163 (33.5)	
Other reasons.	126 (25.9)	
Not sure or refuse to answer	46 (2.1)	
(For participants who prefer clinician-collected sampling) If clinician-collected sampling were not available, would you choose self-sampling?	Yes—699 (41.6)	Maybe—153 (9.1)
	No—775 (46.2)	Not sure—61 (3.6)
Which do you prefer: urine self-sampling or vaginal self-sampling? Why?		
Urine self-sampling	785 (35.5)	
More convenient.	756 (96.3)	
Vaginal self-sampling may hurt oneself.	54 (6.9)	
Vaginal self-sampling equipment may not clean.	30 (3.8)	
Other reasons.	13 (1.7)	
Vaginal self-sampling	243 (11.0)	
More reliable.	229 (94.2)	
More convenient.	24 (9.9)	
Other reasons.	27 (11.1)	
Both	1,167 (52.8)	
Not sure or refuse to answer	17 (0.8)	

The results are presented as n (%).

TABLE 7 Participants' acceptance of self-sampling ($n = 2,212$).

Acceptance	No. of women n , %	
The difficulty level of urine self-sampling		
1—very easy	1,608 (72.7)	
2—relatively easy	572 (25.9)	
3—somewhat difficult	24 (1.1)	
4—very difficult	2 (0.1)	
Difficult to describe or refuse to answer	6 (0.3)	Mann–Whitney U test Z: −34.0 P < 0.001
The difficulty level of vaginal self-sampling		
1—very easy	672 (30.4)	
2—relatively easy	1,026 (46.4)	
3—somewhat difficult	419 (18.9)	
4—very difficult	61 (2.8)	
Difficult to describe or refuse to answer	34 (1.5)	
Place for self-sampling		
At home.	337 (15.2)	
A private room in the hospital.	188 (8.5)	
Guided by medical staff.	1,365 (61.7)	
All the above.	272 (12.3)	
Not sure.	42 (1.9)	
Refuse to answer	8 (0.4)	
Would you be willing to send the self-collected samples to the hospital or testing institution?	Yes—1,907 (86.2)	No—183 (8.3)
	Not sure—199 (5.4)	Refuse to answer—3 (0.1)
Would you be willing to recommend self-sampling technology to others?	Yes—1,790 (80.9)	No—274 (12.4)
	Not sure—138 (6.2)	Refuse to answer—10 (0.5)

The results are presented as n (%).

need further refinement to increase sensitivity and specificity. Additionally, multicenter, large-scale prospective studies are needed to provide higher-level evidence for the adoption of HPV self-sampling.

5 Conclusion

Self-collected urine and vaginal samples for the detection of hrHPV and CIN2+ demonstrate high diagnostic accuracy and acceptability, making them viable alternatives to clinician-collected samples. Self-sampling methods may improve screening accessibility and compliance, especially in resource-limited settings, thereby supporting the prevention and early detection of CIN2+.

Data availability statement

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

Ethics statement

The studies involving humans were approved by the Peking Union Medical College Hospital (PUMCH) (approval code ZS-3293) and West China Second University Hospital (WCSUH). 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

SX: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing, Software, Visualization. XZ: Conceptualization, Data curation, Methodology, Project administration, Writing – review & editing, Investigation. JL: Conceptualization, Writing – review & editing, Data curation, Methodology. LK: Conceptualization, Data curation, Methodology, Software, Writing – review & editing. MX: Conceptualization, Funding acquisition, Project administration, Resources, Supervision,

Writing – review & editing. LX: Investigation, Writing – review & editing. PF: Investigation, Project administration, Writing – review & editing, Supervision. MZ: Investigation, Writing – review & editing. MA: Investigation, Writing – review & editing. XY: Investigation, Writing – review & editing. DL: Investigation, Writing – review & editing. GL: Conceptualization, Data curation, Methodology, Project administration, Resources, Supervision, Writing – review & editing.

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