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Chlamydia trachomatis infection: Epidemiology, prevention, clinical, and basic science research

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Published in
Frontiers in Public Health

ISSN 1664-8714
ISBN 978-2-83251-976-9
DOI 10.3389/978-2-83251-976-9

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Chlamydia trachomatis infection: Epidemiology, prevention, clinical, and basic science research

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Citation

Wang, C., Ong, J., Tang, W., eds. (2023). *Chlamydia trachomatis infection: Epidemiology, prevention, clinical, and basic science research*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-83251-976-9

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frontiers | Frontiers in Public Health

TYPE Editorial
PUBLISHED 07 March 2023
DOI 10.3389/fpubh.2023.1167690

OPEN ACCESS

EDITED AND REVIEWED BY
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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 16 February 2023
ACCEPTED 22 February 2023
PUBLISHED 07 March 2023

CITATION
Li C, Ong J, Tang W and Wang C (2023) Editorial: *Chlamydia trachomatis* infection: Epidemiology, prevention, clinical, and basic science research.
Front. Public Health 11:1167690.
doi: 10.3389/fpubh.2023.1167690

Editorial: *Chlamydia trachomatis* infection: Epidemiology, prevention, clinical, and basic science research

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KEYWORDS

review, epidemiology, prevention, clinical, chlamydia

Editorial on the Research Topic
Chlamydia trachomatis infection: Epidemiology, prevention, clinical, and basic science research

Chlamydia trachomatis (*C. trachomatis*) infection is a global health concern due to its serious reproductive health consequences, such as pelvic inflammatory disease (PID), ectopic pregnancy, and tubal infertility (1). It also facilitates HIV infection and transmission (2). In 2020, WHO estimated nearly 129 million new cases of *C. trachomatis* infection worldwide each year (3). Early detection and treatment have been implemented in high-income countries (HICs) for many years, potentially reducing the incidence of PID and tubal infertility (4). However, routine screening has been lacking in low- and middle-income countries (LMICs). The appropriate screening strategy remains unclear due to a lack of evidence on epidemic patterns, the cost-effectiveness of screening for *C. trachomatis* infection, and the different socioeconomic contexts in these regions. In this issue of Research Topic, "*Chlamydia trachomatis infection: Epidemiology, prevention, clinical, and basic science research*," the authors reported epidemic characteristics, the cost-effectiveness of screening, and improved treatment (Hu et al.; Wang et al.; Shi et al.; Pérez-González et al.; Sun et al.; Montes-Olivas et al.; Ning et al.; Liu et al.; Weng et al.; Yu et al.; Xu et al.; Huston et al.).

Although the high incidence and morbidity rates of *C. trachomatis* infection among women of reproductive age have been reported, there are few studies on the epidemic in this population in LMICs. Three studies from China reported a high prevalence of *C. trachomatis* infection of 9, 6, and 4, respectively, among 8,324 patients attending STI clinics, 306 men who have sex with men (MSM), and 3,580 female sex workers (FSWs) (Wang et al.; Hu et al.; Shi et al.). Consistently, all three studies found that individuals aged <25 years, those with low levels of education, and those who had ever or currently had sexually transmitted infections (STIs) were more likely to be infected with *C. trachomatis*. In addition, a cohort study in northwestern Spain suggested that PrEP users have a higher risk of *C. trachomatis* infection, especially among individuals who engage in chemsex (Pérez-González et al.). Further, a study from China reported that pre-pregnant couples should also be given more attention due to the higher risk of adverse pregnancy outcomes in *C. trachomatis*-infected women (32%, 9/28) and men (25%, 4/16) (Sun et al.). The above studies highlight the need for targeted interventions (e.g., routine screening) in pre-pregnant couples and key populations, including attendees of STI clinics, MSM, and FSWs, especially those aged <25 years, with low education and who have ever or currently had STIs.

Early detection and timely treatment are effective interventions to prevent reproductive harm from *C. trachomatis* infection. Routine *C. trachomatis* infection screening has been implemented in the UK, USA, and some European countries. However, the cost-effectiveness of screening is controversial, and the optimal screening strategy for each key population is poorly understood. A modeling study compared the cost-effectiveness of universal screening and targeted screening in the high-risk population of Hong Kong, China (Montes-Olivas et al.). It found that the most effective strategy was targeted screening with contact tracing for individuals with multiple partners. The ICER for targeted screening with contact tracing at 20 and 40% effectiveness was $4,634, and $7,219 per QALY gained, respectively (10-year time horizon). Before the screening, the clinical symptom may provide clues to identify the target population and improve the intervention strategy (Ning et al.). The responding symptoms include frequent urination/urgency/urodynia/itching, balanitis, and inguinal lymph node enlargement in men, and vaginal secretion increase or odors and vaginal itching in women, respectively. For example, another study found that *C. trachomatis* genotype H may be a sign for the target population because of its higher risk of cervical intraepithelial lesions (Liu et al.). The location of taking biospecimen is one of the important determinants of screening effectiveness.

Regarding the positivity rate, the rectal specimen is more appropriate for MSM (Weng et al.). A study in China found that the prevalence of *C. trachomatis* infection using rectal samples was almost six times higher than the prevalence in urine among MSM. Still, only 44% of MSM accepted, and 96% (128/133) of them successfully provided a valid rectal specimen.

Clinical treatment of *C. trachomatis* infection is considered convenient and safe, but the development of antibiotic-resistant strains and other treatment failures are often observed in patients. A study in China found that Rhein (4, 5-dihydroxyanthraquinone-2-carboxylic acid, a monomer primarily extracted from rhubarb) could improve the treatment of *C. trachomatis* infection (Yu et al.). Experiments *in vitro* and *in vivo* showed that Rhein could inhibit the growth of *C. trachomatis* by regulating pathogen-host cell interactions and synergizing the inhibitory effect of azithromycin (Xu et al.). Besides treating *C. trachomatis* infection cases, current guidelines recommend patient-delivered partner therapy to reduce chlamydia re-infection. Meanwhile, a study from Hong Kong examined the impact of screening only and screening plus accelerated partner therapy and showed that accelerated partner therapy did not significantly affect overall chlamydia prevalence and caused overtreatment (Montes-Olivas et al.).

Re-testing for chlamydia 3 months after treatment to detect possible re-infection has been recommended in HICs. However, re-testing does not appear to be universally implemented. A study of 5,806 heterosexuals with chlamydia in Melbourne, Australia, showed that only 36% were re-tested for chlamydia within 1 year, and 15% were reinfected (Xu et al.). Another cohort study of 305 women with urogenital chlamydial infection showed that 12% had recurrent infection after treatment with azithromycin and that recurrent infection was associated with sexual contact (Huston et al.). The low rate of chlamydia re-testing and the high rate of chlamydia re-infection highlight the need to optimize partner management and encourage testing for re-infection at 3 months.

Compared to primary infection, chlamydia DNA load was higher in women who experienced recurrent infection (Huston et al.). Vaginal chlamydial gene expression (ompA, euo, omcB, htrA, trpAB) was significantly higher at the time of recurrent infection or repeated positive tests during follow-up compared to baseline; two of the selected immune genes analyzed and had significantly lower expression at the time of recurrent infection (Huston et al.). The results suggest that repeat infections with chlamydia may be more transcriptionally active at certain genes after azithromycin treatment. There may be immunological changes after treatment that interact with repeat exposures to establish active infection. Therefore, more regular testing of women at the highest risk is needed to reduce the risk of sequelae.

Chlamydia is a significant threat to the population's sexual and reproductive health. The high prevalence and impact on reproductive health by the studies reported in this issue underscore the need for targeted CT screening of high-risk populations. Given differences in epidemiology and socioeconomic contexts, more research on the cost-effectiveness evaluation of routine screening, case management, and basic science is needed to improve prevention and clinical strategies.

Author contributions

CL drafted the first manuscript, other co-editors provided comments, and prepared the final version, which all co-authors approved. CW, JO, and WT contributed to formulating key lessons. All authors contributed to the article and approved the submitted version.

Conflict of interest

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

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 27 July 2022
DOI 10.3389/fpubh.2022.932096

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases – Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 29 April 2022
ACCEPTED 04 July 2022
PUBLISHED 27 July 2022

CITATION
Montes-Olivas S, Ozten Y, Homer M, Turner K, Fairley CK, Hocking JS, Tse D, Verschueren van Rees N, Wong WCW and Ong JJ (2022) Evaluating the impact and cost-effectiveness of chlamydia management strategies in Hong Kong: A modeling study. *Front. Public Health* 10:932096. doi: 10.3389/fpubh.2022.932096

Evaluating the impact and cost-effectiveness of chlamydia management strategies in Hong Kong: A modeling study

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Objectives: To illustrate the epidemiologic and cost-effectiveness impact of shifting the focus from population-based screening toward a targeted management approach for genital chlamydia infection.

Design: Modeling study, implementing an individual-based, stochastic, dynamic network model.

Setting: Hong Kong.

Population: A hypothetical sample network of 10,000 people with a partnership distribution based on Hong Kong's sexually active population of reproductive age (age 18–49 years).

Interventions: In this study, we present several scenarios with different implementations of universal vs. targeted screening (based on partner numbers). We also explored the impact of (1) screening only, (2) screening plus expedited partner therapy, and (3) screening plus partner testing.

Primary outcome measures: Change of chlamydia prevalence before and after implementing the different strategies. The cost-effectiveness analysis reports total direct cost from a health provider perspective, the QALYs gained, and incremental cost-effectiveness ratios (ICER).

Results: In comparing the effects of universal screening only and targeted screening of the high-risk population, the mean prevalence during the 10th year of intervention was 2.75 ± 0.30% and 2.35 ± 0.21%, respectively (compared with 3.24 ± 0.30% and 3.35 ± 0.21% before the interventions, respectively). The addition of contact tracing to the latter targeted screening scenario reduces the mean prevalence during the 10th year of intervention

to 1.48 ± 0.13% (compared with 3.31 ± 0.33% at baseline) in the best-case of testing before treatment and maximal contact-tracing effectiveness (40%). Overall, the most effective scenarios were those for which interventions focused on the high-risk population defined by the number of partners, with contact tracing included. The ICER for targeted screening with contact tracing at 20% and 40% efficiency was $4,634 and $7,219 per QALY gained, respectively (10-year time horizon). Expedited partner therapy did not significantly impact overall chlamydia prevalence and caused overtreatment.

Conclusions: Our study suggests that targeted screening with strengthened contact tracing efforts is the most cost-effective strategy to reduce the prevalence of chlamydia in Hong Kong.

KEYWORDS

chlamydia, cost-effectiveness, economic evaluation, dynamic network models, stochastic model

Introduction

Chlamydia trachomatis is the most common bacterial sexually transmitted infection (STI) globally (1). Untreated infections can result in long-term reproductive health consequences such as pelvic inflammatory disease (PID), ectopic pregnancy and infertility in women and infection during pregnancy may result in neonatal blindness, pneumonia or death (2, 3). In men, chlamydia can cause urethritis, proctitis and epididymo-orchitis. Although there is some evidence to support screening to reduce the incidence of chlamydia and hence the complications (4, 5), population-based screening of asymptomatic individuals has been questioned. Opportunistic screening of at-risk populations has not effectively reduced the overall prevalence of chlamydia (6, 7).

Recent discussions have shifted the focus from population-based screening toward strengthening patient management (1, 7). Thus, there are recommendations to move toward a targeted approach instead and strengthen methods to control and prevent the disease (7). For example, best practice sexual health guidelines suggest strengthened case management using contact tracing, retesting for reinfection between 3 and 6 months after chlamydia treatment, and expedited partner therapy (1, 8, 9). Thus, there is a need to evaluate how these additional practices may aid in achieving reductions in chlamydia compared with efforts to increase population-based screening. Although the overall *Chlamydia trachomatis* prevalence was low in Hong Kong at 1.4% (95%CI 0.8–2.5%), sexually active young (18–26 years) women had a relatively high prevalence (5.8%, 95%CI 1.7–18.2%) and a unique U-shape disease burden was observed with peaks in younger and older (40–49 years) women (10). There is currently no national chlamydia screening policy in Hong Kong. Since most chlamydia cases are asymptomatic, control of the disease is a significant challenge even though it can be cured with a course of antibiotics. At the same time, poor awareness, stigma and discrimination associated with STIs in Chinese societies such as Hong Kong prevents contact tracing and stops many from seeking testing and treatment (11).

Mathematical models can explore potential control strategies and incorporate the non-linear dynamics of infectious diseases to optimize interventions. Additionally, these models can give an insight into the cost-effectiveness of these strategies. The model used in this research is an individual-based dynamic transmission model, which represents each individual as a set of states that change over time, allowing large-scale behavior to emerge from small-scale processes.

This study aims to use data from Hong Kong in an individual-based model of chlamydia transmission to illustrate the epidemiologic and cost-effectiveness impact of shifting the focus away from population-based screening toward a targeted approach.

Methods

We adapted an individual-based, stochastic, dynamic network model of a sexually transmitted infection, previously developed and parameterised for gonorrhea among men who have sex with men (MSM) in the UK (12). This model was originally used to examine antimicrobial-resistant gonorrhea strain development, and we adapted it to represent chlamydia transmission dynamics in a Hong Kong context. The main adaptations were modifying the partnership network to allow only heterosexual partnerships, the presence of a single strain of chlamydia (as opposed to two gonorrhea strains in the previous model), and a single drug therapy treatment.

The stochastic, discrete-time Markov model follows the Susceptible-Infected-Susceptible paradigm (SIS), such that individuals are susceptible to infection through interaction with a sexual partner. In this model, individuals correspond

to nodes in a network that accounts for sexual partnerships. Every individual can be infected or susceptible to a single strain of chlamydia. Susceptible individuals become infected though contact with infected partners, with a probability β (per contact per day). Following recovery from the illness after treatment or spontaneous natural recovery, an individual becomes susceptible again. A range of different treatment and intervention pathways are considered, described in Section Intervention scenarios below. We have modified the partnership network to generate a bipartite network that only allows heterosexual partnerships. Similar to the MSM model, the adapted model assumes a scale-free network that obeys a power-law degree distribution, but here each sex has a different power-law exponent (α), as reported by Schneeberger et al. (13). In addition, a small external influx of disease (η) has been added to include the possible input from the mobility of nodes outside the network.

The model was completely re-parameterised to account for the different disease epidemiology, and change in modeled population. By updating sexual behavior parameters according to Hong Kong's population, and implementing various interventions consistent with the Hong Kong setting, we used our updated model to understand the impact and cost of screening interventions. The parameters modified to fit the model to our target population are described in the baseline section and their values can be found in Table 1. We considered the local healthcare perspective for costs and benefits associated with disease prevention to evaluate a setting-specific impact and cost-effectiveness analysis.

Model structure

The individualized model has been previously published (12), and further technical details are provided in the Supplementary Material (12). The model has dynamic, stochastic sexual partnership dynamics: individuals are explicitly represented as nodes. Each individual is represented by a (time-varying) vector, indicating their infection status, symptoms or lack thereof, whether they require testing/treatment, flags to represent whether they have been traced or screened, and their associated delays. There are two sexes (male and female), and each node has its own assigned degree denoting its associated number of sexual partners per year. An important part of the model is the ability to represent the dynamics of highly connected individuals or "super-spreaders". A network restriction algorithm is employed to mimic time-varying partnership networks while preserving long-term network structure; see Zienkiewicz et al. (12) for more details.

The model does not incorporate age structure or site-specific infection. It does contain flexible options for treatment-seeking due to symptoms and asymptomatic screening and includes variable delays according to service provision in time to test and time to receive treatment, as well as options for contact

TABLE 1 Parameter values, and reference, used in the model scenarios presented in this study.

Parameter	Symbol	Baseline	Scenarios	Reference
Total population size	N	10,000	Maintained	Selected
Power law exponent	α	Females: 2.8 Males: 2.5	Maintained	Fitted using (10, 14) and MATLAB "polyfit"
Transmission probability	β	0.0016 per sexual partner per day	0.0016–0.0020	Fitted to achieve suitable prevalence value
Natural recovery rate (asymptomatic)	R	0.0027 per day	Maintained	(15)
Fraction symptomatic	P_{symp}	10%	Maintained	(16)
Symptomatic testing	P_{seek}	62%	Maintained	(17)
Screening rate	γ	0	0.00027–0.0012 per day	Assumed
Tracing efficiency	Ψ	0%	2–40%	Assumed
Extra-network CT import probability	η	Males: 9×10^{-5} Females: 0 per person per day	Maintained	Assumed
Interval between screening and returning for treatment (Lab delay + Seek delay)	$\delta_L + \delta_S$	N/A	13 days	(18)
Time frame for partnerships network update		1 week	Maintained	Selected
Maximum partners during time frame		10	Maintained	Assumed
Initial chlamydia prevalence		3–4%	Maintained	Fitted

tracing. Model parameters are explained in more detail in the Supplementary Material and are summarized in Table 1.

Sexual partnership network construction

The underlying sexual partnership network is described using a small number of parameters. This has the advantage of reproducibility and is both fast and straightforward to parameterise. Firstly, the cumulative degree distribution, i.e. the number of sexual partners per person over a given timeframe (the network refreshes once a week), is used to calculate the power-law coefficient. If the partnerships reported are discrepant between men and women (e.g., men typically report a higher mean number of partners than women), this must be adjusted to create a balanced partnership structure across the entire population. Once this static cumulative distribution is described, the whole network of contacts is created for the year using an algorithm to join male and female nodes.

The second stage consists of the time-dependent network generation. This requires two additional parameters: the timeframe over which partnerships can be formed or dissolved, and the restricted maximum degree within the selected timeframe. As this timeframe increases, the simulation approaches the static network. As it decreases, it takes longer to run but can incorporate more realistic partnership dynamics. The original MSM model analysis showed that the underlying annual partnership network was well captured over wide ranges of the partnership timeframe and degree restriction parameters (12). For this study, we updated the partnership network once a week.

Baseline

We informed our choice of scenarios from recent challenges made to previous control strategies and expert knowledge of the Hong Kong health care system (Table 1). The baseline scenario was obtained according to current chlamydia prevalence and treatment statistics in Hong Kong. The population of interest in the present study are those sexually active, age between 18- and 49-years, whom in the case of Hong Kong was approximately 3,195,400 people in 2020, according to the demographic statistics of the census and statistics department (19). However, a network of that size would require a high computational cost. Thus, we compared different sized networks of 10,000, 20,000 and 1% of the population of interest (31,954) and observed a similar partnership distribution at time 0. Hence, a sample size network of 10,000 people was selected to efficiently run the scenarios presented in this study. The percentage of symptomatic patients was estimated from Korenromp, Sudaryo and de Vlas' study (16), who estimated that symptomatic chlamydia is 11% in males and 6% in females. Since the current model does not differentiate between sexes in relation to the proportion of symptomatic infections, we set the baseline of the symptomatic proportion at 10%. Treatment is given to all those who are symptomatic and seek treatment; we assume that only a proportion of the symptomatic population will seek treatment.

We used data from two surveys performed in Hong Kong (10) to estimate the slope parameters for the power-law distribution of the sexual partnership network. The distribution of the number of partners for both males and females estimated from the surveys was fitted using the 'polyfit' function in MATLAB.

This baseline was used on all intervention scenarios, with a burn-in period of 20 years.

Intervention scenarios

All intervention scenarios started in endemic equilibrium, in which we had a mean prevalence value in the range of 3–4% in a year. The model provided a range of control options. We first give an overview of the different control option scenarios before describing them in full detail below; Table 2 summarizes the strategies and relevant parameter values. The scenarios present different implementations of three main programs: (1) screening only with no contact tracing; (2) screening plus contact tracing, with treatment of all successfully traced contacts; and (3) screening plus contact tracing, with testing before treatment of successfully traced contacts. We also tested the additional impact of re-testing within 3–6 months, and targeting the screening plus contact tracing to a higher risk population (defined here as those reporting multiple sexual partners in one week) for the above programs.

The screening programme referred to an approach in which a selected population (either the entire modeled population or a selected sub-population) was tested for chlamydia according to a daily screening rate. This type of test was offered randomly to the selected population without further stratification by age or additional risk factors (e.g., sex workers). In the case of the contact tracing programme, all screened patients and those who voluntarily sought treatment were asked for a list of their partners to trace and notify them and provide treatment depending on the scenario provided. These recent contact partners were traced according to a specific tracing efficiency to account for different compliance scenarios. Afterwards, the traced partners would proceed through two routes: the first one successfully traced partners who would receive treatment without a laboratory test to confirm infection. This type of treatment has been recommended to reduce frontline delay times (20). In the second route, all successfully traced partners were tested and received treatment only when a positive result was returned.

10.3389/fpubh.2022.932096

TABLE 2 Summary of scenarios.

Scenario	Programs				Variables			
	Universal screening	Targeted screening						
	Only screening	Only screening	Screening plus partner tracing		Fraction symptomatic	Follow-up period	Screened population proportion in a year	Partner trace efficiency
			Treatment to all partners	Testing partners before treatment				
Non-targeted								
Ai	X				10%	-	≈10%	-
Aii	X				10%	-	≈30%	-
Targeted: Follow-up testing of patients seeking treatment								
Bi		X			10%	3 months	-	-
Bii		X				6 months	-	-
Biii		X				12 months	-	-
Biv			X			3 months	-	40%
Bv				X			-	
Ci		X			30%		-	-
Cii			X				-	40%
Ciii				X			-	
Targeted: Population with two partners or more								
Di		X			10%	-	≈10%	-
Dii			X			-	-	2%
Diii			X			-	-	10%
Div			X			-	-	20%
Dv			X			-	-	40%
Ei				X		-	-	2%
Eii				X		-	-	10%
Eiii				X		-	-	20%
Eiv				X		-	-	40%

Scenarios A implements non-targeted screening programs. Scenarios B/C target the symptomatic population seeking treatment by performing follow-up testing. Scenarios D/ E target the population with two or more partners.

Universal screening (scenarios A)

In these scenarios, we applied a universal screening with a screening rate that covers around ∼10 and ∼30% of the sexually active population per year (see Table 2, scenarios Ai and Aii, respectively). This type of screening is offered randomly in the population without considering factors like the number of partners or symptoms.

Targeted screening (scenarios B to E)

Follow-up testing of patients seeking treatment (scenarios B and C)

Symptomatic patients with chlamydia who sought treatment would have a follow-up test at a certain period after their appointment/treatment. The waiting period was 92 days (∼3 months) for most of the scenarios presented in this study. However, 6 months and 1 year of waiting period were also included (see Table 2, scenarios Bii and Biii, respectively). For the scenarios using this definition of targeted screening, we also tested two different fractions of the symptomatic population: a worst-case scenario with only 10% of the symptomatic population (see Table 2, scenarios B) and a more realistic scenario with a fraction of symptomatic population at 30% (see Table 2, scenarios C).

Targeting a higher-risk population (scenarios D and E)

In these scenarios, we changed the screened population to aim at those considered a higher risk. The "higher risk" subpopulation was defined as people in the network with two or more partners in the last week. Scenarios D and E (see Table 2) worked similarly to the universal screening, with treatment to all successfully traced partners (scenario D) or testing before

treatment (scenario E), but instead focused on the proportion of the network defined as higher risk. The screening rate was modified to obtain a similar proportion of screened patients as the universal screening scenario (∼10% per year). This type of targeted screening did not consider if the patient was symptomatic or not; thus, all scenarios were implemented in a network with 10% symptomatic population. We considered a range of partner trace efficiencies (the proportion of the index case's partners that are traced, on average): 2% (see Table 2 Dii, Ei), 10% (see Table 2 Diii, Eii), 20% (see Table 2 Div, Eiii), 40% (see Table 2 Dv, Eiv).

All simulated scenarios presented in Table 2 were applied during 10 simulated years and replicated 100 times.

Cost-effectiveness analysis

A cost-effectiveness model was constructed, which takes the outputs of the dynamic transmission model, and uses them to estimate the costs and benefits associated with each of the scenarios described above. The individual-based model returns a time-series of each infection, clinic attendance, treatment, contact tracing, and testing event. So, we can calculate the total costs of each event, for any period, given appropriate costs per intervention. We use the direct costs shown in Table 3. We calculate the direct cost of the interventions in each scenario, in a given year of the control period, as the mean difference between the total direct costs in that year and those in the final pre-control year (across the ensemble of simulations for the relevant scenario.

The cost analysis method implemented is similar to the one used by Turner et al. (22), in which the direct costs associated with testing, clinic attendance, treatment and contact tracing are estimated and complemented with the saved costs of averted follow-on complications of untreated chlamydia infections. We also model the saved costs of averted follow-on complications of untreated chlamydia infections as a result of the interventions in each scenario and the resulting quality adjusted life year (QALY) gained. We considered six sequalae: four in females–pelvic inflammatory disease (PID), chronic pelvic pain (CPP), ectopic pregnancy (EP), and tubal factor infertility (TFI)–and two in males –epididymitis and urethritis. We take a pragmatic approach, as in Adams et al. (23) to calculate the benefits. Time-series of untreated chlamydia infections by gender are output by the dynamic transmission model, from which each follow-on infection has a probability of occurrence p, and associated health state utility value (HSUV) H & duration T, giving a QALY gained (per complication, per untreated chlamydia infection, p(1-H)T. The mean numbers of sequelae are calculated in post-processing; the model dynamics are independent of any follow-on infections. The values of the parameters used to evaluate each complication, and their associated treatment costs in Hong Kong, are given in Table 4. There is no evidence of the willingness to pay threshold for Hong Kong, thus we present our findings (Table 5) according to 1–3 times the GDP per capita, as per WHO guidance.

TABLE 3 Estimated average direct costs of modeled infection events.

Event	Cost (HKD)	Reference and assumptions
Clinic consultation (per attendance)	445	(21)
Testing (per chlamydia test)	700	Hong Kong Department of Health
Contact tracing (per index case)	44.5	Assumed to be 10% of the consultation cost
Treatment (per course of antibiotics)	361	Private practice

Results

Figure 1 compares the effect of universal screening, scenarios Ai and Aii (Figures 1.1–3 and 1.4–6 respectively) and targeted screening of high-risk population defined by the number of partners, scenario Di (Figures 1.7–9). The change

TABLE 4 Follow-on complication parameters: probability of occurrence (per untreated chlamydia infection), health state utility value (HSUV), duration and direct financial cost.

Complication	Probability p	HSUV H	Duration T	Cost (HKD)	Reference and assumptions
Pelvic inflammatory disease	10%	0.9	11 days	6,000	(24–27)
Chronic pelvic pain	1.8%	0.69	5 years	4,000	(24–26, 28)
Ectopic pregnancy	0.76%	0.79	4 weeks	20,000	(24–26, 28)
Tubal factor infertility	0.998%	0.76	15 years	50,000	(24–26, 28)
Epididymitis	2%	0.92	1 week	4,000	(25, 27, 29)
Urethritis	75%	0.93	1 week	445	(29)

TABLE 5 Results of the cost-effectiveness analysis: costs per QALY gained.

Scenario	Newly incurred direct cost/QALY gain		Net cost/QALY gain	
	Year 1	Year 10	Year 1	Year 10
Non-targeted				
Ai	$512,499	$343,590	$487,348	$318,438
Aii	$596,685	$423,059	$571,533	$397,907
Targeted: Follow-up testing of patients seeking treatment				
Bi	−$110,978	$54,265	−$136,130	$29,113
Bii	$55,798	$67,573	$30,646	$42,421
Biii	−$1,978	$19,254	−$27,130	−$5,898
Biv	$40,943	−$87,493	$15,791	−$112,645
Bv	$90,293	$219,212	$65,142	$194,060
Ci	$115,551	$125,699	$90,399	$100,547
Cii	$79,901	$100,692	$54,749	$75,540
Ciii	$354,610	$255,767	$329,458	$230,615
Targeted: Population with two partners or more				
Di	$362,043	$185,584	$336,891	$160,432
Dii	$45,335	$49,740	$20,183	$24,588
Diii	$29,622	$31,921	$4,470	$6,769
Div	$28,511	$30,398	$3,359	$5,246
Dv	$28,845	$30,612	$3,693	$5,460
Ei	$356,063	$200,862	$330,911	$175,710
Eii	$521,497	$322,297	$496,345	$297,145
Eiii	$593,423	$422,323	$568,271	$397,171
Eiv	$634,844	$540,549	$609,692	$515,397

For each scenario (labels as defined in Table 2), the newly incurred direct costs in HKD per QALY gained are shown in columns 2–3 (years 1 and 10 of the control period respectively), and the net change in costs in HKD per QALY gained are shown in columns 3–4 (years 1 10 of the control period respectively). The color scale is from 0 (green) to 3 (red) times HK GDP per capita (HK$357,076 in 2020) (30); negative results are uncolored. Direct costs (columns 2–3) include clinic attendance, treatment, tracing and testing costs as appropriate for each scenario. Net costs (columns 3–4) additionally include the (reduction in) costs due to averted complications; see the text for details.

in chlamydia prevalence before and after implementing the two approaches is displayed in Figures 1.1,4,7. The change in the number of treatments provided per month is presented in Figures 1.2,5,8. The main difference between the effect of both approaches shown here is that the targeted approach can reach a higher number of infected individuals at the beginning when reaching a similar number of screenings per year. This means that more treatment is provided during the first months, further decreasing the prevalence before reaching a steady state again. Here we observe that, even when the average number of people being screened a year was similar (see Figures 1.3,9), the prevalence could be reduced further by targeting a specific higher-risk subpopulation of the network. During the last year of the burn-in period, the mean prevalence for scenario Ai (10% universal screening) was 3.24 ± 0.31%, and 2.75 ± 0.30% during the 10th year of the intervention. For scenario Aii (30% universal screening), the mean prevalence was 3.35 ± 0.31% at the end of the burn-in period and 2.01 ± 0.20% during the 10th year of the intervention. On the other hand, the mean prevalence of the burn-in period for scenario Di (targeted screening: 2 partners or more, 10% of the population screened per year) was 3.35 ± 0.38% during its last year and decreased to 2.35 ± 0.21% during the 10th year of intervention.

The targeted scenarios in which there was a follow-up screening for those patients' seeking attention and all their combinations were also analyzed. However, as shown in Figure 2, we did not observe a significant change in the overall prevalence due to the limited population who presented with symptoms and sought attention. It is important to highlight that this targeted intervention was analyzed by increasing the percentage of the population presenting with symptoms from 10% (Figures 2.1–4) to 30% (Figures 2.5,6) and by changing the follow-up periods on the population of 3 months (Figure 2.1), six months Figure 2.2) and a year (Figure 2.3).

On the other hand, the targeted scenarios in which the intervention was focused on the higher-risk population defined by the number of partners provided a more significant reduction of the mean chlamydia prevalence in the sample network. Figure 3 summarizes the main results obtained from the combination of these interventions. For scenario Di, in which screening only was applied as an intervention, the mean prevalence at equilibrium was 3.35 ± 0.38% (see Figure 3.1). After the intervention, this mean prevalence was reduced to 2.35 ± 0.21%. For the best-case scenario of screening plus contact tracing with an effectiveness of 40%, Dv, the mean prevalence changed from 3.27 ± 0.34% to 1.73 ± 0.17% (see Figure 3.5). For comparison, scenario Eiv, which is similar but without over-treating all the traced partners, has a mean prevalence of 3.31 ± 0.33% that was reduced to 1.48 ± 0.13% after the intervention period (see Figure 3.9).

Table 5 reports the results of the cost-effectiveness analysis. Two sets of cost/benefit results are shown for each intervention scenario, based solely on the costs of additional chlamydia treatment, testing, and tracing (columns 2–3) or also including the (saved) costs of averted complications (columns 4–5). The color scale in Table 5 indicates the ratio of the cost per QALY gained to the HK GDP per capita (in 2020), from 0 (green) to 3 (red), for ease of identification of good (green) to poor (red) cost-effectiveness. Note that some cost-effectiveness results returned a negative value (and are not color-scaled), particularly in the case of targeted scenarios in which there was a follow-up screening for those patients seeking attention (Bi, Biii and Biv). These results are caused by the negligible change in incidence (see Figure 2) and consequent negligible change in QALY gain; the effect is that the cost-benefit ratio in these cases is particularly sensitive to small statistical variation (as the ratio of two near-zero quantities) and should be viewed accordingly. Results are shown for years 1 and 10 of the control period, indicating the annual cost-effectiveness in the transient and steady-state, respectively.

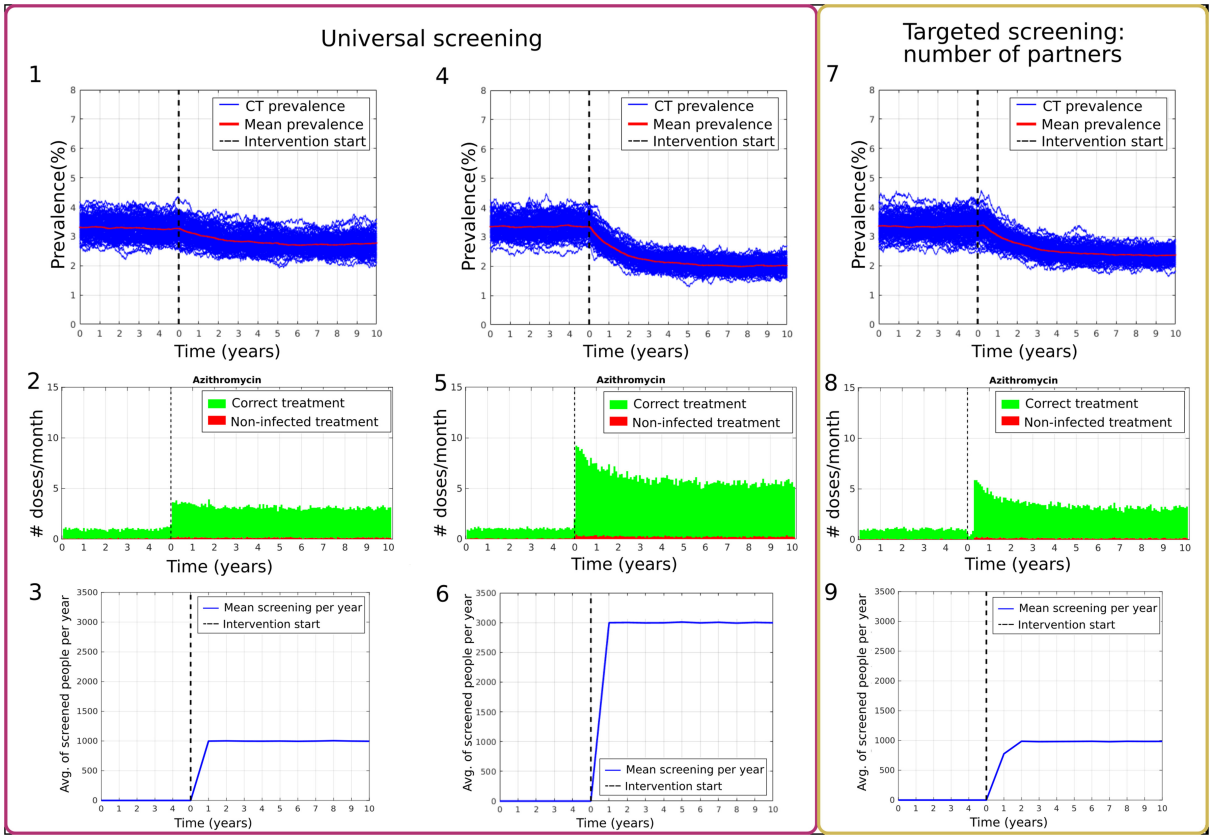

FIGURE 1
Comparison of program effects between universal screening with 10% and 30% of the population screened per year (**1–3** and **4–6** respectively) and targeted screening according to the number of partners with 10% of the population screened per year **(7–9)**. **(1,4,7)** show prevalence results over 100 simulations (blue) and the mean percentage of prevalence (red). **(2,5,8)** present t the number of doses provided per month, where the individual had a chlamydia infection (green) or was not infected (red). **(3,6,9)** show the average number of people screened per day.

In broad terms, the results in Table 5 suggest that the most cost-effective scenarios are those for which the intervention was focused on the higher-risk population defined by the number of partners, with contact tracing included (Dii-Dv). Reasonable-to-good cost-effectiveness can also be obtained in some targeted scenarios in which there was a follow-up screening for those patients seeking attention (Bii, Cii). However, the results in this case are vulnerable to the ratio of small values issue described above and should be interpreted with caution. There is an important cost that is not included in these results, which must be included in any future decision-making. In scenarios where there is contact tracing with treatment to all such traced partners (Biv, Cii, and Dii-Dv), there is extensive over-treatment (i.e., treatment of un-infected traced individuals)–see, for example, Supplementary Figure S8–which would potentially be a driver of antimicrobial resistance. Scenarios where testing precedes treatment for traced individuals (Bv, Cii, and Ei-Eiv) almost eliminate the over-treatment problem but are less cost-effective due to the high testing costs.

Table 6 summarizes the cost-benefit results in an incremental cost-effectiveness ratio (ICER) league table. The process by which scenarios are selected to be reported in the table follows the methodology of Paulden (31) scenarios are ranked by cost and removed if they are "dominated" (have higher cost and smaller or equal benefits than at least one other scenario) or "extendedly dominated" (similarly for a combination of two other scenarios). The results are shown for year 1 of the control period and years 1 to 10, the latter both with and without discounting. The discounting analysis considered both costs and benefits to be subject to discounting, at the same rate of 5%, over the 10-year time horizon of the scenario analysis period, starting at the beginning of the control period. In all cases, three scenarios remain. In order of increasing cost-effectiveness, these are scenarios Biii (follow-up testing of patients seeking treatment after 12 months), Div and Dv (targeted screening of population with two partners or more, with contact tracing at 20 and 40% efficiency, respectively). The corresponding ICERs for scenarios Div and Dv are $3,369 and $6,577 (in year 1), $4,634 and $7,219 (across years 1–10 without

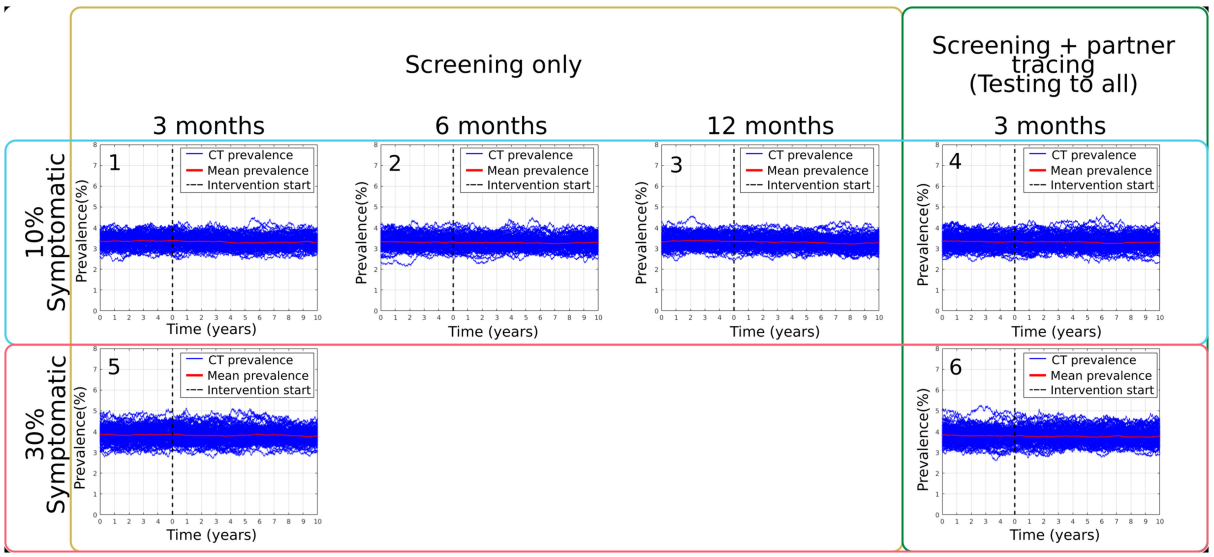

FIGURE 2
Summary of main results obtained from targeted: follow-up screening simulations, varying follow-up waiting periods, scenarios Bi **(1)**, Bii **(2)**, Biii **(3)**, the modeled percentage of symptomatic population present in the model, as well as the addition of one version of partner tracing on scenarios Biv **(4)**, Ci **(5)** and Cii **(6)**.

discounting), and $4,534 and $7,160 (across years 1–10 with discounting). Other plausible choices of discounting rates lead to the same three scenarios with broadly similar ICER values.

Discussion

This modeling study evaluates the epidemiologic and economic impact of chlamydia screening approaches in Hong Kong. We add to the literature by providing further evidence for the value to shift resources from a population-based universal screening toward more targeted testing with strengthened patient management. This is consistent with recent calls to rethink strategies to control chlamydia globally (1, 7).

Despite the potential for universal screening to reduce stigma because screening is offered to all irrespective of symptoms or risk behavior, our model showed this was not cost-effective compared to targeted testing with strengthened patient management. Universal screening (which may include opportunistic testing) is recommended in several countries (32–34) but is challenging to scale up and sustain without heavy investments and may not be effective in reducing chlamydia prevalence at the population level. For example, in a cluster-randomized controlled trial in Australia, increasing screening rates from 8 to 20% among 93,828 men and women aged 16 to 29 years had no significant impact on chlamydia prevalence in the community (35).

Our study demonstrated that targeting chlamydia testing toward high-risk individuals, as defined by those with multiple partners, was more cost-effective than universal screening for controlling chlamydia in Hong Kong. Practically, this could be challenging as people may not accurately disclose their sexual activity or under-report the number of sexual partners due to fear or stigma and discrimination. In addition, individuals with multiple partners may be less likely to present to traditional health facilities. There is an urgent need to understand the values and preferences for chlamydia testing and management among high-risk individuals (36). These preference data are helpful to better target individuals with multiple partners to improve the efficiency and cost-effectiveness of chlamydia testing programs. Spurred on by learnings from the COVID pandemic, implementation of novel models of service delivery should be considered, such as improving access points for testing such as point-of-care diagnostics (37), self-testing (38), telehealth or online testing services (39), express STI clinical services (40), and testing in non-clinical settings (41). Concurrently, measures to decrease stigma such as normalizing sexual health checks and routinising sexual health history taking among health providers to better identify those who would benefit from screening the most will be critical for improving the uptake of chlamydia testing among these higher-risk individuals.

Contact tracing of the partners of infected individuals is a cornerstone for STI control. Contact tracing can be patient referral or provider referral (11). Comparing screening only interventions and screening plus contact tracing interventions, we found that the combination of these interventions performs better in reducing the prevalence (and becomes more cost-effective) as the partner tracing effectiveness increases. Prompt

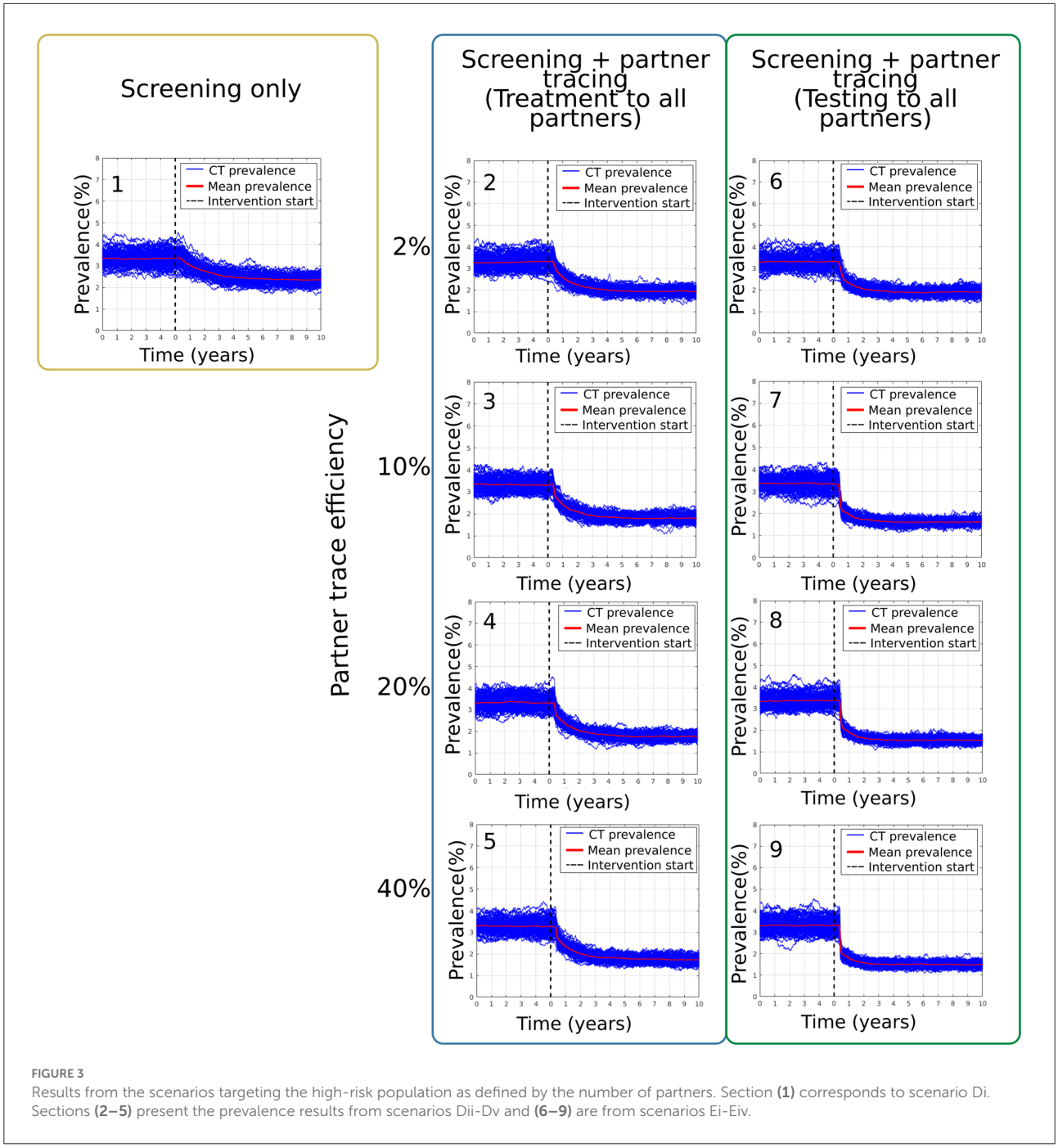

FIGURE 3
Results from the scenarios targeting the high-risk population as defined by the number of partners. Section **(1)** corresponds to scenario Di. Sections **(2–5)** present the prevalence results from scenarios Dii-Dv and **(6–9)** are from scenarios Ei-Eiv.

evaluation and treatment of sexual contacts are important to interrupt transmission, prevent reinfection of the index case and prevent sequelae from STIs. However, contact tracing has been challenging globally due to the stigma associated with STIs, feeling uncomfortable disclosing an STI diagnosis to sexual partners, fear of relationship breakup, and violence. For example, a qualitative survey from Hong Kong exemplifies the complex struggles of contact tracing for chlamydia (42). There are best practice guidelines to overcome some of these barriers using anonymous notification via SMS or email, assisted by the provider with counseling or contact tracing officers (43).

Providing extra antibiotics or prescriptions for sexual partner(s) of heterosexuals may prevent reinfection of chlamydia in the index patient (8). However, our model demonstrated that expedited partner therapy did not significantly impact overall chlamydia prevalence and caused

TABLE 6 Results of the cost-effectiveness analysis: ICER league table.

Scenario	Net cost	QALY gain	Incremental cost	Incremental QALY	ICER
Year 1					
Only screening with a 12 months follow-up (Biii)	–$884	0.03	-	-	-
Targeted screening with 20% contact tracing efficiency (Div)	$333,661	99.32	$334,545	99.29	$3,369
Targeted screening with 40% contact tracing efficiency (Dv)	$409,345	110.83	$75,683	11.51	$6,577
Years 1–10 total					
Only screening with a 12 months follow-up (Biii)	-$5,708	1.72	-	-	-
Targeted screening with 20% contact tracing efficiency (Div)	$4,120,737	892.16	$4,126,446	890.44	$4,634
Targeted screening with 40% contact tracing efficiency (Dv)	$4,807,866	987.34	$687,129	95.18	$7,219
Years 1–10 total, with discounting of costs and benefits					
Only screening with a 12 months follow-up (Biii)	-$3,949	1.29	-	-	-
Targeted screening with 20% contact tracing efficiency (Div)	$3,265,235	722.27	$3,269,184	720.98	$4,534
Targeted screening with 40% contact tracing efficiency (Dv)	$3,822,986	800.17	$557,751	77.90	$7,160

The table shows all non-dominated and non-extendedly-dominated scenarios, reporting the total net cost and incremental costs (in HKD) and QALY gains, and the ICER (incremental cost/QALY). The results are shown for year 1 of the control period, and for the total of all years 1 to 10 of the control period, the latter both with and without discounting with a discount rate of 5%.

overtreatment. We showed that partners should ideally be tested before treatment to avoid overtreatment and the potential to worsen antimicrobial resistance. This is particularly important with macrolide use causing a selection pressure on *Mycoplasma genitalium*, syphilis, and Shigella (8, 44, 45). In addition, we found that scenarios that included contact tracing with treatment for all had a slower prevalence reduction in time than those that included screening to all those traced. This could be due to the ability of the model to detect positive patients among those traced who subsequently had their own list of partners to be tested as well. Thus, this allowed for faster detection of infected individuals within sexual networks. Like expedited partner therapy, retesting infected individuals may have benefits at the individual level to detect reinfection earlier, but the population effect was minimal. Together, we show that neither expedited partner therapy nor retesting infected individuals sooner significantly impacted the overall chlamydia prevalence but could still benefit the index patient.

The main strength of this paper is the use of an individual-based model which could more accurately capture sexual partnership dynamics to explore the epidemiologic and cost-effectiveness impact of universal screening compared to strengthening partner management via contact tracing, expedited partner therapy and re-testing for re-infection. We used the latest available data from Hong Kong to contextualize the model. Our findings should be read in light of several limitations. First, consistent with all economic evaluations, the ranking of cost-effective interventions is context-specific and generalisable only to similar countries to Hong Kong. However, we have made the codes of our model freely available so that others can adapt the model to their country's setting. Second, we did not specifically model other populations at higher risk for chlamydia, e.g., sexual minorities or female sex workers. There is uncertainty in Hong Kong regarding whether there is a significant bridging of chlamydia transmission between subpopulations, e.g., between heterosexuals and men who have sex with men. Our model allowed for the seeding of new infections that may account for this potential bridging effect or importation of infections outside the modeled heterosexual population. Similarly, the model does not explicitly account for variations in sexual activity and mixing across different age groups and is parametrised only to population-level data. Last, all models are a simplification of reality, so our findings must be verified in larger population-based studies where possible.

In conclusion, we found that targeted screening with strengthened contact tracing efforts is more effective and cost-effective than universal screening to reduce the prevalence of chlamydia in a Hong Kong context.

Data availability statement

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

Author contributions

WW, JO, JH, CF, and KT conceived and designed the research and secured the funding. NV integrated the heterosexual network into the model. SM-O and YO designed and applied the model's simulation scenarios and data analysis. MH and JO performed the cost-effective analysis. All authors

contributed to manuscript editing and revision, read and approved the submitted version.

Funding

Financial support for this study was provided by a grant from the Hong Kong Medical Research Fund (18171282). The funding agreement ensured the authors' independence in designing the study, interpreting the data, writing, and publishing the report. JO is supported by an Australian National Health and Medical Research Council (NHMRC) Emerging Leadership Investigator Grant (GNT1193955). CF is supported by an NHMRC Leadership Investigator Grant (GNT1172900). JH is supported by an NHMRC Senior Research Fellowship (GNT1136117).

Acknowledgments

This work was carried out using the computational facilities of the Advanced Computing Research Center, University of Bristol–http://www.bris.ac.uk/acrc/.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.932096/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 26 September 2022
DOI 10.3389/fpubh.2022.1002029

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 24 July 2022
ACCEPTED 07 September 2022
PUBLISHED 26 September 2022

CITATION
Yu X, Xu Q, Chen W, Mai Z, Mo L, Su X, Ou J, Lan Y, Zheng H and Xue Y (2022) Rhein inhibits *Chlamydia trachomatis* infection by regulating pathogen-host cell. *Front. Public Health* 10:1002029. doi: 10.3389/fpubh.2022.1002029

Rhein inhibits *Chlamydia trachomatis* infection by regulating pathogen-host cell

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The global incidence of genital *Chlamydia trachomatis* infection increased rapidly as the primary available treatment of *C. trachomatis* infection being the use of antibiotics. However, the development of antibiotics resistant stain and other treatment failures are often observed in patients. Consequently, novel therapeutics are urgently required. Rhein is a monomer derivative of anthraquinone compounds with an anti-infection activity. This study investigated the effects of rhein on treating *C. trachomatis* infection. Rhein showed significant inhibitory effects on the growth of *C. trachomatis* in multiple serovars of *C. trachomatis*, including D, E, F and L1, and in various host cells, including HeLa, McCoy and Vero. Rhein could not directly inactivate *C. trachomatis* but could inhibit the growth of *C. trachomatis* by regulating pathogen-host cell interactions. Combined with azithromycin, the inhibitory effect of rehin was synergistic both *in vitro* and *in vivo*. Together these findings suggest that rhein could be developed for the treatment of *C. trachomatis* infections.

KEYWORDS

Chlamydia trachomatis*, antibacterial activity, rhein, host-directed therapy, azithromycin, *in vivo

Introduction

Chlamydia trachomatis (C. trachomatis), a Gram-negative bacterial pathogen, is a causative agent of sexually transmitted infections in humans. There are an estimated 127 million new cases of *C. trachomatis* genital infection annually worldwide (1). *C. trachomatis* is classified into 15 serovars, with serovars D to K causing urinary or genital tract infections and serovars L1 to L3 being associated with lymphogranuloma venereum (2, 3). The developmental cycle of *C. trachomatis* is unique and biphasic, featuring an infective, metabolically inactive, elementary body (EB) and a metabolically active, intracellular, reproductive reticular body (RB) (4). Many individuals infected with *C. trachomatis* are asymptomatic, but chlamydia

infections can have serious consequences. Untreated or recurrent chlamydial urogenital infections can lead to severe complications such as pelvic inflammatory disease and infertility (5). Genital infection with *C. trachomatis* also facilitates other sexually transmitted infections such as HIV (6) and human papillomavirus (7, 8). Thus, the potential risk chlamydia poses to human health should not be underestimated.

Currently, 1 g azithromycin (AZM) or 100 mg doxycycline twice a day for 7 days is the unanimously recommended, first-line treatment for uncomplicated *C. trachomatis* infections of the urogenital tract in the United States (9), China (10, 11), Europe (12), and Australia (13). Nevertheless, antibiotic resistance or treatment failure is not uncommon in *C. trachomatis* infections (10, 14, 15). For example, AZM treatment failure has been reported in 5% to 23% of *Chlamydia*-positive men with non-gonococcal urethritis and women with cervicitis not at risk of reinfection (16). A partner-treatment study reported that 8% of women had persistent chlamydial infection despite stating they had no sexual contact after treatment (17). These treatment failures may be due to resistance in members of the genus *C. trachomatis* and persistent infection. The *tet (M)* gene confers resistance to tetracycline antibiotics, while mutations in the 23S rRNA gene confer resistance to macrolides (18–20). The rates of 23S rRNA gene mutations and the abundance of *tet (M)* in *C. trachomatis* were higher in a treatment-failure group than in a treatment-success group (21). Furthermore, following exposure to antimicrobial drugs at sub-inhibitory concentrations, *C. trachomatis* can transform into a surviving reticulate with an almost persistently quiescent metabolism, which further increases the resistance to antibiotics (22). The emergence of antibiotic resistance and treatment failure indicated the need to identify novel anti-chlamydial agents.

Phytochemicals have attracted attention in recent years because of their therapeutic potential against a wide variety of pathogenic microorganisms (23). Compounds extracted from biomaterials and phytochemicals include flavones (24), terpenoids (25), alkaloids (26), and essential oils (27), and many of these compounds have antimicrobial properties. In a previous study, rhubarb inhibited *C. trachomatis* infection (28). Rhein (4, 5-dihydroxyanthraquinone-2-carboxylic acid; Figure 1A) is a monomer primarily extracted from rhubarb (29, 30). This lipophilic, naturally occurring compound has numerous pharmacological properties, including antitumor, antioxidant, anti-inflammatory, antimicrobial, hepatoprotective, and nephroprotective activities (31). As an antimicrobial, rhein has effects against *Staphylococcus aureus* (32, 33), *Pseudomonas aeruginosa* (34), *Porphyromonas gingivalis* (35), and influenza virus (36), among others. In the current study, the effects of rhein on *C. trachomatis* replication in cell culture and in mice were investigated with the aim of determining whether rhein had potential as a novel therapeutic against *C. trachomatis* infections.

Materials and methods

Cell culture and *C. trachomatis* strains

Human epithelial carcinoma cells (HeLa) (ATCC CCL-2.1), mouse fibroblast cells (McCoy) (ATCC CTL-1696) provided by Dr. Lifang Jiang (Sun Yat-sen University, China) and African green monkey kidney cells (Vero) (CCTCC GDC062) were maintained in Dulbecco's modified Eagle's medium (Gibco, St. Louis, MO, USA) with 10% heat-inactivated fetal bovine serum (Gibco) at 37°C in an incubator supplied with 5% CO_2 (Sanyo, Tokyo, Japan). *C. trachomatis* serovars D, E, F and L1 were provided by Dr. Joke Spaargaren (Public Health Laboratory of the Municipal Health Service of Amsterdam, Netherlands).

To obtain sufficient quantity of *C. trachomatis*, confluent HeLa cells were infected with *C. trachomatis* and centrifuged for 60 min at 1,000 ×*g*. After centrifugation, the supernatants were replaced with 1 ml maintenance medium supplemented with 1.0 μg/ml cycloheximide (MedChemExpress, Monmouth Junction, NJ, USA). At 48 hpi, infected cells were sonicated and resuspended in sucrose–phosphate–glutamate. Stocks were divided into small aliquots and stored frozen at −70°C.

Compounds and drugs

Rhein (MedChemExpress) and AZM (North China Pharmaceutical Group Corporation, Hebei, China) were dissolved according to the manufacturers' instructions and stored at −70°C. DMSO (Sigma, St. Louis, USA) was stored at 4°C.

Cytotoxicity assays with rhein

Cytotoxicity of rhein in HeLa cells was assessed using a Cell Counting Kit-8 (CCK-8) (Dojindo, Tokyo, Japan) according to the manufacturer's instructions. Briefly, HeLa cells were seeded at 1×10^4 cells per well in 96-well plates and incubated overnight. Cell monolayers were exposed to various concentrations of rhein (0, 5, 10, 20, 40, 80, and 160 μM) for 48 h, then the CCK-8 kit was utilized, measuring the absorbance of the cells, and the results were expressed as percent viable cells.

Immunofluorescent staining

Confluent HeLa cells were centrifuged at 1,000 ×*g* with *C. trachomatis* for 1 h and then placed at 37°C in an incubator supplied with 5% CO_2 for 1 h. The medium was then changed to maintenance medium in the presence of 40 μM rhein or DMSO. Infected HeLa cells were cultured on cell slides for 48 h and fixed with 4% paraformaldehyde for 20 min at room

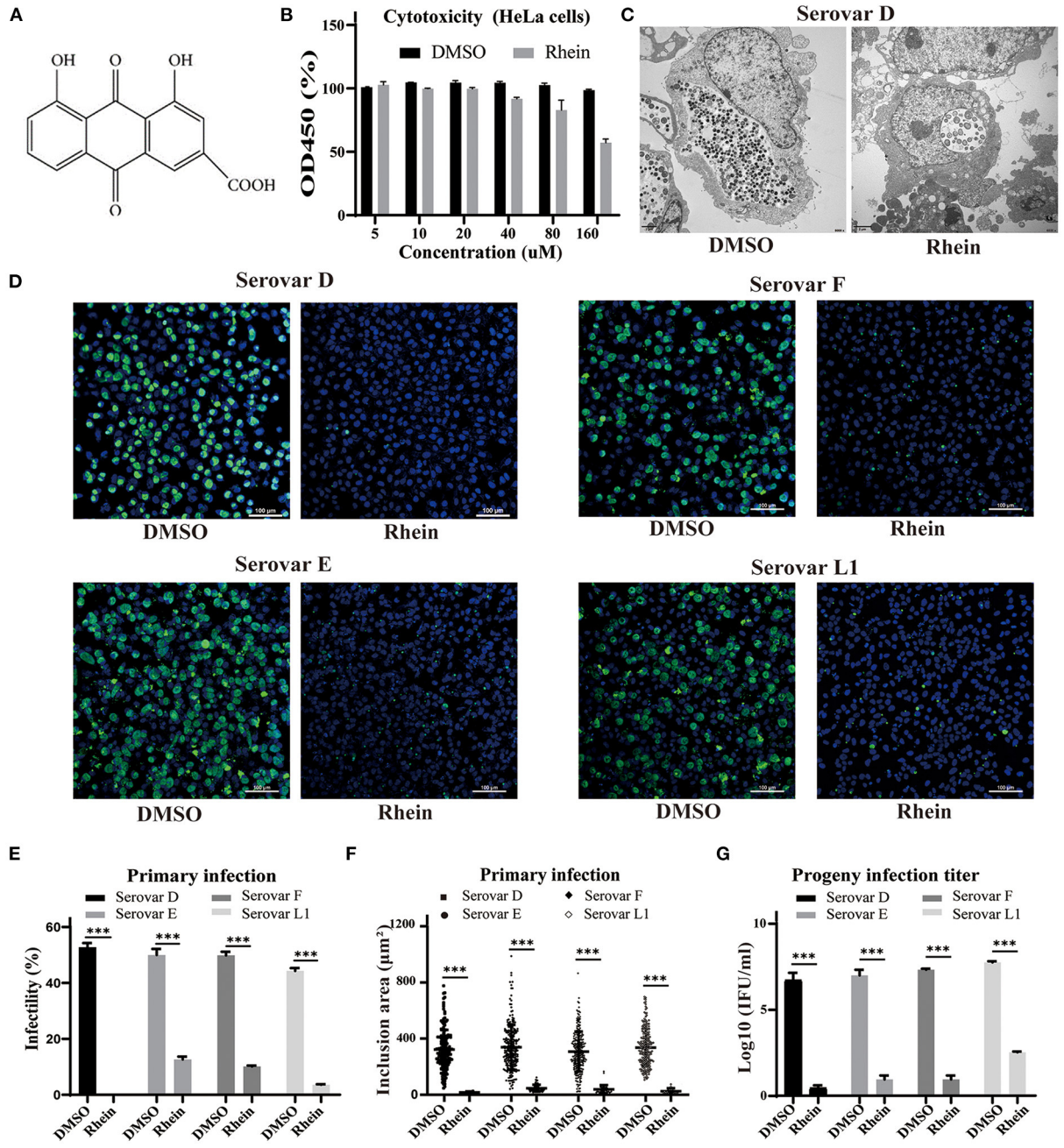

FIGURE 1
Rhein efficiently inhibited *C. trachomatis* replication. **(A)** Chemical structure of rhein. **(B)** Cytotoxic effect of rhein on HeLa cells assessed using a Cell Counting Kit-8. **(C)** Representative transmission electron micrographs of HeLa cells infected with *C. trachomatis* serovar D and exposed to 40 μm rhein or DMSO. Scale bars, 2 μm. **(D)** Immunofluorescent staining of HeLa cells infected with *C. trachomatis* serovars D, E, F, and L1 and exposed to 40 μM rhein for 48 h. *C. trachomatis* inclusions were stained with FITC-conjugated MOMP antibody (green) and nuclei were counterstained with DAPI (blue). Fluorescent images were captured on a confocal microscope at ×200 magnification. Scale bars, 100 μm. **(E)** Infectivity. **(F)** Inclusion area. **(G)** Infectious progeny titer. Data bars in b, e, and g represent the mean ± standard deviation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

temperature. Cells were permeabilized with 0.1% Triton X-100 for 20 min at room temperature and were then incubated with 1% bovine serum albumin in phosphate-buffered saline with Tween (PBS + 0.1% Tween 20) for 60 min to block non-specific binding of the antibodies. Cells were incubated with a fluorescein isothiocyanate (FITC)-conjugated antibody against

the major outer membrane protein (MOMP) of *C. trachomatis* (Abcam, Cambridge, UK) and were then counterstained with DAPI (Solarbio, Beijing, China).

Confocal microscopy

Samples were examined under a confocal microscopy at ×200 magnification (Nikon, Tokyo, Japan) and the number of inclusions and nuclei were counted in 15 random fields from triplicate samples in each experiment. The number of inclusions and nuclei were used to assess infectivity by calculating the inclusion/nuclei percent with Nikon AR NIS 5.02.00 software. The software was also used to measure the area of inclusion bodies.

Electron microscopy

Infected HeLa cells were centrifuged at 1,000 ×*g* for 1 h and then placed at 37°C in an incubator supplied with 5% CO_2 for 1 h. The medium was then changed to maintenance medium in the presence of 40 μM rhein or DMSO. At 48 hpi, cells treated with rhein or DMSO were collected, pelleted by centrifugation at 1,000 ×*g* for 5 min, and were then embedded and examined by transmission electron microscopy (Japan Electron Optics Laboratory, Tokyo, Japan).

Titer of infectious progeny assay

C. trachomatis-infected cells were collected and sonicated. EBs were released from the cells and used to infect fresh cell monolayers. Total inclusions were counted, and numbers of inclusion-forming units (IFU/ml) were calculated at 48 hpi.

Direct interaction assay

EBs of *C. trachomatis* were co-incubated with 40 μM rhein for 12, 24, 36, or 48 h at 4°C before infection (37); DMSO was used as a positive control. *C. trachomatis* was washed with PBS to remove the residual rhein and was then used to infect HeLa cells in 24-well plates. At 48 hpi, cells were fixed with paraformaldehyde and observed by confocal microscopy.

Influence-on-cell assay

HeLa cells were seeded in 24-well plates at 1×10^5 cells/well and 40 μM rhein was added to the culture medium and incubated for 24 h. Cell monolayers were washed three times with PBS, then the pretreated cells were infected with *C. trachomatis*; DMSO treatment served as a positive control. At 48 hpi, cells were stained with MOMP antibody and observed using confocal microscopy.

Influence-on-adsorption assay

HeLa cells were infected with *C. trachomatis* and simultaneously exposed to 40 μM rhein in the culture medium; a control group received the equivalent amount of DMSO. The culture plate was centrifuged at 1,500 ×*g* for 1 h and then placed at 37°C in an incubator supplied with 5% CO_2 for 1 h. The medium containing rhein was then discarded, and cells were washed with PBS three times before addition of the maintenance medium. Immunofluorescence staining was conducted at 48 hpi.

Western blotting

Treated cells were incubated for 0, 12, 24, 36, or 48 h, then the cellular proteins were lysed by RIPA (Invitrogen, 89900) supplemented with a protease and phosphatase inhibitor cocktail (Invitrogen, 78440), and incubated with SDS-PAGE loading buffer (Reducing) (Cwbio, CW0027) at 100°C for 10 min. Antibodies used for western blotting were as follows: anti-RSK1 p90 (phospho T359 + S363) antibody (1:1,000, ab32413, Abcam), anti-RSK1 p90 antibody (1:1,000, ab32114, Abcam), anti-Phospho-p44/42 MAPK (Erk1/2) (1:1,000, 4370S, Cell signaling), anti- p44/42 MAPK (Erk1/2) (1:1,000, 4695S, Cell signaling), anti-cHSP60 (1:2,000, sc-57840, Santa Cruz), anti-GAPDH (1:1,0000, ab181602, Abcam), anti-rabbit IgG-HRP-linked antibody (1:5,000, 7074S, Cell signaling), and anti-mouse IgG-HRP-linked antibody (1:5,000, 7076S, Cell signaling). Blots were imaged on a ChemiDoc MP Imaging System (Bio-Rad).

Animals

Female BALB/c mice (4–6-week-old) were purchased from the Southern Medical University (Guangzhou, China). At 10 and 7 days before infection, all mice were injected subcutaneously with 2.5 mg medroxyprogesterone acetate (Bayunshan Pharmaceutical Company, Guangzhou, China) to synchronize estrus (38). After treatment, the mice were vaginally infected with 1×10^7 *C. trachomatis* IFU or an equal volume of sucrose–phosphate–glutamate. Experiments were conducted in the Experimental Animal Center of South China Agricultural University and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. All procedures performed in studies involving experiments on animals were approved by the Ethics Committee of South

China Agricultural University (SCAU, Guangzhou, China and approval number: 2020c035).

Drug treatment *in vivo*

Mice were divided into five groups: negative control, positive control, rhein treatment, AZM treatment and rhein + AZM combined treatment. Rhein was dissolved in DMSO at 10 mg/ml, and AZM was dissolved in ethanol at 0.084 mg/ml. Mice were treated with 120 mg/kg rhein, 1.0 mg/kg AZM or a combination of 120 mg/kg rhein and 1.0 mg/kg AZM in 0.5% carboxymethylcellulose sodium (CMC-Na) once daily by gavage from day 4 to day 10. Control mice were gavaged with 0.5% CMC-Na. Vaginal swabs were taken for cell culture on day 4 (before gavage), day 7 and day 10 after infection, and the number of inclusions were measured.

Statistical analyses

GraphPad Prism 8 (GraphPad Software, La Jolla, CA, USA) was used to generate graphs, and statistical analyses were conducted using SPSS 15.0 (SPSS Inc., Chicago, IL, USA). Quantitative data are presented as mean ± standard deviation. The Shapiro–Wilk test was used to test the normality of quantitative data. Fisher's exact test and Bonferroni's multiple comparisons were used to assess infectivity. Kruskal–Wallis and Dunn's multiple comparisons tests were used to evaluate the area of inclusions. An unpaired *t*-test was used to analyze the difference in EB titer between groups. *P*-values were calculated using one-way ANOVA followed by Bonferroni correction for multiple comparisons. A nonparametric Wilcoxon test was used for mouse model statistics. Differences were considered significant at $P < 0.05$ (*), $P < 0.01$ (**) and $P < 0.001$ (***).

Results

Rhein effectively inhibited *C. trachomatis* replication

Cell viability was approximately 95% in samples exposed to 40 μM rhein (Figure 1B). The anti-chlamydial effects of rhein were investigated in HeLa cells infected with the more prevalent serovars of *C. trachomatis* (serovars D, E and F) and the L1 serovar that can lead to venereal lymphogranuloma (39). A few aberrant RBs were observed by transmission electron microscopy in HeLa cells infected with *C. trachomatis* serovar D and treated with 40 μM rhein, compared with many small, mature EB particles within the inclusion of DMSO-treated cells at 48 hpi (Figure 1C). Immunofluorescent staining revealed that the inclusion bodies became smaller and the infectivity, inclusion size and infectious progeny decreased in the presence of 40 μM rhein (Figures 1D–G). These results demonstrated that rhein effectively inhibited the growth and reproduction of different serovars of *C. trachomatis* in HeLa cells.

The effect of rhein on *C. trachomatis* was dose- and time-dependent

The effect of different concentrations of rhein (0, 5, 10, 20, 40, and 80 μM) on HeLa cells infected with *C. trachomatis* was examined, and the infectivity, inclusion area and infectious progeny are decreased in the presence of rhein in a dose-dependent manner (Figure 2A). HeLa cells infected with *C. trachomatis* were also exposed to 40 μM rhein at various time points (0, 6, 12, 18, and 24 h) after infection. *C. trachomatis* inclusions were larger and more numerous with rhein exposure at 24 hpi compared with rhein exposure at 0 hpi (Figure 2B). The titer of infectious progeny also increased with the delay in exposure to rhein (Figure 2B). These findings indicated that rhein inhibited the replication of *C. trachomatis* in a dose- and time-dependent manner, and suggested that the earlier cells are treated with rhein, the better the inhibition of *C. trachomatis*.

Rhein did not directly inactivate *C. trachomatis* elementary bodies

Rhein and other anthraquinone drugs, including emodin and aloe-emodin, have been extracted from rhubarb. Emodin and aloe-emodin have antibacterial or virucidal activity by destroying the envelope of bacteria or viruses (40–43). Rhein was previously demonstrated to directly inhibit the growth of *S. aureus* (33). To determine whether rhein could directly impair *C. trachomatis* activity, 40 μM rhein was co-incubated with *C. trachomatis* serovar D for 12, 24, 36, and 48 h, respectively (36, 40). The infectivity and inclusion area of *C. trachomatis* exposed to rhein were not significantly different from those of the corresponding DMSO control ($P > 0.05$; Figure 3). This suggested that rhein did not directly inactivate *C. trachomatis* EBs.

Rhein inhibited *C. trachomatis* through regulation of host cells

C. trachomatis is an obligate intracellular parasitic pathogen that needs to combine with host surface receptors to enter a cell. The pathogen then uses host cell nutrients to replicate and reproduce by regulating the interaction with the host cell (44). Rhein did not have a direct inactivation effect on *C. trachomatis*. To elucidate the potential inhibitory mechanism

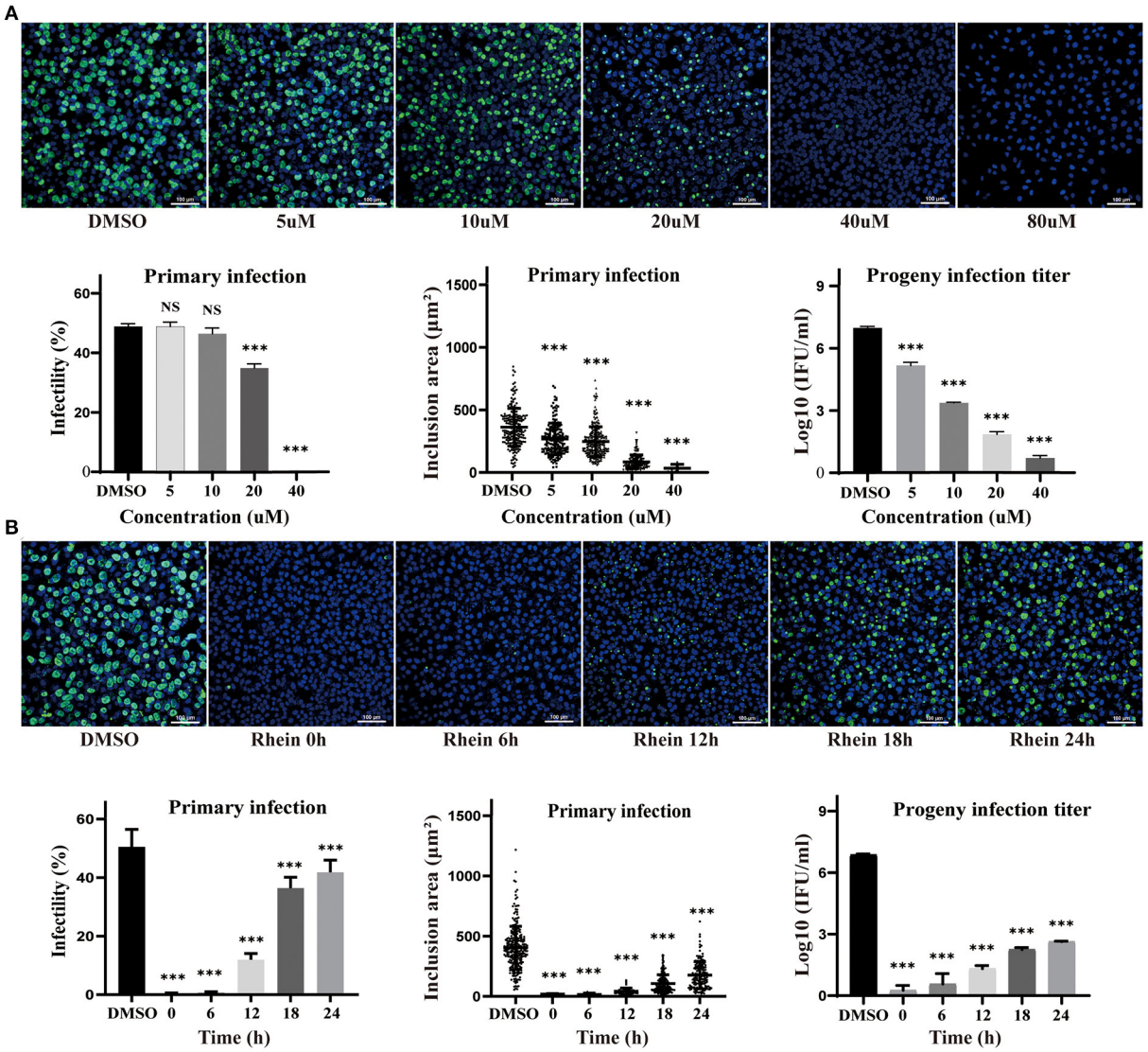

FIGURE 2
Inhibitory effects of rhein on *C. trachomatis* infection were dose- and time-dependent. **(A)** HeLa cells were infected with *C. trachomatis* serovar D at multiplicity of infection (MOI) 5 and were exposed to various concentrations of rhein (5, 10, 20, 40, and 80 μM) or DMSO for 48 h before fixation and immunostaining. **(B)** HeLa cells infected with *C. trachomatis* were exposed to rhein (40 μM) at 0, 6, 12, 18, and 24 hpi. Cells were fixed, and *C.trachomatis* were stained with a FITC-conjugated anti-MOMP antibody (green), while host cell nuclei were counterstained with DAPI (blue). Scale bars, 100 μm. Data bars in the graphs represent the mean ± standard deviation. NS, not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

of rhein, a set of influence-on-cell, influence-on-adsorption, and influence-on-post-adsorption assays were designed (37, 45) (Figure 4A). The first two assays were used to determine whether rhein affected the adhesion and binding of EB particles to cell membranes, while the third assay was used to determine whether rhein inhibited *C. trachomatis* during its replication stage. The influence-on-post-adsorption assay showed a significant inhibitory effect of rhein (Figures 4B–D). Our previous study demonstrated that the extracellular signaling-regulated kinase (ERK)/ribosomal S6 kinase (RSK) signaling pathway was important in *C. trachomatis* infection (46). To investigate the mechanism of action of rhein in *C. trachomatis* infection, the protein of ERK and RSK were performed by western blotting in the current study on *C. trachomatis*-infected cells treated with rhein for different times post-infection. P-RSK expression was up-regulated at 12 h after *C. trachomatis* infection in the presence or absence of rhein. P-ERK and P-RSK were both down-regulated in the presence of rhein at 36 h and 48 h post-infection (Figures 4E,F). The total ERK and RSK remain constant. Moreover, in cell lines of murine (McCoy) and

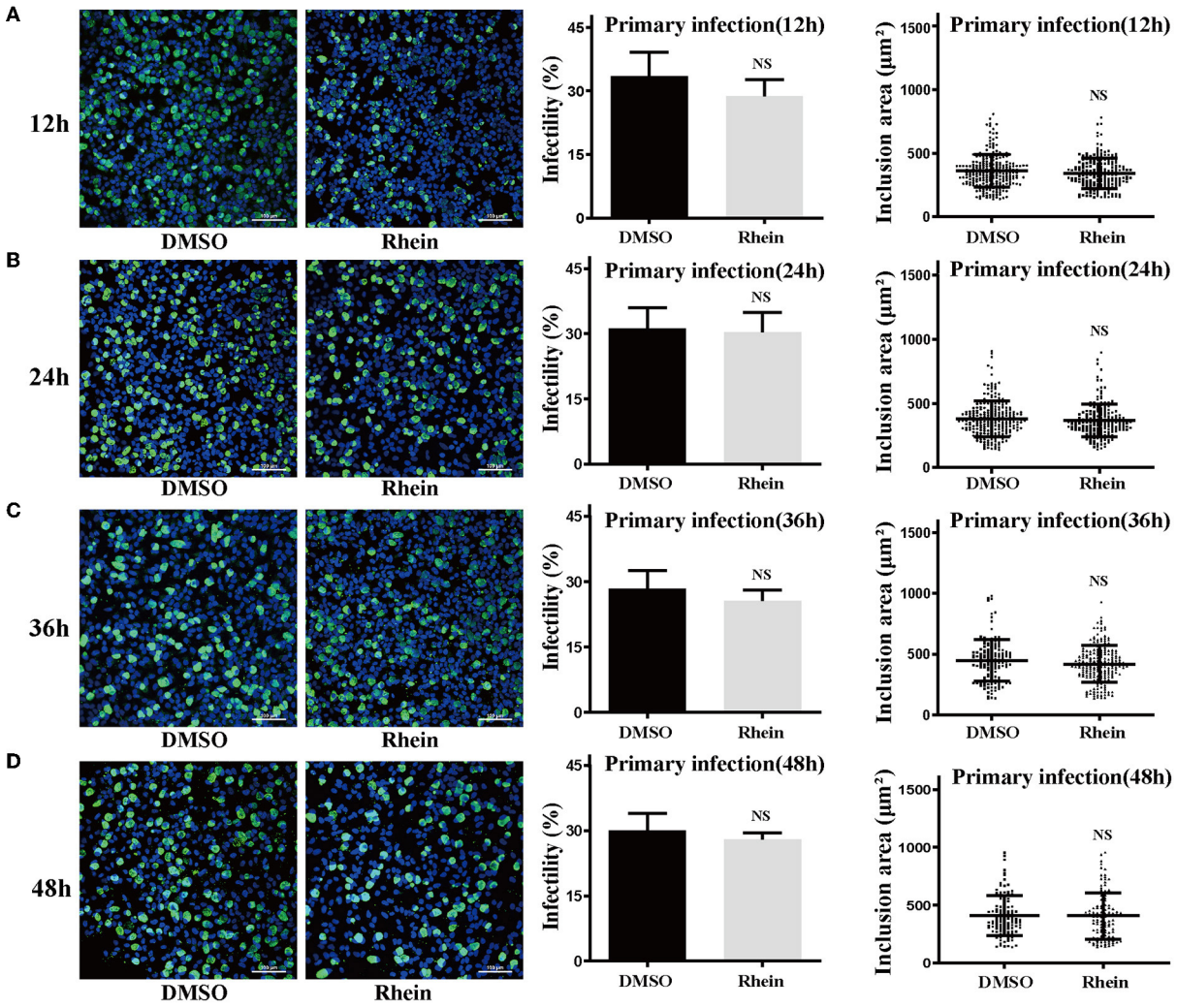

FIGURE 3
Pretreatment with rhein did not impair *C. trachomatis* particles. Elementary bodies of *C. trachomatis* were respectively co-incubated with 40 μM rhein for 12, 24, 36, or 48 h at 4°C before infection. At 48 hpi, *C. trachomatis* inclusions, infectivity, and inclusion areas were observed by confocal microscopy. **(A)** 12 h; **(B)** 24 h; **(C)** 36 h; and **(D)** 48 h. Images were captured at ×200 magnification. Scale bars, 100 μm. Data represent the mean ± standard deviation of triplicate samples. NS, not significant. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

primate (Vero) origin, the antibacterial activity of rhein was also exerted during the replication stage of *C.trachomatis* (47) (Supplementary Figure S1). These observations suggest that the inhibitory activity of rhein may not be host cell-specific and that rhein may regulate host cells and change the environment to inhibit *C. trachomatis* replication.

Rhein and AZM had a synergistic inhibitory effect against *C. trachomatis*

AZM is a first-line drug for treating *C. trachomatis* infections, but treatment failure has been reported (15, 17). Although rhein alone impaired growth of *C. trachomatis*, an experiment was conducted to investigate whether rhein and AZM had a synergistic suppressive effect on *C. trachomatis* infection. Sub-inhibitory concentrations of 20 μM rhein and 0.005 μg/ml AZM were tested. The infectivity, the area of inclusions and infectious progeny of *C. trachomatis* were reduced by the two individual treatments (rhein alone and AZM alone) (Figure 5). However, a greater inhibitory effect on *C. trachomatis* replication was observed when rhein was combined with AZM compared with rhein alone and AZM alone (Figure 5). Thus, the combination of rhein and AZM potentially has great clinical value.

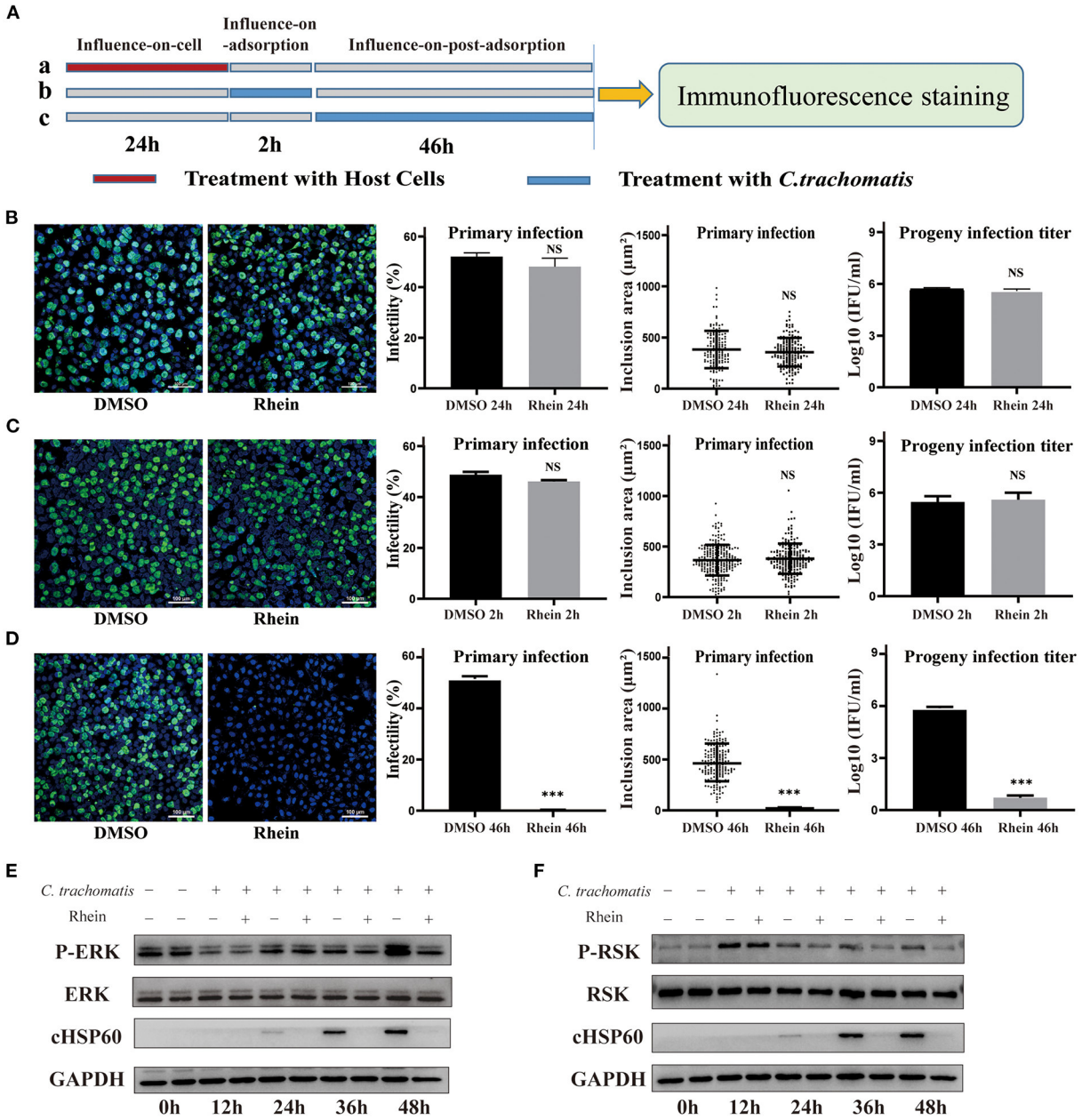

FIGURE 4
Rhein inhibited *C. trachomatis* infection by regulating host cells. **(A)** Three treatment conditions (each row is a treatment): **(A)** influence-on-cell, cells were pretreated with 40 μM rhein for 24 h; **(B)** influence-on-adsorption, cells were exposed to 40 μM rhein for 2 h during the period of adsorption; **(C)** influence-on-post-adsorption, cells were exposed to 40 μM rhein for 46 h after adsorption. **(B–D)** Immunofluorescent images (×200 magnification), infectivity, inclusion area, and infectious progeny titer. DMSO was used as positive control. **(B)** Cells were pretreated with 40 μM rhein for 24 h (treatment a). **(C)** Cells were exposed 40 μM rhein for 2 h during the period of adsorption (treatment b). **(D)** Cells were exposed 40 μM rhein for 46 h after adsorption (treatment c). **(E)** Western blots of p-ERK, ERK, cHSP60 and GAPDH protein expression in *C. trachomatis*-infected cells with or without rhein at different time points post-infection. The bands were cropped from different parts of the same gel. **(F)** Western blots of p-RSK, RSK, cHSP60 and GAPDH protein expression in *C. trachomatis*-infected cells with or without rhein at different time points post-infection. The bands were cropped from different parts of the same gel. Data in the graphs represent the mean ± standard deviation of triplicate samples. NS, not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

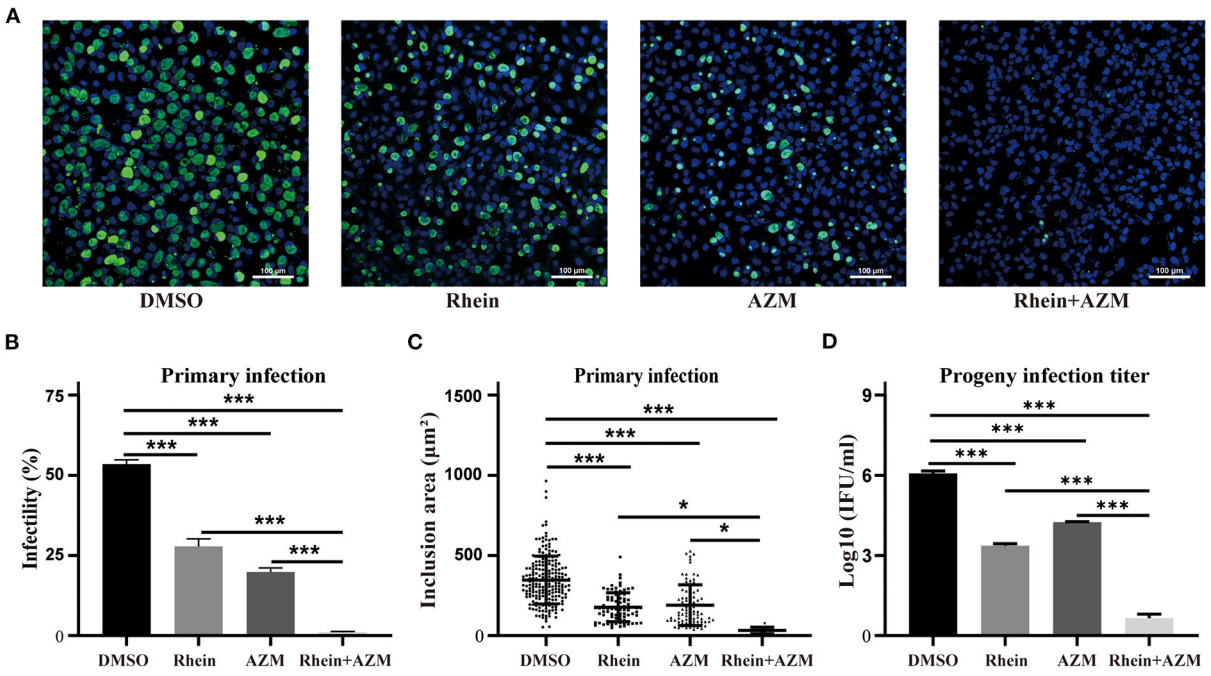

FIGURE 5
Rhein and AZM combined had synergistic inhibitory effects on *C. trachomatis*. **(A)** Immunofluorescence images (×200 magnification; scale bars, 100 μm) of control (DMSO), 20 μM rhein, 0.005 mg/l AZM, and 20 μM rhein + 0.005 mg/l AZM treatments are shown from left to right. **(B)** Infectivity, **(C)** inclusion area, and **(D)** infectious progeny titer of *C. trachomatis* according to treatments. Data represent the mean ± standard deviation from three independent experiments. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Rhein combined with AZM inhibited *C. trachomatis* infection in mouse models

The *in vitro* experiments demonstrated that rhein effectively inhibited *C. trachomatis* infection, and when combined with AZM, there was a synergistic inhibitory effect. The inhibitory effect of rhein on *C. trachomatis* was therefore tested *in vivo* in a mouse model. Six-week-old female BALB/c mice were infected with *C. trachomatis* serovar D, then DMSO, AZM, rhein and AZM + rhein were administered orally from day 4 to day 10 post-infection. Vaginal swabs were taken on days 4, 7 and 10 for cell culture and to determine the number of infectious progenies. The number of infectious progenies in the DMSO control and rhein-treated group was not significantly different between days 4, 7, and 10 (Figures 6A,B). However, the number of infectious progenies in the AZM-treated group decreased significantly from day 4 to day 10 (Figure 6C), and the number of infectious progenies in the AZM + rhein treatment group decreased significantly from day 4 to day 7 (Figure 6D). Murine tissues were examined by hematoxylin and eosin (H&E) staining on day 22 after *C. trachomatis* infection. Edema and hypertrophy were observed in the uterus of infected mice (Figure 6E), but the uterine edema was relieved in the rhein and/or AZM treatment groups. There were no pathological changes in the heart, liver, spleen, or kidney of mice in any treatment group as revealed by H&E staining (Supplementary Figure S2).

Discussion

Rhein significantly inhibited *C. trachomatis* replication across various serovars and in HeLa, McCoy and Vero host cells. In combination with AZM, rhein exerted a synergistic suppressive effect on *C. trachomatis* infection, both *in vitro* and *in vivo*. In addition, rhein may regulate host cells and change the environment to inhibit *C. trachomatis* replication. Taken together, the findings of this study suggest that rhein may be a potential treatment for *C. trachomatis* infection.

Rhein was previously reported to have effective antibacterial and antiviral activity against *S. aureus*, *Helicobacter pylori*, influenza A virus, and hepatitis B virus (HBV) (33, 36, 46). The mechanism of action of rhein was shown to involve direct impairment of pathogens or regulation of host cell signaling pathways. Rhein increased the transcription of genes encoding the iron-regulated surface determinants system and genes involved in the ribonucleotide reductase systems of *S. aureus* (33). In addition, rhein exerted its antimicrobial activity against *S. aureus* by reducing the transcription of genes responsible for

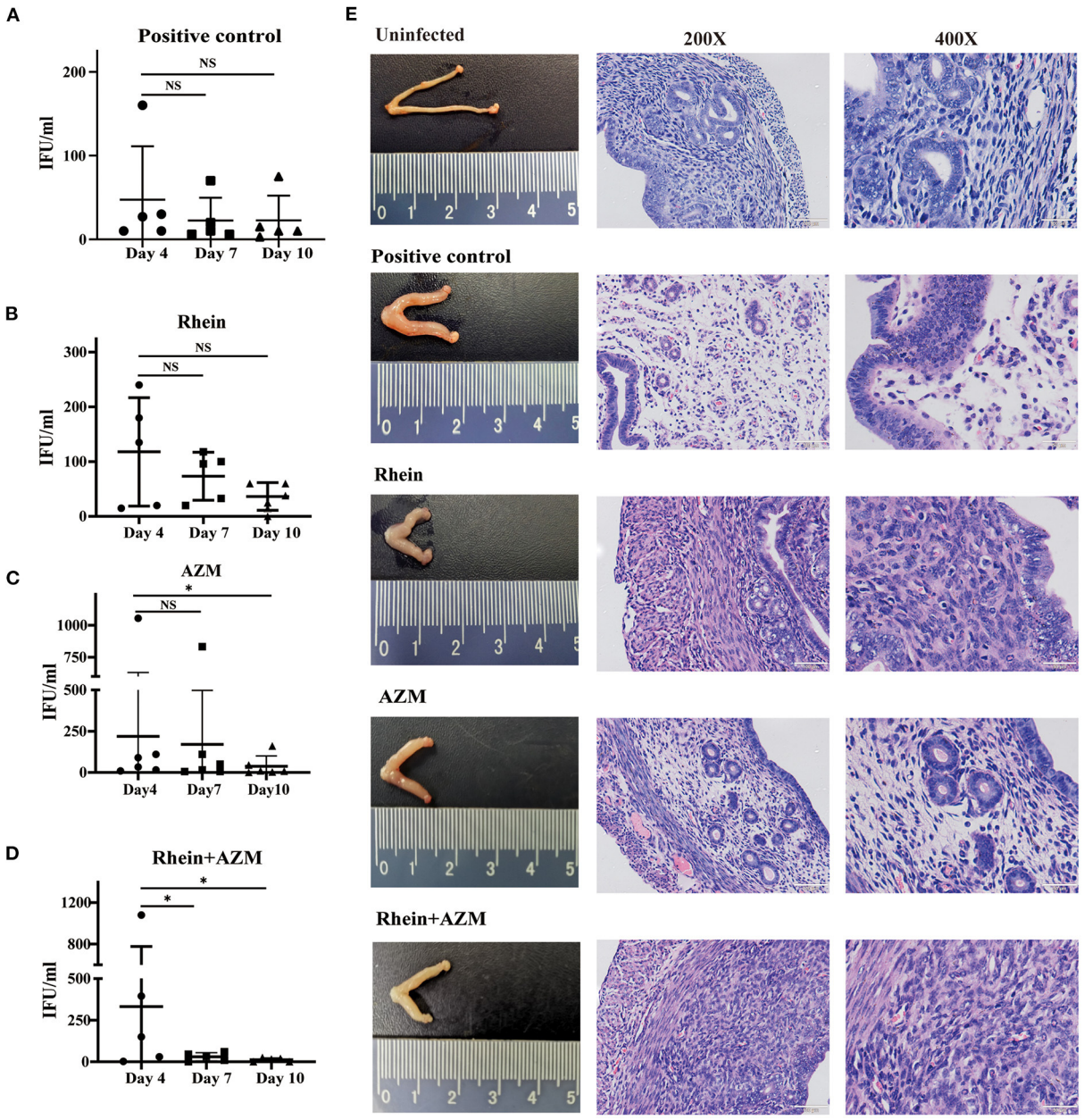

FIGURE 6
Rhein combined with AZM inhibited *C. trachomatis* infection in mouse models. Vaginal swabs were taken on days 4, 7, and 10 after infection for cell culture and determination of the number of inclusion bodies. **(A)** Positive control group. **(B)** Rhein treatment group. **(C)** AZM treatment group. **(D)** AZM + rhein treatment group. **(E)** Pathological changes in the gross morphology of the uterus (×200 magnification; scale bars, 100 μm. ×400 magnification; scale bars, 50 μm). The nonparametric Wilcoxon test was used for statistical analysis. NS, not significant; *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

anaerobic respiration and fermentation (33). Rhein inhibited DNA polymerase activity in HBV (48). In above studies, the mechanisms of action of rhein involve direct impairment of pathogens. However, rhein also significantly inhibited influenza A virus-induced oxidative stress and decreased influenza A virus-induced expression of Toll-like receptor 2 (TLR2), TLR3 and TLR4. Moreover, rhein suppressed influenza A virus-induced activation of host signaling pathways including the Akt, p38/JNK MAPK and NF-κB pathways in A549 cells (36). In the current study, rhein did not have a direct inactivating effect on *C. trachomatis*, but rather inhibited this pathogen in a post-adsorption replication stage. *C. trachomatis* is an

intracellular pathogen that is heavily dependent on host cells, thus the mechanism of rhein inhibition of *C. trachomatis* may be similar to that of influenza A virus whereby host cells are regulated to affect the growth and development of pathogens (49).

Rhein has multiple targets and consequently regulates multiple pathways at the molecular level, including the MAPK signaling pathway, the PI3K-AKT signaling pathway, and the Wnt signaling pathway (31). Among these pathways involved in the pharmacological activity of rhein, the MAPK signaling pathway can be considered one of the most interactive pathways and rhein can regulate the Ras/Raf/MEK/ERK pathway to inhibit the phosphorylation of ERK1/2 (50, 51). The ERK pathway is considered crucial in cell proliferation and migration and RSK is an important downstream effector of the Ras/Raf/MEK/ERK signaling pathway (52, 53). Phosphorylated substrates of RSK are involved in diverse cellular processes including gene transcription, protein synthesis, cell cycle regulation, and cell survival (54, 55). ERK signaling pathways are the most prominent kinase signaling network utilized by *C. trachomatis* and have been characterized as being instrumental in nutrient acquisition, host cell apoptosis resistance, immune responses, and even pathology associated with chlamydial infections (56–58). Moreover, our previous study suggested that ERK/RSK may be a novel target for *C. trachomatis* therapeutics (46). In this study, phosphorylated ERK/RSK was reduced upon exposure to rhein, suggesting that rhein may inhibit *C. trachomatis* infection by regulating the ERK/RSK pathway.

In the process of infectious disease treatment and drug development, host-directed therapy (HDT) is a novel strategy for treating bacterial and viral infections. Biological products or small molecules are used to interfere with replication or persistence of the pathogen by regulating host factors (59). Currently, small-molecule drugs have been proposed for the management of tuberculosis, HBV and HIV by HDT (60–62). *C. trachomatis* development requires host cell energy and nutrients and may therefore be a suitable pathogen for the development of HDT (63–65). The small molecule mycophenolate mofetil was recently demonstrated to effectively inhibit *C. trachomatis* growth by targeting the rate-limiting enzyme inosine-5′-monophosphate dehydrogenase in the biosynthesis of guanine nucleotides in host cells (66). In addition, our research team reported that inhibitors targeting ERK/RSK had potential in the treatment of *C. trachomatis* infection (46). Findings from the current study indicated that rhein may regulate host cells and change the environment to inhibit *C. trachomatis* replication. Moreover, rhein and AZM had a synergistic inhibitory effect on *C. trachomatis in vitro* and *in vivo*. Rhein may therefore be a potential drug for a HDT strategy of managing chlamydial infections.

Although rhein was demonstrated to inhibit *C. trachomatis* infection, the precise molecular mechanism of rhein on *C. trachomatis* has not yet been elucidated. Current research suggests that rhein inhibits *C. trachomatis* survival most likely through targeting host factors. Future work will explore the molecular mechanism by which rhein affects *C. trachomatis* replication.

In summary, this study provided evidence that rhein reduced *C. trachomatis* replication in *vitro* and *in vivo* and indicated that rhein may have potential in drug development for the treatment of *C. trachomatis*.

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 animal study was reviewed and approved by the Ethics Committee of South China Agricultural University (SCAU, Guangzhou, China and approval number: 2020c035).

Author contributions

XY and QX performed most of the experiments in this study and jointly wrote the draft manuscript. WC and ZM were responsible for the initial data analysis. XS and LM compiled figure preparation and statistical analysis. JO and YL provided experimental assistance and constructive comments to this study. HZ and YX had the leading contribution to the design of studies and interpretation of the whole dataset. All authors read and approved the manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (81974307), the Natural Science Foundation of Guangdong Province (2018A030313662, 2019A1515011827, and 2021A1515012255), Research Foundation of Department of Education of Guangdong Province (2021KTSCX014), Science and Technology Program of Guangzhou (202201011579), and Guangdong Province Bureau of Traditional Chinese Medicine Scientific Research Project (20211276).

Acknowledgments

We thank Dr. Bin Yang for helpful scientific discussions.

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.2022.1002029/full#supplementary-material

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TYPE Original Research
PUBLISHED 10 October 2022
DOI 10.3389/fpubh.2022.1012835

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases–Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 05 August 2022
ACCEPTED 23 September 2022
PUBLISHED 10 October 2022

CITATION
Huston WM, Lawrence A, Wee BA, Thomas M, Timms P, Vodstrcil LA, McNulty A, McIvor R, Worthington K, Donovan B, Phillips S, Chen MY, Fairley CK and Hocking JS (2022) Repeat infections with chlamydia in women may be more transcriptionally active with lower responses from some immune genes.
Front. Public Health 10:1012835.
doi: 10.3389/fpubh.2022.1012835

Repeat infections with chlamydia in women may be more transcriptionally active with lower responses from some immune genes

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Chlamydia trachomatis, the most common bacterial sexually transmitted infection worldwide, is responsible for considerable health burden due to its significant sequelae. There are growing concerns about chlamydial treatment and management due to widely documented increasing burden of repeat infections. In the current study, a cohort study design of 305 women with urogenital chlamydial infections demonstrated that 11.8% of women experienced repeat infections after treatment with azithromycin. The chlamydial DNA load measured by quantitative PCR was higher in women who experienced a repeat infection ($p = 0.0097$) and repeat infection was associated with sexual contact. There was no genomic or phenotypic evidence of azithromycin resistance within the chlamydial isolates. During repeat infection, or repeat positive tests during follow up, vaginal chlamydial gene expression (*ompA, euo, omcB, htrA, trpAB*) was markedly higher compared to baseline, and two of the selected immune genes analyzed had significantly lower expression at the time of repeat infection. Overall, there are two implications of these results. The results could be generalized to all recent infections, or repeat positive events, and indicate that chlamydial infections are have higher transcriptional activity of select genes early in the infection in women. Alternatively, after azithromycin treatment, repeat infections of *Chlamydia* may be more transcriptionally active at certain genes, and there may be post-treatment immunological alterations that interplay into repeat exposures establishing an active infection. The potential that recent infections may involve a higher level of activity from the organism may have implications for

management by more regular testing of the most at risk women to reduce the risk of sequelae.

KEYWORDS

***Chlamydia*, sexually transmitted infection, repeat infections, genomics, gene expression, azithromycin, antibiotic**

Introduction

Chlamydia trachomatis continues to be the most commonly diagnosed bacterial sexually transmitted infection (STI) globally (1). It can have adverse health consequences particularly for women [reviewed (2)] with an estimated 17% of cases leading to pelvic inflammatory disease (3) and an estimated 45% of tubal factor infertility being attributable to past *Chlamydia* infection (4). Several studies have reported high *Chlamydia* repeat infection rates among young women re-tested following treatment. An Australian cohort of 1,116 young women found that among those women testing positive at baseline, 18% tested positive again at 3 months following treatment (5). Other studies have reported similarly high rates of repeat infection, ranging from 21% within 6 months in New Zealand (6) to 29.9% per year after treatment in the UK and 34% per year (7) in the USA.

Repeat infections may represent: (1) re-infection due to unprotected sexual contact with an infected partner; (2) treatment failure as a result of noncompliance with treatment, poor absorption of the drug, reduced antimicrobial susceptibility, or antimicrobial resistance; (3) persistence due to host or microbial factors such as immune response or other undefined host factors; or (4) auto-inoculation from a persistent rectal chlamydia infection that has not been effectively treated (8–12). There is increasing concern that treatment failure or auto-inoculation from a persistent rectal infection may account a significant proportion of repeat urogenital infections in women (13, 14). Rectal chlamydia is detected in about 80% of women who are diagnosed with urogenital infection (15). Azithromycin is still widely used to treat urogenital infection but is substantially less effective at clearing rectal chlamydia (16), increasing the risk that any concurrent rectal infection could subsequently auto-inoculate causing a repeat urogenital infection. There have not been any confirmed azithromycin resistant isolates, although there are some conflicting reports (17, 18), and there is laboratory evidence that such isolates are unfit and unlikely to be maintained in the population (19). A partner treatment study found that among female participants who reported no sexual intercourse after treatment, 22 of 289 (8%; 95% CI: 5–11%) had persistent infection at follow up, suggestive of treatment failure (20). A cohort of adolescent females also found a treatment failure rate of 7.9% (95% CI: 4–10.1%) (7). There is also *in vitro* evidence that *Chlamydia* can enter a persistent form where infected cells exposed to β-lactam antibiotics, interferon-γ or deprived of iron or amino acids, can exhibit persistence (8, 9, 11, 21, 22). This allows chlamydia to remain dormant, non-infectious and undetectable by culture but, on removing the stressful conditions, it can be recovered by culture. There is also evidence that latent infection may not be detectable, even using PCR, if only cells shed from the mucosal surface are sampled (22–24). It is not known how often this persistent state occurs *in vivo* and whether removal of treatment can trigger reactivation.

We conducted a cohort study of women diagnosed with urogenital chlamydia to investigate factors associated with repeat infection in women treated with azithromycin, including examining human and microbial factors. Here we report genotypic and phenotypic analysis of the chlamydial isolates and some host responses from this cohort study.

Materials and methods

Cohort design and analysis

This study was a cohort study to examine repeat infections in women with urogenital chlamydial infections treated with Azithromycin, the Australian Chlamydia Treatment Study (25). In brief 305 women aged ≥ 16 diagnosed with NAAT positive genital chlamydia were recruited from two large sexual health clinics in Sydney and Melbourne Australia between October 2012 and October 2014. Women were eligible for inclusion if they had adequate English language skills to give consent and remained in the local area for 8 weeks. Women were recruited when they returned to the clinic for treatment for their initial chlamydia infection. They completed a survey and provided four clinician collected high vaginal swabs for testing. Women were excluded if they had a concomitant STI, had concurrent PID, were commercial sex workers, had taken another antibiotic within the last 2 weeks, did not have a mobile phone or an address to which parcels could be posted, were HIV positive or had a macrolide allergy or were taking other medications likely to interact with azithromycin. The proportion and 95% confidence intervals of those who had a repeat infection was calculated using exact binomial methods accounting for clustering at the clinic level. The incidence or repeat infection and 95% confidence intervals were calculated using poisson methods. Cox proportional hazards regression was used to calculate factors associated with time till repeat infection.

Factors investigated included socio-demographic and behavioral variables. Given relatively small number of cases of repeat chlamydia, only unadjusted Cox regression was performed.

Associations between repeat positive testing events and chlamydia organism at the index by IFU or PCR load were investigated for all those participants who had a test of cure at week 4 using *t*-tests where measures of load were log transformed. It was not always possible to measure load for all available baseline samples due to factors such as heavy contaminants of blood or other organisms interfering in assays, low chlamydial load, or poor swab collection quality (see Supplementary Table S1).

All repeat positive index and follow up samples were cultured where possible and of these, 19 samples from index eight samples from follow up were able to be purified from other contaminants and included in the minimum inhibition concentration (MIC) measures. A further 26 index samples from women who were negative on follow up (follow up negative, FoN) were also able to be cultured and purified from other contaminants and included in the MIC analysis. These isolates represented 26 samples at indexfrom women with no repeat positives (FoN), 19 samples from index from women who experienced a FoP and eight samples at a test of cure (4 weeks), from women who experienced positive follow up results (FoP).

MIC (minimum inhibitory concentration) was conducted on chlamydial cultures in McCoy B cell monolayers following the protocol previously described (12). Azithromycin was added to the cultures in a twofold dilution dose series (μg/ml), cultures conducted, fixed and labeled using immunocytochemistry to examine chlamydial inclusions using our in-house method [described (26, 27)]. MIC_{tp} or transition point MIC, was defined as the dose at which 90% or more of the inclusions were altered in size and morphology. The presence of 10% or less visible standard inclusions was used to determine the MIC_{tp}, with the MIC considered to be the next higher dose in the series from the MIC_{tp}. Resistance is considered to be a MIC of 4 μg/ml or greater for *Chlamydia* (12). The MIC of cultured isolates to azithromycin at baseline were calculated and compared between those with and without repeat chlamydia infection using *t* tests.

Gene expression analysis

A nested Case-Control study was conducted within the cohort study where a group of women from the study (12 case- Follow up Positive, control- Follow up Negative) were selected based on matching for age and contraceptive usage (two controls per case) and included in the gene expression analysis. See Supplementary materials for further characteristics of these cases and controls (Supplementary Table S2). Total RNA was then extracted and purified from each sample using the Purelink™ RNA Mini Kit (Thermo Fisher Scientific), in accordance with the manufacturer's instructions. The concentration and purity of each RNA sample was assessed using the NanoDrop™ One/One C UV-Vis spectrophotometer (Thermo Fisher Scientific). Complementary DNA (cDNA) synthesis with random hexamer priming was conducted using the SuperScript™ III First Strand Synthesis Reverse Transcription kit (Thermo Fisher Scientific). The relative human gene expression levels in cDNA samples were analyzed using reverse-transcription quantitative polymerase chain reaction (RT-qPCR). The genes of interest included in the analysis were IDO1, IRF-1, FTH-1, IL-6, IL-8, IL-1α, TNF-α, IL10, IFN-γ. The expression levels of each human gene of interest was determined relative to the geometric mean of glyceraldehyde-3-phosphate dehydrogenase (*gapdh*) and phosphoglycerate kinase 1 (*pgk1*) cDNA levels. Chlamydial genes were analyzed by normalization against 16srRNA DNA levels (as an indicator of chromosome counts). Primers are provided in Supplementary Table S3. The oligonucleotide sequences used to amplify and measure chlamydial *16S rRNA* (28), *euo* (29) and *ompA* (30) cDNA were from previously published studies, while those used to amplify *htrA, trpBA* and *omcB* cDNA were designed using the NCBI Primer Blast tool. The genome of *C. trachomatis* type strain D/UW-3/Cx (accession number NC_000117) was used as the template for primer design; however, primers were only selected if they amplified from control cultures of in-house stocks of all genovars in the present study.

Each of the primers used were validated by conventional PCR and agarose gel electrophoresis, before the efficiency of each primer set was determined. Only primers with an efficiency of between 90 and 110% were used during this study. The mean cycle threshold (Ct) value of each of the participant cDNA samples was calculated from technical replicates, which were repeated or excluded if the mean Ct value had a standard deviation greater than 1.0.

Raw RT-qPCR data was exported from the Rotor-Gene Q Series software platform and stored and transformed using Microsoft Excel. For each sample, the mean Ct value of each gene of interest was subtracted from the geometric mean of *gapdh* and *pgk1*, to obtain the delta cycle threshold (ΔCt) value. These ΔCt values were then transformed using means and standard deviation for each gene to $\Delta\Delta$Ct using negative log transformation. The chlamydia genes were first normalized to the quantity of chromosomal DNA of the 16S rRNA chromosome for that sample before transformed using means and standard deviation for each gene to $\Delta\Delta$Ct using negative log transformation. The analysis was Baseline: No Repeat – Baseline: Repeat infection, and Baseline: Repeat Infection – Repeat Infection. The transformed $\Delta\Delta$Ct datasets were graphed in Graphpad Prism 7. Statistical comparisons were conducted using Graphpad Prism 7, with the Mann-Whitney U used test to determine whether any significant differences between two groups using the non-transformed $\Delta\Delta$Ct datasets. Paired sample analysis was conducted using

the Wilcoxon test prior to log transformed ΔΔCt for graphical display.

Genomic analysis

Genome sequences of the *Chlamydia* were examined using two approaches. One approach was whole genome sequencing of cultured isolates on a small selection of the specimens collected. The cases of repeat infection where chlamydial isolates were able to be cultured were included in this genomic analysis, were selected to ensure representation across a variety of *ompA* genotypes, and able to be cultured and selected away from contaminants during the culture.This included 15 isolates (from 11 participants); eight were the index and FoP events from four participants, four were associated with FoP but from index isolates only, and 3 from index swabs from women who did not experience repeat positive (FoN) (Supplementary Table S1).

The second approach, using sequence capture and enrichment, was attempted on index and follow up positive samples from all participants who experienced a follow up positive test. The aim was to sequence and analyse *chlamydial* DNA directly from high vaginal swabs from both events in each participant to characterize any genomic features that might associate with repeat infection. All swabs from index and FoP event in the same participant were included in the sequence capture genomics methods, although several failed to recover adequate DNA or sequencing read depth for genomic analysis and were excluded from further analysis (Supplementary Table S1).

The baseline and repeat infection swabs collected in SPG (chlamydial storage solution) were used to extract DNA for this analysis. The reads were filtered with a Q20 threshold for quality, and stringent read filtering thresholds were used in bowtie2 (31) and samtools (32) to remove reads from other bacteria, and to allow mapping of reads against a reference from *C. trachomatis* D/UW. A selection of 15 isolates were cultured and sequenced from DNA extractions from the cultures (as many as 12 culture passages from the primary swab were used for these extractions). The DNA extracts were sequenced using an Illumina MiSeq using the TruSeq v3 reagents generating 300 bp long paired-end reads. The sequence library was prepared using and Illumina NexteraXT Library Preparation Kit. Paired end reads were quality checked for consistency in base qualities using FastQC v0.11.2 and were trimmed using Trimmomatic v0.32 (33). Reads were analyzed using a sliding window of 4 bp and trimmed once the average base quality across the window dropped below 28. A minimum read length of 50 bps was required after trimming. Only bases 5 to 200 of each read was retained due to the lower average quality of the bases outside this range. Reads were mapped to a reference published *C. trachomatis* strain D/UW/3-CX genome using both Shrimp v2.2.3 (34) and Bowtie v.2.2.3 (31) with only the best match reported for each read. SNPs and indels were filtered, called and annotated using the Nesoni data analysis toolset (Harrison, unpublished). Reads were assembled with Velvet v1.2.10 (35). Optimal *k*-mer length for De Brujin graph construction was determined using the VelvetOptimiser v2.2.5 wrapper script (Gladman & Seeman, unpublished). Assemblies were optimized using PAGIT to close gaps by comparing against a reference genome (36). Assembled genomes were annotated using Prokka v1.10 (37).

A phylogeny was constructed using kSNP v2.1.2 (38) with a *k*-mer of 17, as estimated by Kchooser to the relationships of the unassembled isolates relative to 62 other *C. trachomatis* strains. The genome sequences of these strains were retrieved from the NCBI RefSeq database in October 2014. A whole genome alignment was performed using Mugsy v1.2.3 (39) using the default parameters and a Maximum-Likelihood phylogeny estimated using RAxML (v8.1.15) using the rapid hill-climbing algorithm (-f a) and the GTR model of nucleotide substitution with a gamma distribution of rate variation among sites (-m GTRGAMMA). The resulting alignment contained 5,854 phylogenetically informative sites.

Biospecimen handling

Biospecimen collection was conducted as previously outlined (25), unless otherwise described here. Diagnostic PCR, genovar profiling PCR (40), and *Chlamydia* culture and enumeration (41) were all conducted in accordance with previous publications. These samples were selected firstly be identifying a variety of cases that were associated with distinct chlamydial *ompA* genomes to get representation across the range of pathogens that could then be matched to two controls by age and contraceptive type in usage. Chlamydial genome sequences were analyzed either directly from the participant swabs or from isolates cultured from the swabs as outlined in the genomic analysis.

Results

Chlamydial burden in the absence of *in vitro* resistance, was associated with repeat infections

A total of 305 women were enrolled into the cohort; 271 (88.9%) had a test of cure at week 4, and 223 (73.1%) were followed until study end at week 8. The median time for follow up was 56 days (IQR: 44–58 days), with a total of 2,154 weeks of follow up. Overall, 36 (11.8%; 95%CI: 9.4, 14.7) had a repeat infection during follow up, with an incidence of 1.7 repeat infections per 100 weeks (95%CI: 1.6, 1.8). Time to repeat positive tests during follow up was associated

10.3389/fpubh.2022.1012835

TABLE 1 Factors associated with positive tests on follow up among women in the cohort.

Variable	No. repeat infections*	Person time (weeks)	Unadjusted HR (95%CI)
Age			
16–20	8	355.1	1.0
21–25	18	1,182.9	0.6 (0.3, 1.5)
26–30	6	436.6	0.5 (0.2, 1.6)
30+	4	179.9	0.7 (0.2, 2.4)
Country of birth			
Other	22	1,402.4	1.0
Australia	13	747.9	1.2 (0.6, 2.4)
Use of hormonal contraception			
No	10	689.7	1.0
Yes	26	1,464.7	1.4 (0.7, 3.0)
Use of condoms as contraception			
No	14	764.1	1.0
Yes	22	1,390.3	0.8 (0.4, 1.6)
Number of partners 12 month before study			
1 partner	2	152.3	1.0
2–4 partners	14	876.6	1.1 (0.2, 4.7)
5–9 partners	13	877.1	0.9 (0.2, 4.1)
10+	7	248.4	2.1 (0.4, 10.2)
Number of partners in last week			
0	13	1,131.4	1.0
1	19	937.7	1.4 (0.7, 2.8)
2 or more	4	85.3	3.4 (1.1, 10.4)
At least one episode of anal sex last week			
No	34	2,101.9	1.0
Yes	2	52.6	3.4 (0.8, 14.3)
Condom used with all vaginal sex partners last week			
No	16	704.1	1.0
Yes	8	347.1	1.3 (0.5, 2.7)
No sex	12	1,103.1	0.6 (0.3, 1.3)

*Some missing data so numbers will not always add up to 36 cases.

with 2 or more partners in the last week compared with no partners (HR = 3.4; 95%CI:1.1, 10.4) (Table 1). No other socio-behavioral variables were significantly associated with repeat infection.

Analysis of organism burden by molecular assay identified a higher load of *Chlamydia* in women at index who experienced positive test during follow up (FoP) (Figures 1A,B, Table 2). MIC of azithromycin against the chlamydial isolates was determined to be within the susceptible range for all isolates that were cultured ($n = 53$) (see Materials and methods, results see Table 2). One isolate showed an elevated MIC (0.125 μg/ml) when cultured from repeat infection compared to the baseline culture but remained in the range considered susceptible (Figure 1C, Table 2). All isolates were susceptible to azithromycin.

Chlamydial repeat infection isolates do not have genomic resistance or a unique genotype

Whole genome sequencing and phylogenetic analysis of *Chlamydia* genomes from 28 women included in the analysis show that the *C. trachomatis* identified in these specimens did not belong to a specific lineage and represented a phylogenetically heterogeneous population likely reflective of the organism's global transmission.

Index and follow up positive swabs were successfully sequenced and analyzed from 25 participants, a total of 50 paired samples, using the sequence capture approach. In the case of six participants only one swab was able to be sequenced and analyzed using the sequence capture approach. This provided

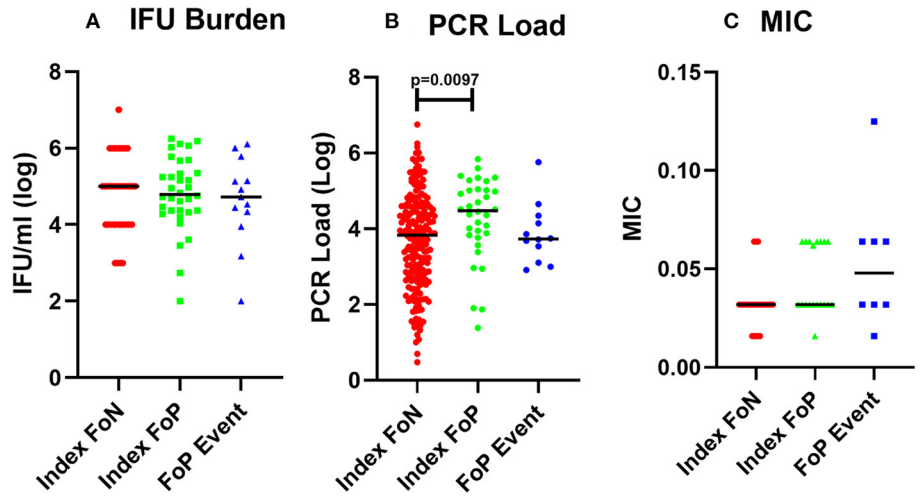

FIGURE 1
Chlamydial burden and Minimum inhibitory concentration of azithromycin against isolates from participants who experienced a positive test during follow up. **(A)** cultured inclusion forming units per ml from high vaginal swabs (log), participant data included includes Index FoN (n = 235), Index FoP (n = 36), FoP Event (n = 31). **(B)** Quantitative PCR assay for CT organism load (log). Data includes participant results from; Index FoN (n = 224); Index FoP (n = 34); and FoP Event (n = 36). **(C)** MIC from cultured chlamydia isolates. The data include the MIC for isolates from participants in the following groups; Index FoN (n = 26 isolates); Index FoP isolates (n = 19); and FoP Event isolates (n = 8). Data are shown by these groups as indicated on the x axis. The measures are shown on the y-axis. Summary data are also provided in Table 1.

genomic insights into 25 individuals who experienced repeat infections (Supplementary Table S4).

The genomics datasets were analyzed from both approaches using phylogeny analysis of the whole genome sequence and there was no clustering of chlamydial genomes associated with repeat infections (Figure 2). The sequences of genetic loci previously associated with resistance to azithromycin, 23s rRNA (ribosomal structural gene and target site of azithromycin), L4 (a ribosomal associated with SNPS identified in azithromycin resistance), and L22 (a ribosomal associated with SNPS identified in association with azithromycin resistance), were examined and no polymorphisms corresponding to known resistance mechanisms previously described in other organisms were present, regardless of if they were associated with follow up positive event or not.

Analysis of the genomics of 25 paired isolates from the women who experienced a positive test result during follow up (Table 3, Figure 2), was conducted in conjunction with the datasets reported by each individual for sexual behavior. The follow up positive sequence capture analysis had sometimes lower read depths compared to baseline (Supplementary Table S4). In three cases identical chlamydial genomic sequences were detected for the baseline and repeat infection events. Two of these cases reported exposure to a repeat sexual partner and a recent history of/or current anal sex. One of these cases reported no current or recent history of anal sex and no sexual contact during the study period. There were three cases with a low number of genomic variations between the index and positive follow up event, with a variety of sexual behavior from no activities to repeated sexual activities with a recent partner reported. 19 cases had clearly different isolates present in the index and follow up positive events with 301 or greater genomic distinctions. In these cases, where the genomics clearly indicated the presence of a genetically distinct chlamydial isolate from the isolate at baseline, there were some participants who reported no sexual exposure, and no recent history of anal sex.

The genomics data and these scenarios was used to further consider the role of infectious burden at the index time point in the outcome of the follow up positive test events. Among women who were likely to have had the same infection, chlamydial DNA load at time of index case was significantly higher than for those women who did not have a positive test during follow up (p = 0.0338; mean 3.65 vs. 4.72 log10). In contrast, there was less evidence of a difference in chlamydial DNA load at time of index case between those women who had positive or negative follow up results (0.0781; mean 3.65 versus 4.19 log10).

Chlamydial genes were more highly expressed in the repeat infection event, and some immune genes were down regulated

The high vaginal expression of some immune genes was analyzed in a subset of participant samples using a nested Case-Control study design (22 no-repeat infection [controls] and 12

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TABLE 2 Microbial features associated with positive follow up test.

	Index FoN	Index FoP	*p* value (Index FoP – Index FoN) f[a]	FoP Event	*p* value (Index FoP vs. FoP event)
IFU/ml Raw data					
Mean	1, 918 53.1	280, 511.8	*index expression of any of the selected* = 0.7168	109, 079.4	$p = 0.6561$
SD	496, 048.7	471, 819.4		297, 597	
Median	10, 858	55, 104.35		0	
IQR	0–120, 342.8	19, 517.25–221, 548.4		0- 33, 659.8	
Range	0–3, 474 560	0–1, 769 854		0- 1, 262 243	
n	235	36		31	
Organism Burden by DNA detection (PCR load)					
Mean	9, 2456.06	86, 024.57	$p = 0.0097$	18, 937.44	$p = 0.5968$
SD	424, 404.1	139, 072.3		95, 166.28	
Median	4,330	28, 000		0	
IQR	339.5–31, 650	5, 780–104, 200		0-2910.53	
Range	0- 5, 620 000	0–684, 000		0–571, 900	
n	224	35		36	
MIC of cultured isolates to Azithromycin[b]					
Mean	0.033	0.044	$p = 0.0216$	0.054	$p = 0.7198$
SD	0.013	0.017		0.034	
Min-Max	0.016–0.064	0.016–0.064		0.016–0.125	
n	26	19		8	

[a]Compared using t-test, [b]compared using Mann-Whitney U test.
Note statistical analysis was conducted on log transformed data as shown graphically in Figure 1.
*Some missing data.

repeat infection [cases]). The gene expression of *IRF-1* (42), *CXCL9* (43), *FTH1* (44), *IL-6* (45), *IL-8* (46), *IDO1* (47), *TNF-α*, *IL-1α*, *IL-10* (48), and *ifn-α* were determined by normalizing to the geometric mean of *GAPDH* and *PGKI* [established for the female reproductive tract (49)]. These genes were selected for the following hypothetical implications; indicative of a pro-inflammatory response needed to clear chlamydial infections (*CXCL*-9, IFN-γ, *TNF-α*, *IRF*-1, *IL*-8, *IL*-1α); biological role which may implicate host conditions compatible with chlamydial persistence (*IDO*-1, *FTH*-1); pleiotropic or reduced pro-inflammatory responses (*IL*-10 and *IL*-6) (50–52). There were no differences in the index expression of any of the selected genes between those that experienced positive or negative follow up test results. However, there was significantly lower gene expression observed at the time of follow up positive of *IL-10* and *CXCL9* when comparing to index for the same women ($p \leq 0.01$ and ≤ 0.05, respectively) (Figure 3).

The expression of selected chlamydial genes was also analyzed from the high vaginal swabs including, the major outer membrane protein (*ompA*) (53), a repressor of genes important for late chlamydial developmental cycle stages (*euo*) (54), an outer membrane complex protein (*omcB*) (55), a protease essential for the replicative phase (*htrA*) (56), and the gene encoding the enzyme that synthesize tryptophan from indole (*trpBA*) that could be important and implicate the potential for chlamydia persistence if a host IFN-γ-IDO1 response was active in the presence of indole producing microorganisms (all normalized to 16S rRNA) (51, 57, 58). There was no difference in the expression of these genes at index comparing specimens from women experiencing FoP to FoN results, but all were found to have higher levels in women at the time of FoP compared to the same women at index (Figure 3).

Discussion

Chlamydia trachomatis, as an obligate intracellular organism, has evolved over long periods of evolutionary time to adapt and survive in the cervical epithelia, a dynamic site influenced by many factors. In addition to the burden associated with testing and treating the sexually transmitted infection, *Chlamydia* is also associated with a range of serious reproductive health outcomes (2). Described here is a cohort study of chlamydial repeat infection, the aim of exploring the potential microbiological and immunological factors associated with positive tests during follow up from a treated infection in women. In this study, positive follow up test results with chlamydial vaginal infections, in women treated with azithromycin, occurred in 11.8% of women within 8 weeks of treatment. There was no evidence for chlamydial antibiotic

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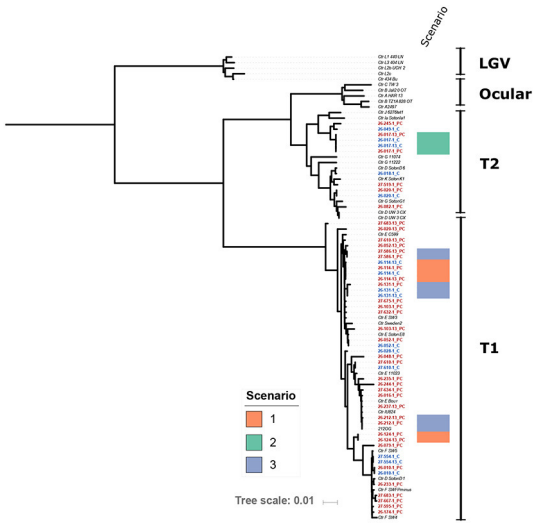

FIGURE 2
Maximum-Likelihood core genome phylogeny of the study isolates with 29 reference *Chlamydia trachomatis* (Ctr) genomes (*italicized*) based on 5,854 phylogenetically informative core SNPs. Isolates from this study are highlighted with red and blue font; red font indicates probe capture (PC) isolates and blue indicates cultured genomes. The position of isolates from Scenarios 1, 2, and 3 are indicated as shown by the color key. Study isolates that do not have a scenario indicated on the figure are those from Scenario 4 as described in the discussion, or where there was not enough resolution in one swab to define the Scenario. Scenario 1 is defined as identical isolate genotypes and the participant reported either/or both sexual contact with a repeat sexual partner and a history of anal sex. Scenario 2 is defined as identical isolate genotypes and the participant reported no sexual contact and no history of anal sex. Scenario 3 is defined as low genetic differences, and participants reported a variety of sexual behaviors from no sexual contact to sexual contact with a repeat partner and a history of anal sex. Scenario 4 indicates where the genomics supports a completely different isolate, in these scenarios participants reported a variety of sexual behaviors from no sexual contact to sexual contact with a repeat partner and a history of anal sex.

resistance, but some indication that follow up positive infections were transcriptionally active at a higher level compared to the index (presumably longer term) infections. This higher transcriptional level of the genes tested may indicate a much more active transcription of the organism at these specific loci in response to the host-pathogen interactions in the context of repeat infections. This suggests that after treatment there may be an increased risk of repeat exposures establishing an infection, or that newly acquired infections regardless of whether repeat infections are more transcriptionally active likely due to the host not having activated an immune response.

Overall, the burden of chlamydia was higher in women who experienced positive follow up events at index by PCR, which is consistent with other studies (59, 60). In the chlamydial genomics analysis, for the 25 women where data was obtained from paired index and follow up positive eent specimens, 19 of these were clearly new infections, 3 had a small number of genotypic differences, and 3 had identical genomes.

This analysis supported four scenarios that could explain repeat infection. In the first scenario for two women in this study, genomics confirmed that the same isolate at both time points (i.e., genotypically identical) was detected (scenario 1, Table 3). In these two cases, the participant reported sexual contact (anal sex) with a repeat sexual partner who had been treated, suggesting these two cases could be accounted for by either repeat exposure, or anal auto-inoculation from an infection derived from anal sex. In the second scenario (scenario 2, Table 3), involving a single participant, the index and follow up positive isolates were genotypically identical, and this individual reported no sexual contact or no anal sex history in the time frame of the repeat infection event. This second scenario could be a treatment failure event or auto-inoculation from the anal site, having potentially acquired the anal infection *via* the hypothesized oral-anal route (13). An alternative explanation could be chlamydial persistence. A third scenario was apparent where a low number of genotypic variations was detected between the index and follow up positive sequences (24-91 SNPs, this included three participants, scenario 3, Table 3). In this third scenario two participants reported repeat sexual contact with a recent partner, and one did not report sexual contact. Given the number of genetic differences these three events could be explained by a newly acquired infection, a repeat exposure with some genetic drift over time, or anal autoinoculation with some genetic drift in host. These isolates from these three scenarios where limited genetic differences were apparent, are clearly their closest relatives, as shown in the phylogeny (Figure 2), but are not clustered together in the overall phylogeny, indicating that there is not a particular genovar or strain associated with these outcomes. The remainder of the follow up positive events from the women included in this paired genomic analysis had between 301 and 4,725 SNP differences between the index and FoP event (with repeat infections observed from 28 to 56 days, including 19 women), these cases have such a degree of genomic difference they are clearly new infections. This data also demonstrates that the genome sequencing approach may have benefits for other studies (such as future vaccine trials) where monitoring new exposure compared to clearance of organisms could be critical to monitor efficacy. This approach whilst costly presents a robust alternative to reliance on self-reported sexual behavior which is well known to have limited validity (61).

Selected chlamydial genes were highly expressed during repeat infections, compared to index. Two immune genes were observed in the gene expression analysis, *IL-10* and *CXCL9,* to be lower during the repeat infection compared to baseline. Il-10 is associated with reduced pro-inflammatory responses and immune suppression, in particular suppression of IFN-γ and other pro-inflammatory cytokine production, and in the past has been associated with reduced protection against chlamydia

TABLE 3 Genomics analysis of positive follow up events in the context of sexual behavior.

Genomic Differences (range)	Number of participants	Read depth	Sexual behavior[!]	Scenario (outlined in discussion)
0	2	Mean = 1,191.5 SD = 409.6 Range = 718–1,566 *n* = 4	Repeat sexual exposure, recent anal sex	1
0	1	Mean = 1528.5 SD = 95.5 Range = 161–1,596 *n* = 2	No repeat sexual exposure, no recent anal sex	2
24–94	3	Mean = 710.8 SD = 628.1 Range = 54–604 *n* = 6	Repeat sexual exposure, recent anal sex reported	3
301–4,725	19	Mean = 651 SD = 593 Range 8–1,570 *n* = 38	Repeat sexual exposure/reported no repeat sexual exposure, recent anal sex/no anal sex	4

[!]Repeat exposure is defined as sexual contact with a repeat sexual partner during study time frame, recent anal sex includes in recent past or during study timeframe.

infection, or reduced cell mediated responses to chlamydia in murine models (48, 62–64). It was surprising to see lower levels of IL-10 in the FoP event compared to index in these women, although IFN-γ gene expression was not significantly increased relative to index in the same participants, so this reduced IL-10 gene expression may not be translating to a biological impact on the cytokine levels. The chemokine CXCLl9 has been associated with pro-inflammatory responses and immunopathology in murine models when higher levels are present or the cognate receptor is inhibited but has not been associated with clearance of the infection (65, 66). The significantly lower levels here were consistent with low (but not significant) levels of other pro-inflammatory factors during repeat infections. It has been reported that a IFN-γ producing CD4+ T cell response is associated with protection against re-infection in women (67). Although we did not detect a significant change in IFN-γ gene expression levels when comparing index with FoP specimens, we do not have subsequent gene expression data from women who were FoN (67). Whilst there were no significant differences in IFN-γ gene expression levels, there did appear to be a trend toward reduced expression of IDO-1 in the repeat infection events, which would be consistent with an overall reduction in pro-inflammatory or cell mediated response in these women with repeat infections. The gene expression analysis included some host and chlamydial genes that have been associated with biological functions or distinct expression profiles during chlamydial persistence *in vitro*. However, as the genomics analysis indicated that most follow up positive events involved new isolates or genetic drift over time indicating a related isolate, this rules out chlamydia persistence as an explanation for most cases. The host genotypes were not examined here, but it is relevant to acknowledge that the genotype of the women likely factor into repeat infection, as previously reported that HLADQB1*06 genotype in African American women was associated with chlamydial reinfection risk (68).

The observation of lower immune gene expression corresponding with higher chlamydia gene expression during follow up positive events should be explored further in future repeat infection studies, as this may have implications for management of women at risk of repeat infections. Three possible explanations are proposed for this observation, 1. the immune suppression from azithromycin (69) may result in higher susceptibility to repeat infection upon exposure; 2. the impact of a recent previous chlamydial infection, treatment, and any subsequent alterations of the microbiome due to varied susceptibility to the therapy used could enable a more active growth of chlamydia (and potentially could also benefit other pathogens); or 3. Incident (new) infections of *Chlamydia* may in general be more active in the transcription of these specific genes (and perhaps a selection of others), regardless of recent treatment or not (unable to explore in this dataset due to the inability to reliably ascertain the duration of any infections for women at index).

There are limitations in the study that should be considered. Firstly, the study had reduced statistical power to assess associations as the sample size was limited to only 36 cases of repeat infection in the cohort. The case-control sub-analysis was implemented to allow some level of control for hormonal

10.3389/fpubh.2022.1012835

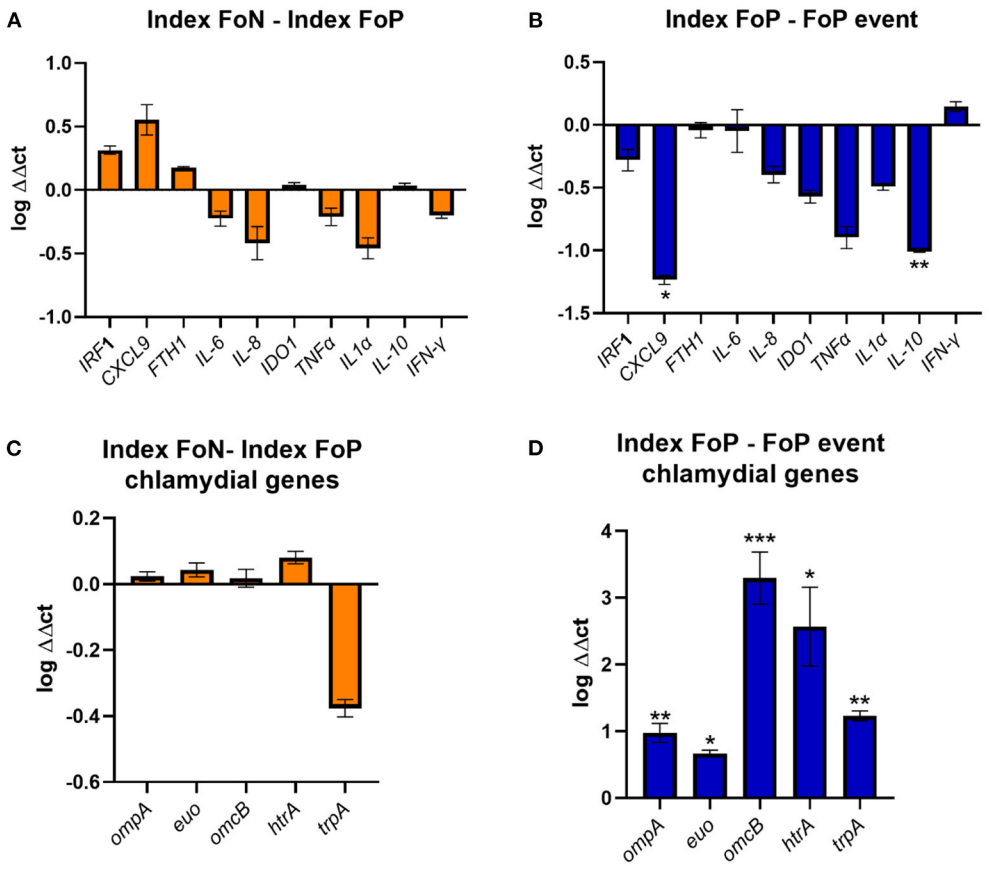

FIGURE 3
Expression of immune genes from vaginal swabs. **(A)** The figure shows the vaginal immune gene expression shown as ΔΔct log transformed data comparing the Index Follow up Negative (Index FoN) to Index Follow up positive (Index FoP) (orange) for the human genes analyzed. **(B)** The graph shows the vaginal immune gene expression shown as ΔΔct log transformed data comparing the Index FoP with the FoP event in the same participants for the human genes analyzed. **(C)** The figure shows the vaginal immune gene expression shown as ΔΔct log transformed data *C. trachomatis ompA*, *euo*, *omcB*, *htrA*, and *trpBA* normalized to *Ct* 16S rRNADNA copies as a normaliser for the comparison of the Index FoP to Index FoN. **(D)** The figure shows the vaginal immune gene expression shown as ΔΔct log transformed data *C. trachomatis ompA*, *euo*, *omcB*, *htrA*, and *trpBA* normalized to *Ct* 16S rRNA DNA levels comparing the Index FoP to the FoP event. Data included here is sourced from biospecimens from participants in the nested case-control study (controls $n = 22$, and cases $n = 12$). The y-axis is log transformed relative expression and x-axis indicates the name of each gene of interest. Statistical significance is denoted by * ($p \leq 0.05$), ** ($p \leq 0.01$), and *** ($p \leq 0.005$).

contraceptives, chlamydial genovar, and age of the participants. A much larger study using a multivariate approach to control for each of these factors may provide greater insights. Much of the analysis here was contingent on molecular or biological assays from specimens which was not always possible due to low organism load, lack of chlamydia present in the specimens, contaminants or other unknown factors resulting in data not being collectable from all the swabs collected. There is an inability to explore anal infection carriage to assess the likely role of anal auto-inoculation, as anal swabs were not consistently collected from women as this was an optional component. Hypothetical factors relating to immune responses, or host factors known to drive persistence were analyzed in the gene expression analysis. When gene expression levels were observed to differ between groups this may be interpreted to indicate a cellular response impacting on gene expression, or an RNA stability change for that transcript has varied. Transcript levels do not necessarily indicate biological function that the detection of the protein would imply. Further, as only a small number of genes (and samples) analyzed it is not possible to draw conclusions with respect to these potential biological responses. Finally, as this study did not include an uninfected control group it is difficult to interpret the observations here and how these various factors may vary naturally.

Overall, these data could be interpreted to indicate that post-treatment, due to a direct impact of azithromycin or the impact of the therapy on the local tissue and microbial environment, conditions may be altered, resulting in repeat chlamydial infections having distinctive gene expression profiles. In support of these scenarios microbiome shifts have been reported after

azithromycin therapy (70), and distinct impacts of azithromycin on metabolism and cytokine responses have also been recently reported (71). Alternatively, it is possible that new infections are highly transcriptionally active, regardless of whether they are repeat exposures or not, as we do not know the timeframes of the infections from the index samples. Nonetheless, repeat, or new infections being highly active in women, supports the importance of timely testing and treating of chlamydia as a public health consideration. Further study is needed to understand *in vivo* the impact of treatment, how treatment may impact risk of repeat infections, and if the reproductive health consequences may vary for these women, given the known increased occurrence of adverse outcomes associated with repeat infections (72, 73).

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 below: https://www.ebi.ac.uk/ena, PRJEB12313.

Ethics statement

The study was reviewed and ethical approval granted by the Alfred Hospital Ethics Committee and the Southern Eastern Sydney Local Health District Human Research Ethics Committee (Southern Sector). The patients/participants provided their written informed consent to participate in this study.

Author contributions

WH: data analysis and interpretation, study design, and manuscript drafting. AL: cultured and biological analysis of chlamydia culture and conducted the MIC analysis. BW: conducted the genomics analysis and interpretation. MT: conducted and analyzed the immunological measures. PT: data analysis and interpretation and study design. LV: study design, ethics, and conduct. SP: Chlamydia PCR genovar determination and PCR load. AM, CF, and MC: study design and conduct. RM and KW: clinical recruitment. JH: study design and conduct, epidemiological analysis, data interpretation, and participant questionnaire analysis. All authors contributed to the article and approved the submitted version.

Funding

This study was supported by National Health and Medical Research Council Project grant number APP1023239.

Acknowledgments

The ACTS Investigators acknowledge the participants for their valuable contribution to the study. The contributions from the teams at Melbourne Sexual Health Centre and Sydney Sexual Health Centre are gratefully acknowledged. We thank M. Humphreys and J. Ravel, IGS, University of Maryland for insights into sequence capture sequencing methodologies.

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.2022.1012835/full#supplementary-material

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 11 October 2022
DOI 10.3389/fpubh.2022.1006254

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases–Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 29 July 2022
ACCEPTED 21 September 2022
PUBLISHED 11 October 2022

CITATION
Hu H, Chen Y, Shi L, Liu X, Xu Z, Sun L, Zhao X, Zhou Y, Lu J, Zhang Z, Liu X and Fu G (2022) Prevalence of syphilis and chlamydia trachomatis infection among men who have sex with men in Jiangsu province, China: A cross-sectional survey.
Front. Public Health 10:1006254.
doi: 10.3389/fpubh.2022.1006254

Prevalence of syphilis and chlamydia trachomatis infection among men who have sex with men in Jiangsu province, China: A cross-sectional survey

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Background: Epidemics of sexually transmitted infections (STIs) among men who have sex with men (MSM) are major global public health concerns. This study aimed to examine the prevalence of syphilis and chlamydia trachomatis (CT) infection and associated factors among MSM in Jiangsu province, China, hoping to provide updated data for the formulation of relevant policies.

Methods: A cross-sectional survey was conducted among MSM from April to July 2021 in four cities in the province. Socio-demographic characteristics and behavioral information were collected through a face-to-face questionnaire interview. Venous blood specimens were collected for HIV, hepatitis C (HCV), and syphilis testing using serological testing methods. First-void urine specimens were collected for CT and Neisseria gonorrhoeae (NG) testing using nucleic acid amplification testing (NAAT) methods. Chi-square tests were used to compare differences in syphilis and CT infection between subgroups of variables. Multivariate logistic regression analysis was used to identify factors associated with syphilis and CT infection.

Results: A total of 1,087 participants were enrolled. The prevalence of HIV, HCV, syphilis, CT and NG infection were 6.6, 0.4, 6.3, 4.2, and 0.4%, respectively. MSM recruited online [adjusted odds ratio (aOR) = 2.189, $P = 0.020$], diagnosed with an STI in the past 12 months (aOR = 3.304, $P < 0.001$), and living with HIV (aOR = 4.721, $P < 0.001$) were more likely to have syphilis infection. MSM who were younger than 25 years (aOR = 4.286, $P = 0.020$), had senior high school level education (aOR = 2.521, $P = 0.038$), and were recruited *via* VCT clinics (aOR = 3.455, $P = 0.001$) were more likely to have CT infection.

10.3389/fpubh.2022.1006254

Conclusions: Our study showed a high prevalence of syphilis and chlamydia among MSM in Jiangsu province, China. STI screening, diagnosis, and treatment services promotion should be a top priority on the prevention agenda.

KEYWORDS

syphilis, chlamydia, prevalence, men who have sex with men (MSM), China

Introduction

Sexually transmitted infections (STIs) are among the most common infectious diseases globally and affect the health and lives of people worldwide. According to a report by the World Health Organization (WHO), there were about 373.1 million STI infections (four of the most common curable STIs) in 2020, of which 128 million chlamydia cases, 82 million gonorrhea cases, 156 million trichomoniasis cases, and 7.1 million syphilis cases (1).

These STIs can cause acute urogenital diseases like urethritis, vaginitis, cervicitis, and genital ulceration. Also, their etiological agents sometimes infect the rectum and pharynx. Chlamydia (etiological agent: Chlamydia trachomatis, CT) and gonorrhea (Neisseria gonorrhoeae, NG) can cause serious complications, including pelvic inflammatory disease in women and orchitis in men. Syphilis (etiological agent: Treponema pallidum) can cause dermatological, neurological, and cardiovascular diseases (2, 3). These STIs are curable but can increase the risk of human immunodeficiency virus (HIV) infection, especially in men who have sex with men (MSM), the group most at risk of HIV infection (4–6).

CT, NG, and syphilis infections were associated with an increased risk of HIV infection in men, possibly due to mucosal inflammation and ulceration of local tissues leading to HIV invasion (7). MSM with HIV and urethral STIs may also have higher viral loads of HIV in semen and thus be more likely to transmit HIV to their sexual partners during unprotected anal intercourse (8). Although direct studies have not established a relationship between STI treatment and reduced HIV transmission rates, urethritis treatment in HIV infection may reduce seminal plasma viral load (9). Model studies and at least one randomized study showed that treatment of STIs may reduce HIV transmission (10, 11). However, CT and syphilis infections are sometimes asymptomatic, and infected individuals may not be screened during routine clinical evaluation. This leads to late diagnosis leading to persistent, unintentional STI and HIV transmission (12).

Understanding the prevalence of STIs among MSM for HIV prevention and control is important. A nationwide cross-sectional study conducted in 61 cities in China from February 2008 to September 2009 found that 11.8% of MSM had syphilis infection (13). In a survey involving MSM in two cities in Jiangsu province of China in 2009, the prevalence of STIs was measured, including CT (6.54%), NG (3.63%), and syphilis (20.34%) (6). In 2010, the Chinese government launched a plan to prevent and control syphilis. Many initiatives focused on prevention and control measures that expanded syphilis testing uptake among key populations, including MSM, and early detection and treatment of syphilis infections. A spatiotemporal meta-analysis of syphilis epidemic among men who have sex with men living in mainland China showed that national syphilis prevalence decreased from 12.3% in 2001–2007 to 7.1% in 2013–2015 (14). However, by the end of 2020, reports on CT prevalence and measures on prevention among MSM were few. Located in southeastern China, Jiangsu province is economically developed, with a more than 80 million population. Herein, we conducted this cross-sectional survey to examine the prevalence of syphilis and CT infection and their associated factors among MSM in Jiangsu province, China, to provide more recent data for formulating relevant policies.

Materials and methods

Study design and participants

A cross-sectional survey was conducted among MSM at the AIDS and STD surveillance sites in Jiangsu province from April to July 2021. MSM were enrolled in one of four cities (Zhenjiang, Wuxi, Yangzhou, and Suzhou) in the province that acted as survey sites for the study. Eligible participants were assigned male gender at birth, 18 years of age or older, and self-reported anal or oral sex with another male in the previous year. Each recruited participant had a face-to-face questionnaire interview and specimen collection. All participants provided written informed consent before enrolment. Implementation of AIDS and STD surveillance sites is a routine part of disease control and prevention, so this study was exempt from ethical review.

Measures

Three convenience sampling methods were used to recruit participants: (1) Recruitment at MSM gathering venues:

Staff from local centers for disease control and prevention (CDCs) and volunteers from local MSM-led community-based organizations (CBOs) conducted on-site surveys at popular MSM gathering venues. Venue owners (e.g., bars, clubs, and bathhouses) and volunteers who knew of public places where MSM frequently gathered (e.g., parks or public restrooms) referred interested and eligible participants to the interviewers.

(2) Online recruitment: Volunteers posted recruitment information, including eligibility criteria, survey sites, survey period, and contact numbers through WeChat, QQ, and other apps. Eligible participants came to the designated location to complete the questionnaire and specimen collection.

(3) Recruitment at VCT clinics: Eligible MSM clients attending VCT (HIV voluntary counseling and testing) clinics were recruited for enrollment in the study after receiving counseling from the VCT clinic staff.

The interviews remained anonymous throughout the study, and any information that could identify individual participants was exempted from the final data. Participants' cell phone numbers were obtained for testing results notification and referrals to infection-related resources and services. In order to prevent duplicate participation, same interviewers were staffed at each study site to identify participants (e.g., face, special characteristics). In addition, staff asked participants if they already participated in similar surveys, and used computers to check participants' cell phone numbers for duplication before the interviews. In our study, there were no duplicate participation between venue, online and VCT recruitment.

Participants were interviewed face-to-face by interviewers who completed provincial or municipal surveillance training, especially in STI training. The information obtained with the questionnaire included socio-demographic characteristics (age, marital status, education level, registered residence, duration of living locally, and monthly income) and STI-related behavior (sexual orientation, the main way to find a sexual partner, unprotected anal intercourse in the past 6 months, STI in the past 12 months and HIV testing in the past 12 months). After completing questionnaire interviews by interviewers, the quality controllers who completed training courses checked the quality of the questionnaire. Unprotected anal intercourse (UAI) was defined as inconsistent condom use with male partners. Not having anal sex was equivalent to consistent condom use.

After questionnaire interviews, venous blood specimens collected from each participant were tested for HIV, Hepatitis C virus (HCV), and syphilis using serological testing methods. Plasma HIV and HCV antibodies were tested using an enzyme-linked immunosorbent assay (ELISA) reagent (Zhuhai livzon Diagnostics Inc., Zhuhai, China). For those with positive HIV or HCV test results, the same blood samples were retested with another ELISA reagent (Beijing Wantai Biological Pharmacy Enterprise Co., Ltd., Beijing, China). If both tests were positive, participants were contacted by CDC staff through reserved phone numbers. Participants with positive HCV results were referred to infectious disease hospitals for treatment and care. Participants with positive HIV results by ELISA testing provided new blood specimens for HIV confirmatory testing by western blot assay (MP Biomedical Asia Pacific Pte. Ltd., Singapore). The previous blood samples were used for confirmatory testing if participants were lost to follow-up after the initial testing. The syphilis antibody testing was conducted using an ELISA reagent (Beijing Wantai) and confirmed using a toluidine red untreated serum test (TRUST, Beijing Wantai). Syphilis infection was defined as having two positive tests, and participants were referred to designated facilities for treatment. First-void urine specimens were collected from each participant to test for CT and NG with nucleic acid amplification testing (NAAT) methods (Shanghai Rendu Biotechnology Co., Ltd., Shanghai, China). Auto-SAT automatic nucleic acid testing and analysis system (Shanghai Rendu) was used to test urine specimens for CT and NG nucleic acid. If tests were positive, participants were considered as having CT or NG infection.

Statistical analysis

Questionnaire data at each survey site were double-entered and checked for accuracy using EpiData software (version 3.1). Quantitative variables were grouped, and qualitative variables were merged according to the data distribution. Socio-demographic and STI-related behavioral characteristics of participants were descriptively analyzed using frequencies. Chi-square tests were used to compare syphilis and CT infection differences between subgroups of variables to seek potential associated factors. Variables with P-values < 0.20 were entered into multivariable logistic regression models to identify independent factors associated with syphilis and CT infection using a forward method to determine the adjusted odds ratios (aORs). P-values < 0.05 were considered statistically significant. All analyses were conducted using SPSS software (version 19.0).

Results

Socio-demographic characteristics of participants

A total of 1,087 eligible participants were enrolled in the study per our informed consent, completed questionnaire, and laboratory testing protocol. The average age of participants was 29.6, with a standard deviation of 12.2 (range: 18–77). Participants aged 24 years or younger constituted 45.0% (489), and those aged 25–39 constituted 37.2% (404). About three-quarters of participants (74.8%, 813) were single, divorced, or widowed. Participants' distribution across the three levels of education was similar (30.8, 35.0, and 34.2%, respectively). Most participants (57.9%, 629) were registered residents of

10.3389/fpubh.2022.1006254

TABLE 1 Socio-demographic characteristics of 1,087 MSM in a cross-sectional survey in Jiangsu province, China.

Variables	No. (%)
Age group (years)	
<25	489 (45.0)
25–39	404 (37.2)
≥40	194 (17.8)
Marital status	
Single, divorced or widowed	813 (74.8)
Married or cohabiting	274 (25.2)
Education level	
Junior high school or below	335 (30.8)
Senior high school	380 (35.0)
College or above	372 (34.2)
Registered residence	
Jiangsu province	629 (57.9)
Other provinces	458 (42.1)
Duration of living locally	
<2 years	475 (43.7)
≥2 years	612 (56.3)
Monthly income (CNY)	
<5,000	549 (50.5)
≥5,000	538 (49.5)
Recruitment source	
Venues	706 (64.9)
Internet	189 (17.4)
VCT clinic	192 (17.7)

MSM, men who have sex with men; VCT, HIV voluntary counseling and testing; CNY, China Yuan.

Jiangsu province and had lived locally for more than 2 years (56.3%, 612). Participants with a monthly income of <5,000 CNY (China Yuan) and more than 5,000 CNY were equally represented. 64.9% (706) of participants were recruited from MSM gathering venues, and others were recruited online (17.4%, 189) and at VCT clinics (17.7%, 192) (Table 1).

STI-related behavioral characteristics of participants

Among participants, 41.9% (455) identified as homosexuals, 25.6% (278) were heterosexuals, and 32.6% (354) were bisexuals (self-reported). 38.5% (418) of participants found sexual partners at MSM gathering venues, and 61.5% (669) did through the Internet or dating apps. Only 12.8% (139) engaged in UAI in the past 6 months, 7.3% (79) were diagosed with an STI within the past 12 months, and the majority (55.7%, 605) tested for HIV in the last 12 months (Table 2).

TABLE 2 STI-related behaviors of participants.

Variables	No. (%)
Sexual orientation	
Homosexual	455 (41.9)
Heterosexual	278 (25.6)
Bisexual or indetermination	354 (32.6)
The main way to find a sexual partner	
MSM venue	418 (38.5)
Internet or dating app	669 (61.5)
UAI in the past 6 months	
No	948 (87.2)
Yes	139 (12.8)
STI in the past 12 months	
No	1,008 (92.7)
Yes	79 (7.3)
HIV testing in the past 12 months	
No	482 (44.3)
Yes	605 (55.7)

STI, Sexually transmitted infection; UAI, Unprotected anal intercourse.

Prevalence of syphilis, CT, and other infectious diseases

Among the 1,087 participants, 69 tested positive for syphilis, showing a high prevalence of 6.3% [95% confidence interval (CI): 4.9–7.8%]. Forty six tested positive for CT infection with a prevalence of 4.2% (95% CI: 3.0–5.4%). HIV, HCV, and NG infection prevalence were 6.6% (95% CI: 5.1–8.1%, $n = 72$), 0.4% (0–0.7%, $n = 4$), and 0.4% (0–0.7%, $n = 4$), respectively. Besides, 1.5% ($n = 16$) had HIV-syphilis coinfection; two had syphilis-CT coinfection; one had HIV-HCV coinfection; no HIV-CT coinfection was found.

Factors associated with syphilis and CT infection

Chi-square tests were used to compare syphilis and CT infection differences between subgroups of variables. The results showed that syphilis was significantly associated with UAI in the past 6 months, STI diagnosis in the past 12 months, and HIV infection (all $P < 0.05$). CT infection was significantly associated with age, education level, registered residence, duration of living locally, recruitment source, sexual orientation, UAI in the past 6 months, and HIV testing in the past 12 months (all $P < 0.05$) (Table 3).

In the multivariate logistic regression analysis, MSM who were recruited online (aOR = 2.189, 95% CI: 1.133–4.230, $P = 0.020$), diagnosed with an STI in the past 12 months (aOR =

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TABLE 3 Differences in syphilis and CT infection between subgroups of variables among men who have sex with men.

Variables	Syphilis infection			CT infection		
	No. positive (%)	No. negative (%)	*P*-value	No. positive (%)	No. negative (%)	*P*-value
Age group (years)			0.418			0.000
<25	27 (39.1)	462 (45.4)		35 (76.1)	454 (43.6)	
25–39	26 (37.7)	378 (37.1)		8 (17.4)	396 (38.0)	
≥40	16 (23.2)	178 (17.5)		3 (6.5)	191 (18.3)	
Marital status			0.862			0.052
Single, divorced or widowed	51 (73.9)	762 (74.9)		40 (87.0)	773 (74.3)	
Married or cohabiting	18 (26.1)	256 (25.1)		6 (13.0)	268 (25.7)	
Education level			0.914			0.039
Junior high school or below	22 (31.9)	313 (30.7)		16 (34.8)	319 (30.6)	
Senior high school	25 (36.2)	355 (34.9)		22 (47.8)	358 (34.4)	
College or above	22 (31.9)	350 (34.4)		8 (17.4)	364 (35.0)	
Registered residence			0.815			0.003
Jiangsu province	39 (56.5)	590 (58.0)		17 (37.0)	612 (58.8)	
Other provinces	30 (43.5)	428 (42.0)		29 (63.0)	429 (41.2)	
Duration of living locally			0.970			0.003
<2 years	30 (43.5)	445 (43.7)		30 (65.2)	445 (42.7)	
≥2 years	39 (56.5)	573 (56.3)		16 (34.8)	596 (57.3)	
Monthly income (CNY)			0.146			0.330
<5,000	29 (42.0)	520 (51.1)		20 (43.5)	529 (50.8)	
≥5,000	40 (58.0)	498 (48.9)		26 (56.5)	512 (49.2)	
Recruitment source			0.112			0.043
Venues	37 (53.6)	669 (65.7)		30 (65.2)	676 (64.9)	
Internet	17 (24.6)	172 (16.9)		3 (6.5)	186 (17.9)	
VCT clinic	15 (21.7)	177 (17.4)		13 (28.3)	179 (17.2)	
Sexual orientation			0.582			0.000
Homosexual	31 (44.9)	424 (41.7)		11 (23.9)	444 (42.7)	
Heterosexual	14 (20.3)	264 (25.9)		23 (50.0)	255 (24.5)	
Bisexual or indetermination	24 (34.8)	330 (32.4)		12 (26.1)	342 (32.9)	
The main way to find a sexual partner			0.891			0.051
MSM venue	26 (37.7)	392 (38.5)		24 (52.2)	394 (37.8)	
Internet or dating app	43 (62.3)	626 (61.5)		22 (47.8)	647 (62.2)	
UAI in the past 6 months			0.002			0.028
No	52 (75.4)	896 (88.0)		45 (97.8)	903 (86.7)	
Yes	17 (24.6)	122 (12.0)		1 (2.2)	138 (13.3)	
STI in the past 12 months			0.000			0.375*
No	55 (79.7)	953 (93.6)		41 (89.1)	967 (92.9)	
Yes	14 (20.3)	65 (6.4)		5 (10.9)	74 (7.1)	
HIV testing in the past 12 months			0.919			0.009
No	31 (44.9)	451 (44.3)		29 (63.0)	453 (43.5)	
Yes	38 (55.1)	567 (55.7)		17 (37.0)	588 (56.5)	
HIV infection			0.000*			0.068*
No	53 (76.8)	962 (94.5)		46 (100.0)	969 (93.1)	
Yes	16 (23.2)	56 (5.5)		0 (0.0)	72 (6.9)	
Syphilis infection			–			0.763*
No	–	–		44 (95.7)	974 (93.6)	
Yes	–	–		2 (4.3)	67 (6.4)	

(Continued)

TABLE 3 (Continued)

Variables	Syphilis infection			CT infection		
	No. positive (%)	No. negative (%)	*P*-value	No. positive (%)	No. negative (%)	*P*-value
CT infection			0.763*			–
No	67 (97.1)	974 (95.7)		–	–	
Yes	2 (2.9)	44 (4.3)		–	–	

*Fisher's precision probability test. CT, Chlamydia trachomatis; VCT, HIV voluntary counseling and testing; UAI, Unprotected anal intercourse; STI, Sexually transmitted infection; CNY, China Yuan.

TABLE 4 Factors independently associated with syphilis and CT infection among men who have sex with men in Jiangsu province, China.

Variables	Syphilis infection		CT infection	
	aOR (95% CI)	*P*-value	aOR (95% CI)	*P*-value
Age group (years)				
<25			4.286 (1.259–14.587)	0.020
25–39			1.419 (0.361–5.587)	0.617
≥40			1.000	
Education level				
Junior high school or below			1.818 (0.713–4.640)	0.211
Senior high school			2.521 (1.050–6.052)	0.038
College or above			1.000	
Recruitment source				
Venues	1.000		1.000	
Internet	2.189 (1.133–4.230)	0.020	0.622 (0.178–2.172)	0.457
VCT clinic	1.033 (0.523–2.039)	0.925	3.455 (1.658–7.200)	0.001
STI in the past 12 months				
No	1.000			
Yes	3.304 (1.696–6.436)	0.000		
HIV infection				
No	1.000			
Yes	4.721 (2.404–9.271)	0.000		

CT, Chlamydia trachomatis; VCT, HIV voluntary counseling and testing; STI, Sexually transmitted infection.

3.304, 95% CI: 1.696–6.436, $P < 0.001$), and were infected with HIV (aOR = 4.721, 95% CI: 2.404–9.271, $P < 0.001$) were more likely to have syphilis infection. MSM who were younger than 25 years (aOR = 4.286, 95% CI: 1.259–14.587, $P = 0.020$), had a senior high school level of education (aOR = 2.521, 95% CI: 1.050–6.052, $P = 0.038$), and were recruited at VCT clinics (aOR = 3.455, 95% CI: 1.658–7.200, $P = 0.001$) were more likely to have CT infection (Table 4).

Discussion

In recent years, MSM have become the group at highest risk of STI infection worldwide, higher than female sex workers and much higher than the general population. According to a survey conducted in 61 cities in China from 2008 to 2009, the prevalence of syphilis among MSM was 11.8% (13). Data from the National AIDS sentinel surveillance program showed that the prevalence of syphilis among MSM decreased from 8.6% in 2010 to 6.4% in 2013 (15). A consecutive survey conducted in Jiangsu province showed that the prevalence of syphilis among MSM was 10.2% in 2011 (16), 8.3% in 2015 (17), and 6.3% in our study, showing a significant decline. Our observed declining trend is similar to that of the world (18), China (15), and parts of China (19, 20). A meta-analysis of syphilis incidence among MSM in China yielded similar results (21). As a curable STI, the expansion of syphilis screening, diagnosis, and treatment services play an important role in preventing and controlling the disease (18). Studies on syphilis self-testing among MSM have been conducted in parts of China to evaluate the expansion of accessibility to syphilis prevention services (22, 23).

In our study, the prevalence of urogenital CT infection among MSM was 4.2%. This rate was similar to the prevalence found in Thailand (24), lower than that in Guangzhou, China (25), far lower than that in Papua New Guinea (26), and higher than that in Germany (27) and Wuhan of China (28). In addition, there has been no obvious decreasing trend in the prevalence of CT infection, especially in China. Compared to the syphilis prevention and control strategy, there is no expanded CT screening strategy for high-risk groups in China, which needs to be strengthened in the future. Our study showed a low prevalence of urogenital NG infection among MSM, far lower than that in two other China-based studies (25, 28). Differences in the MSM recruitment sources may explain the contradictions in our findings. In the other two studies, MSM participants were recruited from STI clinics (25, 28), whereas MSM were recruited from MSM gathering venues, online, and VCT clinics in our study. Unlike CT infection, which is mostly asymptomatic, urogenital NG infection in men often presents acute urethral inflammation, which could prompt early testing and treatment. This may be another possible reason for the huge differences in NG infection rates.

Consistent with the findings of some studies (17, 29), HIV infection and online recruitment were independent risk factors for syphilis among MSM in our study. MSM recruited online may use online spaces to find sexual partners more often, increasing their risk of syphilis infection due to multiple sexual partners and reduced self-protection awareness. Therefore, we should pay more attention to this group of MSM, promoting more frequent testing and intervention of syphilis. HIV infection is a risk factor for syphilis infection, which shows that these two diseases are closely related. Interventions for encouraging condom use should be strengthened for people living with HIV. They are less likely to transmit HIV to others after effective antiretroviral treatment but more likely to transmit STIs like syphilis. The prevention and control of STIs and AIDS should be integrated and implemented as a policy.

We found that age, education level, and recruitment source were factors independently associated with CT infection. In line with some studies (25, 28, 29), young MSM are more likely to have CT infection due to more engagement in UAI and limited STI prevention knowledge or awareness. Therefore, there is a need to pay more attention to STI-related health education among MSM, and special efforts should be made to provide services to young MSM. Compared to MSM with college or above education, MSM with senior high school level of education are more likely to be CT infected. There are differences in knowledge of STI prevention and awareness of the need for regular testing between MSM with different levels of education. The health education and testing service for this particular group should be strengthened. MSM in VCT clinics usually have high sexual-risk behaviors, so this group has a high risk of CT infection. Although UAI was not independently associated with syphilis and CT infection in our study, it is well known that UAI is a risk factor for STIs in MSM. Therefore, promoting condom use would still play an important role in preventing and controlling these STIs' transmission.

Through this study, we have determined the current prevalence of syphilis and CT infection among MSM in Jiangsu province and possible risk factors, which are very important for future STI prevention and control. However, several limitations of this study should be noted. First, we did not rely on the STI clinic to recruit MSM. So, we only collected convenient urine specimens and did not collect rectal specimens, which may have shown a higher CT positive rate. Nonetheless, we will continue to observe trends in CT infection rates through multi-year surveys. Second, due to memory bias and face-to-face interview survey patterns, participants might have provided incorrect, fragmentary, or socially expected answers. Third, our study was conducted among some MSM in Jiangsu province, and our results may not be generalizable to all MSM in the province and other regions. Besides, this study was done during the COVID-19 pandemic period. The composition of participants may be affected, for example by the enclosed management of some universities.

Conclusions

This study showed a high prevalence of syphilis and chlamydia among MSM in Jiangsu province, China. Therefore, it is necessary to strengthen the screening and treatment of STIs in MSM to improve their sexual health.

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 Institutional Review Board of Jiangsu Provincial Center for Disease Control and Prevention. The patients/participants provided their written informed consent to participate in this study.

Author contributions

HH, YC, and GF contributed to conception and design of the study. YC, LSh, XiaoxL, ZX, LSu, and XZ organized the database. HH and YC performed the statistical analysis. HH wrote the first draft of the manuscript. YZ, JL, ZZ, and XiaoyL wrote sections of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version.

Acknowledgments

We thank all staff members of the AIDS and STD surveillance sites for their dedicated work. We thank the support from Applied Research Plan for genital infection with Chlamydia trachomatis (Stop CT). We also thank Dr. Gifty Marley for her revision of English writing.

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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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 28 October 2022
DOI 10.3389/fpubh.2022.1005622

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 28 July 2022
ACCEPTED 10 October 2022
PUBLISHED 28 October 2022

CITATION
Pérez-González A, Represa M, Coll P, Potel C, Rodríguez-Rivero S, Flores EV, Vázquez-Estévez C, Ocampo A, Pousada G and Poveda E (2022) Real-life cohort experience after implementing HIV pre-exposure prophylaxis for one year in northwest Spain.
Front. Public Health 10:1005622.
doi: 10.3389/fpubh.2022.1005622

Real-life cohort experience after implementing HIV pre-exposure prophylaxis for one year in northwest Spain

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Introduction: Pre-exposure prophylaxis (PrEP) has become a useful tool to reduce the transmission of human immunodeficiency virus (HIV) in key populations. In this article we assessed the effectiveness, safety, adherence, sexually transmitted infections (STIs) dynamics, and frequency of anal dysplasia among a real-life cohort of PrEP users in Northwest Spain.

Methods: A retrospective cohort study was undertaken in the Alvaro-Cunqueiro Hospital, Vigo which included every individual who started daily emtricitabine/tenofovir-disoproxil-fumarate (FTC/TDF) between November-2019 and October-2021. Clinical and epidemiological data were obtained from the patient's medical records. The effectiveness and safety of FTC/TDF were assessed by HIV serology and renal function monitoring every 3 months. Anal, urethral, and oropharyngeal exudates were collected quarterly after the baseline visit.

Results: A total of 126 individuals were considered eligible, most of the participants had previously been diagnosed with a STI (60.3%), 22% had consumed recreational drugs in the year prior, and 13% had engaged in chemsex. At the end of the follow-up, no cases of HIV infection were detected; 3 patients had discontinued FTC/TDF because of side effects but none of them had presented renal toxicity. In addition, the diagnosis of STIs during the follow-up was common (100 cases in 54 patients). Moreover, engagement in chemsex was more common within this latter group (22 vs. 6%, $p = 0.013$). Among the study population included in the anal screening programme, the frequency of dysplasia was 9%.

Conclusions: FTC/TDF was effective, safe, and tolerable in a real-life cohort; adherence remained high throughout the study period (79%). However, a high number of STIs were diagnosed, especially among patients who engaged in chemsex.

KEYWORDS

HIV, pre-exposure prophylaxis (PrEP), sexually transmitted infections, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, human papillomavirus (HPV)

Introduction

In recent years pre-exposure prophylaxis (PrEP) has become a useful strategy to reduce the transmission of human immunodeficiency virus (HIV) (1). PrEP regimens are based on daily or on-demand administration of antiretroviral drugs (e.g., emtricitabine or tenofovir) in key populations such as men who have sex with men (MSM) or sex workers. The combination of emtricitabine (FTC) and tenofovir-disoproxil-fumarate (TDF) has been proven to reduce the transmission of HIV (2), both when used as daily or on-demand regimens (3). In addition, FTC/TDF showed a good safety profile in previous studies. Nonetheless, some concerns have been raised in relation to a possible increase in the expression of kidney tubule health biomarkers associated with the use of these drugs (4).

Whether FTC/TDF is related to a decline in the estimated glomerular filtration rate (eGFR) remains controversial and so far, contradictory data has been reported in this respect (4, 5). Importantly, kidney tubule and bone toxicity are a common side effect of TDF-based regimens among people living with HIV (PLWH) (6). However, a novel formulation of TDF, tenofovir-alafenamide (TAF), seems to present a safer renal profile (7) and may be more appropriate for future use. Moreover, in a clinical trial involving PrEP users, patients receiving an FTC/TAF regimen showed better renal biomarker profiles compared to those using FTC/TDF (8).

Nevertheless, in the context of PrEP, some concerns have emerged regarding the incidence of sexually transmitted infections (STIs) other than HIV. In this respect, several studies have reported a high incidence of STIs among PrEP users (9), a decrease in condom usage (10), and an increase in the consumption of recreational drugs during sexual encounters (chemsex) (11). Furthermore, human papillomavirus (HPV) infection is more common among MSM, especially those infected by HIV (12), for whom specific anal dysplasia screening programs have been developed in recent years (13). Whether these programs are useful for HIV-uninfected MSM remains unknown (14). Therefore, real-life data is critical to better understand the safety and effectiveness of FTC/TDF, STI dynamics, and the prevalence of anal dysplasia among PrEP users and other key populations (e.g., MSM and sex workers, among others).

In November 2019, an FTC/TDF based PrEP programme was implemented by the Public Health Service in Northwest Spain (Galicia). This plan included the prescription of a daily dose of FTC/TDF, kidney function monitoring, and STI screening every 3 months for individuals at a high risk of HIV infection. Herein, we report our experience with a real-life cohort located in Northwest Spain, including data from the time the use of PrEP was initiated in our health area. The PrEP effectiveness, safety, sexual behaviours, drug consumption, and STI incidence during the study period were all reviewed.

Methods

Study design

An observational retrospective cohort study was undertaken by the Infectious Diseases Department in the University Hospital Complex of Vigo which has a catchment area population of 450,000 inhabitants. The medical records of individuals who requested PrEP administration were reviewed. The Galician PrEP programme (15) started in November 2019 and included MSM and transgender women for whom at least two of the following criteria applied: regular condomless sex, more than 10 sexual partners in the year prior, recreational drug consumption during sexual intercourse, HIV post-exposure prophylaxis prescribed at least twice in the year prior, or a STI diagnosed in the year prior. All the patients completed a baseline visit which included STI screening and HIV serology prior to the FTC/TDF administration. Once HIV-infection was ruled out, daily FTC/TDF was prescribed and from thereon the kidney parameters, HIV status, and presence of STIs were monitored in these patients every 3 months.

Sample collection and laboratory tests

Samples were processed at the Microbiology Service at the University Hospital Complex of Vigo according to laboratory standards. Anal, urethral, and oropharyngeal samples were screened for *Chlamydia trachomatis* (CT) and *Neisseria gonorrhoeae* (NG) using a Cobas® 6,800 system (Roche, Switzerland). Serum samples were tested for HIV

(Cobas HIV-1/HIV-2 Qual; Roche), *Treponema pallidum* (Chemiluminescence Immunoassay, Liason Treponema Screen, LIAISON, Diasorin, Italy Rapid Plasma Reagin, BioMérieux®, France), and hepatitis C virus (HCV; Cobas 6,800/8,800 HCV, Roche). The Roche Cobas HPV test was used to detect high-risk human papillomavirus (HPV) in anal cytology samples and to genotype HPV-16 and HPV-18 as well as a grouped category of high oncogenic risk HPV (pHR-HPV; 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, and 68). The anal cytology was processed in the Pathology Department according to routine procedures and later categorised based on the standard criteria. Biopsies obtained during the anoscopy were reviewed by an expert pathologist and classified according to the Bethesda criteria.

Anal dysplasia surveillance

At the time of their baseline visit all the patients were invited to participate in the Anal Dysplasia Surveillance Programme. For those who accepted the offer, an anal cytology sample was obtained during the anal exam. A high-resolution anoscopy (HRA) was then conducted by an expert surgeon to screen for altered anal exam results or abnormal cytology results (e.g., the identification of condyloma acuminata, i.e., genital warts).

Variable definitions

A past STI was defined as any STI diagnosed prior to the inclusion in the PrEP programme; baseline STI status was defined as a venereal disease diagnosed during the baseline visit (prior to FTC/TDF prescription); and finally, an incident STI was defined as a new episode of a STI that occurred during the follow-up after starting PrEP.

Statistical analyses

The study data were collected and managed using REDCap electronic data capture tools hosted at the *Instituto de Investigación Sanitaria Galicia Sur*. Quantitative variables were expressed as the median and interquartile range. Qualitative variables were shown as absolute values and percentages. Categorical variables were compared using χ-squared tests or the Fischer exact test, as appropriate. Quantitative variables were compared by employing Man–Whitney *U*-tests. *P*-values < 0.05 were considered significant in all cases. The statistical analyses were performed using Statistical Package for Social Sciences (SPSS) software (version 22, IBM Corp., Armonk, NY).

Ethics

This study was approved by the Ethics Committee of Pontevedra-Vigo-Ourense (reference 2021/311). The need for informed consent was waived because of the retrospective design of this work. The STROBE guidelines were used to ensure the reporting of this study.

Results

During the study period, a total of 136 individuals requested the prescription of PrEP in the Infectious Disease Unit. Of these, 10 were excluded in the final study cohort, in 6 cases because the patients had been included in a clinical trial, 3 individuals tested positive for HIV-infection, and 1 patient eventually declined to use PrEP. Thus, a total of 126 patients were finally considered eligible for this study. The baseline characteristics of the study population are shown in Table 1. Briefly, most of the participants were young males (median age 35 years), 22.2% ($n = 28$) had consumed recreational drugs in the year prior, and 12.7% ($n = 16$) had consumed recreational drugs during sex (chemsex). Most of patients had previously been diagnosed with an STI, with syphilis being the most frequent (32.5%, $n = 41$).

Baseline screening

According to the local protocols, a baseline STI screening was performed prior to the administration of FTC/TDF. Three patients tested positive for HIV-infection and were therefore excluded from the PrEP programme. An acute HIV-infection was suspected in the first patient (he referred high-grade fever and odynophagia) while the other two individuals were asymptomatic; two were born in Latin America and the other was born in Spain. The ages were 19, 24, and 28 years old, two of them had a previous history of a STI (one syphilis case and one gonorrhoea, respectively). None of them had previously received post-exposure prophylaxis (PEP). All three patients had tested negative for HIV-infection in the prior year. No HCV infection cases were detected. Among the remaining patients, 30.2% ($n = 38$) tested positive for a bacterial STI, with CT ($n = 17$, 13.5%) and NG ($n = 15$, 11.9%) being the pathogens most commonly isolated.

The safety and effectiveness of pre-exposure prophylaxis

The median follow-up time was 13 months, and no cases of HIV or HCV infections were detected. At the end of the

TABLE 1 Baseline characteristics of the study population.

Total study population	***N* = 126**
Male, *n* (%)	124 (98.4%)
Transgender woman, *n* (%)	2 (1.6%)
Age, mean, in years	35.0 (9.0)
Ethnicity	
Spanish, *n* (%)	102 (80.9%)
Latin American, *n* (%)	22 (17.5%)
Other, *n* (%)	2 (1.6%)
Tobacco consumption	
Active smoker, *n* (%)	36 (28.6%)
Former smoker, *n* (%)	5 (3.9%)
Never smoker, *n* (%)	54 (42.9%)
Unknown, *n* (%)	31 (24.6%)
Drug consumption last year	28 (22.2%)
Chemsex	16 (12.7%)
Intravenous drugs (*slamming*)	1 (0.8%)
Cocaine	12 (9.5%)
Cannabis	8 (6.3%)
Mephedrone	8 (6.3%)
Alkyl nitrites (*poppers*)	5 (4.0%)
PDE5-inhibiting drugs (e.g., sildenafil)	3 (2.4%)
Gamma-Hydroxybutyric acid and derivates (e.g., *GHB or GBL*)	3 (2.4%)
Past STI diagnosis, *n* (%)	76 (60.3%)
Syphilis, *n* (%)	41 (32.5%)
Neisseria gonorrhoeae infection, *n* (%)	27 (21.4%)
Condyloma acuminata (genital warts), *n* (%)	20 (15.9%)
Chlamydia trachomatis infection, *n* (%)	15 (11.9%)
Other (genital herpes, scabies, etc.), *n* (%)	12 (9.5%)
Active HCV infection at baseline	0
Sexual role	
Versatile	61 (48.4%)
Passive	10 (7.9%)
Active	9 (7.1%)
Unknown	46 (36.5%)
Age at the time of first sexual intercourse, in years	17 (3)
Number of sexual partners (in prior year)	15 (12)
Number of sexual partners (lifetime)	100 (250)
Condom use during sexual intercourse	9 (7.1%)
Received PEP at least once	11 (8.7%)

Quantitative variables are expressed as the median and interquartile range; qualitative variables are expressed in numbers and percentages. PDE5, phosphodiesterase type 5; STI, sexually transmitted infection; HCV, hepatitis C infection; PEP, HIV post-exposure prophylaxis.

study period 79% (n = 100) of the participants remained on PrEP, 14% (n = 17) were lost to follow-up, and PrEP was no longer indicated (i.e., the patient had a stable sexual partner or consistently used condoms) in 5% (n = 6) of the cases. FTC/TDF was discontinued because of safety or toxicity concerns in 3.2% (n = 3) individuals, 2 of them because of digestive intolerance and 1 as the result of skin toxicity (severe folliculitis attributed to the drugs). No patients stopped FTC/TDF treatment due to kidney toxicity.

Risk factors for incident sexually transmitted infections

During the follow-up, 43% of patients (n = 54) were diagnosed with at least one STI, with NG (39%, n = 39) and CT (35%, n = 35) infections accounting for most cases (Figure 1), although the anatomical locations affected slightly differed. NG was most often isolated from oropharyngeal samples while CT was more commonly found in the anal region (Figure 2). Syphilis was less common (8%, n = 8) and the majority of cases were of early asymptomatic and latent syphilis (n = 7); only one patient presented a chancre. In addition, genital herpes was detected in n = 7 individuals, while other STIs (scabies or non-gonococcal urethritis) were diagnosed in 10 patients.

Patients were classified into two groups according to whether an incident STI was detected or not; the comparison between these two groups is shown in Table 2. Recreational drug consumption was more common in the STI group (33.3 vs. 13.8%, p = 0.017), especially among those who practiced chemsex (22.2 vs. 5.5%, p = 0.013). Patients who were diagnosed with an STI at the baseline visit exhibited a higher proportion of incident STIs, although this difference was not statistically significant (38.9 vs. 23.6%, p = 0.064). Conversely, the age of the first sexual encounter, number of sexual partners, and prior prescription of a post-exposure prophylaxis (PPE) treatment, did not differ between the two groups.

Human papillomavirus infection and anal dysplasia

During the baseline visit, all the patients were invited to join an anal squamous intraepithelial lesion (SIL) surveillance programme, with 77% (n = 97) of the participants accepting this offer (Table 3). Abnormal cytology results were detected in 23% (n = 22) individuals and HRA was performed in n = 26; 9.3% (n = 9) patients were diagnosed with an anal SIL, with most of them being low-grade (LSIL) except for 1 high-risk (HSIL) case. The prevalence of HPV within this subgroup was common (69.1%), in most of these cases due to pHR-HPV (55.7%), with HPV-16 (17.5%) and HPV-18 (3.1%) diagnoses being less usual. Most patients (86%) had received the nine-valent HPV vaccine prior to their inclusion in the anal dysplasia screening programme.

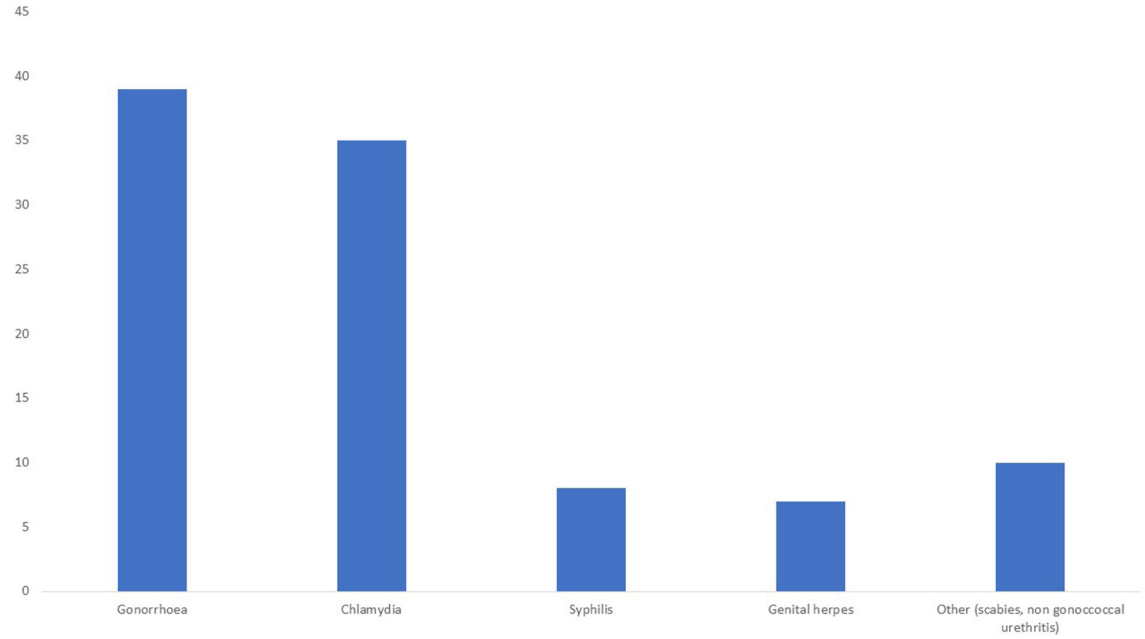

FIGURE 1
Total number of sexually transmitted diseases detected during the follow-up period.

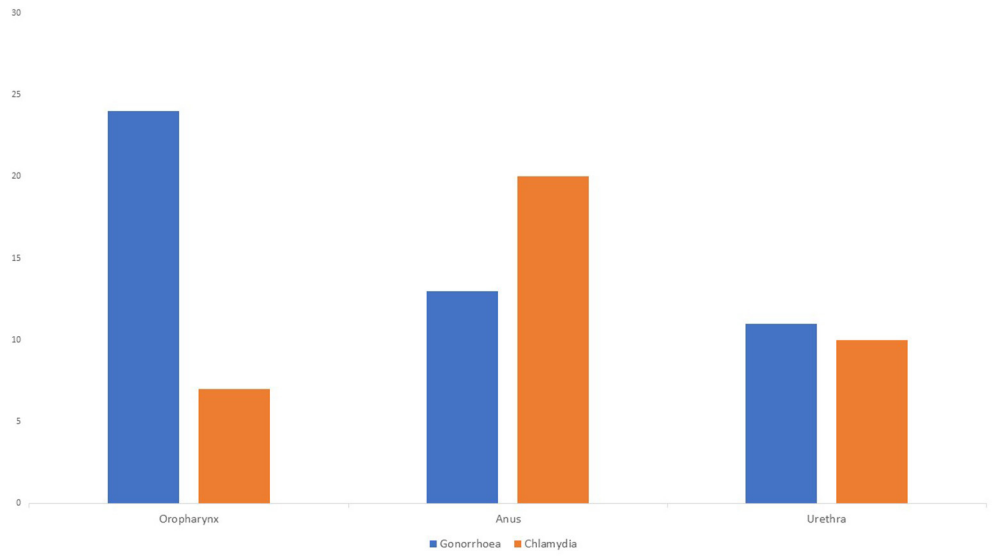

FIGURE 2
Total number of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* cases according to the anatomical location.

Discussion

PrEP has become a useful public health tool to reduce the transmission of HIV in key populations. Thus, several health systems, including those in Spain, have developed PrEP programmes in recent years. In addition to the primary objective of HIV prevention, PrEP programs might serve as useful tools to monitor other public health concerns (e.g., STIs or the prevalence of chemsex). Real-life studies also provide valuable data concerning the safety and effectiveness of FTC/TDF and STI dynamics after the implementation of PrEP programmes worldwide. To the best of our knowledge, this is the first

TABLE 2 Univariant analysis of the incidence of sexually transmitted infections risk factors.

	Incident STI $n = 54$ (42.9%)	Non-incident STI $n = 72$ (57.1%)	*P*-value	RR (95% CI)
Age at the time of PrEP initiation, in years	37 (9)	34 (15)	$p = 0.900$	
Age at the time of first sexual intercourse, in years	17.5 (4)	17.0 (2)	$p = 0.773$	
Number of sexual partners in the year prior	16.5 (10)	15.0 (19)	$p = 0.340$	
Recreational drug consumption	18 (33.3%)	10 (13.8%)	**$p = 0.017$**	1.7 (1.2–2.5)
Chemsex	12 (22.2%)	4 (5.5%)	**$p = 0.013$**	1.9 (1.3–2.8)
Baseline STI	21 (38.9%)	17 (23.6%)	$p = 0.064$	
Received PPE at least once	4 (7.4%)	7 (9.7%)	$p = 0.757$	

Quantitative variables are expressed as the median and interquartile range; qualitative variables are expressed in numbers and percentages. PrEP, pre-exposure prophylaxis; RR, risk ratio; STI, sexually transmitted infection; PPE, HIV post-exposure prophylaxis. Statistically significant *p* values were highlighted with bold letters.

TABLE 3 Prevalence of human papillomavirus infection in the patients included in the anal squamous intraepithelial lesion surveillance programme.

Anal SIL sub study	$n = 97$
HR-HPV	67 (69.1%)
HPV-16	17 (17.5%)
HPV-18	3 (3.1%)
pHR-HPV	54 (55.7%)
Abnormal anal cytology result	22 (22.7%)
HRA performed	26 (26.8%)
Anal dysplasia	9 (9.3%)
LSIL	8 (8.2%)
HSIL	1 (1.0%)

SIL, squamous intraepithelial lesion; HPV, human papillomavirus; HR-HPV, high oncogenic risk HPV; pHR-HPV, high oncogenic risk (grouped); HRA, high-resolution anoscopy; LSIL, low-grade squamous intraepithelial lesion; HSIL, high-grade squamous intraepithelial lesion.

study to report real-life data after the implementation of a PrEP programme in Northwest Spain. In this cohort, FTC/TDF was highly effective with no HIV infections detected during the study period and no treatment discontinuations because of renal toxicity. The main cause for PrEP discontinuation in our study population was the presentation of adverse gastrointestinal events (2.4%); interestingly, these were also more common in the FTC/TDF arm compared to the placebo (14 vs. 5%, respectively) in the IPERGAY clinical trial (2). Adverse skin side-effects were also less frequent than digestive disruptions (<1%), accounting for around 1.7 cases per 1,000 person-years in the United States within PrEP users (16).

In the recent years, access to HIV, STI and PrEP care has become more challenging owed to the COVID-19 pandemic (17, 18). Nevertheless, among our study population, only 31 individuals were on PrEP by 15 March 2020, when a national lockdown was imposed in Spain owed to the COVID-19 pandemic. Those patients included in the programme before the pandemic, remained attending to the unit as usual.

A major concern within key populations is an increasing incidence of different STIs (15). It is concerning that, despite the COVID-19 pandemic, the frequency of STIs continues to increase (19) and the widespread presence of multi-drug resistant sexually transmitted pathogens has been reported (20). Moreover, there is a higher incidence of STIs among PrEP users (21) because condom use is less frequent among this group. Of note, *Chlamydia trachomatis* infection was frequently isolated from anal samples, which has been described as a main risk factor for HIV acquisition (22), thus those patients diagnosed of anal *Chlamydia* might deserve special attention owed to an increased risk of HIV infection Anal HPV infection is also common among MSM, although data concerning its prevalence among PrEP users is scarce. However, a sub study in the IPERGAY clinical trial revealed a higher prevalence of anal HPV infection compared to our study population (92 vs. 69%, respectively), including infection with HPV-16 (22 vs. 18%) (23). Of note, most of our patients (86%) had received an HPV vaccine prior to or during their baseline visit. The main concern regarding HPV infection is the oncogenic potential of this virus to induce several anogenital cancers (e.g., cervical, anal, etc.) (24).

In addition, SIL surveillance programs have been developed for cervix and anal cancer prevention which have led to a decline in the incidence of HPV-driven dysplasia. Anal squamous cell carcinoma is a rare disease among the general population, but its incidence is disproportionally high in HIV-infected MSM (25). Whether anal SIL surveillance is necessary among HIV-uninfected MSM remains controversial, and very little data are available in this respect (14). Owed to our previous experience in PLWH (13), PrEP users were invited to a join an HPV surveillance program which revealed a SIL prevalence of 9% in our cohort, mostly comprising LSIL cases. Nonetheless, prospective studies will be required to assess the

usefulness of anal screening among PrEP users. Meanwhile, HPV vaccination should be encouraged as the main preventive tool for anal dysplasia.

Of note, chemsex has emerged in recent years as a major concern both among PLWH and PrEP users. Chemsex is associated with the consumption of various psychoactive recreational drugs (e.g., gamma-hydroxybutyric acid, mephedrone, or ecstasy), group sex, and condomless intercourse that can last hours or even days (26). The overall prevalence of chemsex participation among MSM varies widely with one meta-analysis estimating an overall prevalence of 2–28% (27). Conversely, Ruiz-Robledillo et al. reported higher rates of chemsex among PrEP users in Spain at 40.6 and 63%, respectively (28, 29). However, chemsex was less frequent (13%) in our study population and only 1 patient reported intravenous recreational drug usage.

Chemsex is also associated with harmful effects in terms of mental health, addiction, and an increased risk of STI/HIV acquisition. Depression, anxiety, and poorer mental health scores are all more common among people who practice chemsex (30). Moreover, the incidence of STIs is also higher among chemsex users (31) with some studies suggesting an increased risk of HIV infection because of a high rate of condomless sexual intercourse among this group (32). Regarding our study population, we detected an increased risk of incident STIs among chemsex users (22.2 vs. 5.5%), although no HIV or HCV infections occurred. Conversely, chemsex was not related to poorer compliance with PrEP regimens (33). Indeed, chemsex users within our study population exhibited similar compliance rates to those who did not engage in chemsex (87.8 vs. 84.6%).

Finally, it is important to note that our study had several limitations. Firstly, the retrospective design of the work might have resulted in some data loss. Our sample size was limited, likely because of the COVID-19 lockdown and social restrictions that may have reduced the demand for PrEP regimens. In addition, in our health area, the implementation of PrEP is restricted to daily treatments while on-demand use of FTC/TDF is discouraged, which might dissuade some patients from using it. Secondly, the sexual habits of the participants (i.e., number of sexual partners and condom usage, etc.) were only recorded at the baseline visit, not during the use of PrEP. Thus, any behavioural changes during the follow-up period were not considered in this current work. Thirdly, no renal biomarkers for TDF kidney toxicity (i.e., proteinuria, phosphaturia, etc.) were measured, although no patients stopped using TDF for renal safety reasons. Finally, at our centre, the screening samples were not routinely tested for *Mycoplasma genitalium* (an emerging sexually transmitted pathogen), and CT isolates were not tested for lymphogranuloma venereum *Chlamydia trachomatis* L1–L3 serovars.

Conclusions

In our study population, the daily use of FTC/TDF was effective and safe. Most patients continued the follow-up for 1 year and treatment discontinuations due to adverse effects were uncommon. Both the incidence and prevalence of STIs were high, especially among chemsex users. Finally, the presence of HPV-driven dysplasia should not be ignored, and long-term studies will be needed to analyse its relevance among PrEP users.

Data availability statement

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

Ethics statement

This study was approved by the Ethics Committee of Pontevedra-Vigo-Ourense (reference 2021/311). Written informed consent for participation was waived for this study in accordance with the national legislation and the institutional requirements.

Author contributions

AP-G: designed data collection tools, monitored data collection, analysed the data, and drafted the manuscript. MR, SR-R, CV-E, GP, and EF: collected data. PC: revised the manuscript. CP and AO: analysed the data and revised the manuscript. EP: designed, analysed, and revised the manuscript. All authors contributed to the article and approved the submitted version.

Funding

AP-G principal investigator is hired under a Río Hortega contract financed by the *Instituto de Investigación Carlos III* (ISCIII) with reference number CM20/00243.

Conflict of interest

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

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 31 October 2022
DOI 10.3389/fpubh.2022.1036264

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 04 September 2022
ACCEPTED 17 October 2022
PUBLISHED 31 October 2022

CITATION
Liu L-l, Sun S, Zhang L, Wu Q-h, Tian L-s, Li B, Chen X-s and Luo Z-z (2022) Distribution of *Chlamydia trachomatis ompA* genotypes and its association with abnormal cervical cytology among women of reproductive age in Shenzhen, China. *Front. Public Health* 10:1036264. doi: 10.3389/fpubh.2022.1036264

Distribution of *Chlamydia trachomatis ompA* genotypes and its association with abnormal cervical cytology among women of reproductive age in Shenzhen, China

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Background: Many studies have focused on the distribution and specific clinical symptoms caused by *Chlamydia trachomatis*. Still, relatively few studies have focused on the associations between *Chlamydia trachomatis* genotypes and cervical intraepithelial lesions.

Objectives: This study was conducted to determine the distribution of *Chlamydia trachomatis* genotypes and its associations with cervical intraepithelial lesions among women of reproductive age. The presence of other STIs coinfection was also evaluated.

Method: 375 *Chlamydia trachomatis* positive cervical swabs collected from women of reproductive age were analyzed though molecular assay. Multivariate logistic regression analyses (covariates include contraception, gravidity (≥1), abnormal vaginal discharge, adverse pregnancy outcomes, reproductive tract symptoms and abnormal cervical cytology) were performed to evaluate the associations between *Chlamydia trachomatis* genotypes and cervical intraepithelial lesions and genital clinical symptoms.

Results: Among 375 *Chlamydia trachomatis* positive cervical swabs, the prevalence of coinfection with *Neisseria gonorrhoeae, Candida albicans, Trichomonas vaginitis, Vulvovaginal candidiasis,* and HPV were 0.8%, 2.7%, 2.4%, 10.1% and 15.5%, respectively. 306 were genotyped successfully, and nine genotypes were identified. The most common genovar was E (25.16%, 77/306), followed by J (22.55%, 69/306), F (17%, 52/306), D (14.4%, 44/306), K (7.2%, 22/306), G (6.9%, 21/306), H (5.2%, 16/306), B (1.0%, 3/306), Ia (0.7%, 2/306). Genotype H was associated with abnormal cervical cytology [$p = 0.006$, aOR = 8.16 (1.86–36.6)]. However, this study observed no association between *Chlamydia trachomatis* genotypes and any genital clinical symptoms.

Conclusions: *Chlamydia trachomatis* genotype H may be a high risk factor for cervical intraepithelial lesions, which is useful for treatment and management measures for patients with cervical intraepithelial lesions.

KEYWORDS

cervical intraepithelial lesion, *Chlamydia trachomatis*, genotyping, human papillomavirus, women of reproductive age

Introduction

Chlamydia trachomatis (*C.trachomatis*), an obligate intracellular bacterium, remains the most frequent causative agent of sexually transmitted infections (STIs) worldwide, with a global incidence of 38 per 1,000 women and 33 per 1,000 men (1). 105 sentinel surveillance sites in China reported that *C.trachomatis* incidence increased from 35.8 per 100,000 in 2011 to 37.1 per 100,000 in 2015. Men who have sex with men (MSM) and female sex workers (FSW) have a higher risk of *C.trachomatis* infection, accounting for 6.5% and 17.3% of infections, respectively (2, 3). The true *C.trachomatis* prevalence may be underestimated because most cases are asymptomatic (about 70% of women and 50% of men don't have any clinical symptoms) (4).

Based on the major outer membrane protein (MOMP) encoded by the *ompA* gene, 19 *C.trachomatis* serovars have been classified into three clusters: genotype A-C (predominantly related to trachoma), genotype D-K (associated with urogenital infections), genotype L_1-L_3 (causing lymphogranuloma venereum) (5). Left untreated, *C.trachomatis* can lead to severe consequences, such as cervicitis, cervical ectopy and chronic pelvic inflammation in females, urethritis in males, and neonatal conjunctivitis in newborns (6).

Cervical cancer (CC) is the fourth most common cancer amongst women worldwide (7). The major risk factors associated with CC development include high-risk human papilloma virus (hrHPV) infection, age, smoking, childbirth, use of oral contraception, and diet (8–13). The association between certain hrHPV of HPV and cervical cancer is well established (14). However, previous studies show that only a few HPV infection cases will develop into CC (15). That means HPV alone is not sufficient for the development of CC.

CC arises from normal cervical epithelium through the progressive development of low grade and high grade cervical intraepithelial lesions (CINs) (16). And *C.trachomatis* is considered as a risk factor for abnormal cervical cytology in previous researches (17–19). However, it is unclear that which are high-risk and which are low-risk for CINs about 19 *C.trachomatis* genotypes, which may facilitate the prevention and treatment of *C.trachomatis* infection. Unfortunately, only a few studies have been conducted to evaluate the association between *C.trachomatis* genotypes and CINs (20). Thus, this study aims to determine the distribution of *Chlamydia trachomatis* genotypes and its associations with CINs among women of reproductive age.

Materials and methods

Study design

From March to August 2017, we recruited participants from 9,249 women who met eligibility criteria and provided informed consent in our previous study (21), and all of the women signed an informed consent to this study. Women who met any of the following exclusion criteria were not enrolled: pregnancy, without a history of sexual activity, sexual intercourse 3 days ago, menstrual period, previous hysterectomy, vaginal bleeding, vaginal douching or using a vaginal suppository, currently suffering from gynecological inflammation. The inclusion criteria were the same as inclusion criteria in our previous study (21): being a female resident, aged 20–60 years and living locally in Shenzhen city Nanshan District during the past 3 months. All participants signed informed consent and were interviewed using a structured questionnaire to collect socio-demographic and clinical information before enrollment. All participants voluntarily agreed to provide a self-administrated 3–5 mL first-catch urine specimen (Chlamydia trachomatis and Neisseria gonorrhoeae tests), a cervical swab (HPV tests), two vaginal swabs (gynecological examinations), and an exfoliated cervical cells specimen (liquid-based cervical cytology test).

Positive endocervical swabs samples

Out of the 9,249 women who met eligibility criteria and signed an informed consent, 9,090 (98.3%) women's specimens were successfully tested, and 375(4.13%, 375/9,090) were *C.trachomatis* positive.

375 positive endocervical swabs samples were used for *C.trachomatis* genotyping to evaluate association with CINs. More detailed study methods and epidemiological information about the study are available in a previously published article (21, 22).

C.trachomatis and neisseria gonorrhoeae DNA test

The method and procedures of *C.trachomatis* and *Neisseria gonorrhoeae* DNA test were described in our previous study (21).

C.trachomatis DNA extraction, *OmpA* PCR amplification, sequencing and genotyping

The positive endocervical swabs were each eluted with 1 ml sterile water and vortexed. Researchers took 200 μl of eluant from the samples for DNA extraction. DNAs were extracted by using QIAamp® cador® Pathogen Mini Kit (Qiagen, Hilden, Germany), according to the manufacturer's instructions. The remaining eluate was stored at −80°C.

Molecular genotyping of *C.trachomatis* was performed by *ompA* gene; the detailed information about *ompA* primers for the current nested PCR system is available at the Uppsala University *C.trachomatis* MLST database (http://mlstdb.bmc.uu.se). The nested PCR cycling protocol methods was previously described (23). The secondary PCR amplifications were purified and sent to be sequenced by HYK-High-throughput Biotechnology Institute (Shenzhen, China). All PCR amplifications were sequenced bidirectionally.

HPV DNA testing and genotype

HPV DNA testing and genotyping (14 HR-HPV including genotype 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68 and 2 low-risk HPV including genotype 6 and 11) were conducted by the Beijing Genomics Institute according to operating instructions (24).

ThinPrep cytological test (TCT)

The cytobrush containing cervical exfoliated cells was collected and stained, and then fixed in TCT cytological solution for 15 min. TCT was performed by a SurePath liquid-based Pap test (BD, United States), according to the manufacturer's instructions. Cervical cytology test results were classified by the Bethesda System (TBS; 2001) criteria as follows: negative for intraepithelial lesion or malignancy (NILM); atypical glandular cells (AGC); squamous intraepithelial lesions (SIL) of low (LSIL) or high (HSIL) grade; atypical squamous cells (ASC) of undetermined significance (ASC-US) or not possible exclude HSIL (ASC-H); and squamous cell carcinoma (SCC) (25). In this study, cervical cytology was dichotomized into normal (NILM) and abnormal (≥ASC). More detailed information is available in our previous study (22).

Vaginal cleanliness evaluation

Vaginal smears on clean glass slides were observed under microscope at the hospital laboratory. The evaluation criteria of vaginal cleanliness was divided into 4 grades by the relative abundance of *Lactobacillus* spp., vaginal epithelial cells, pus cells and white blood cells per high power field (26). Grade I and grade II indicate normal vaginal cleanliness, whereas grade III and grade IV are abnormal, indicating the presence of inflammation or infection (22).

Gynecological examination

Vaginal secretions swab specimens were collected by two skilled gynecologists and were rolled on to a glass slide for Gram staining immediately. Vaginal cleanliness, detection of hyphae and spores of Candidiasis and clue cells were confirmed by Gram staining of vaginal secretions. Trichomonas Vaginalis was diagnosed by microscopic examination of wet mounts immediately once the vaginal secretions swab collected. Amine test, pH of vaginal secretions and leukocytes were further confirmed within 15 min. The diagnosis of bacterial vaginosis was based on Amsel's criteria (27), which was widely adopted to the clinical diagnosis of bacterial vaginosis. A positive diagnosis of bacterial vaginosis was made once three of the four following signs are present: the presence of clue cells, an adherent and homogenous grayish-white vaginal discharge, a vaginal pH exceeding a value of 4.5, a fishy or amine odor after the addition of a 10% potassium hydroxide solution. The method and procedures of gynecological examination were described in our previous study (22).

Statistical analyses

Raw data collection and statistical analysis were performed by Microsoft Excel 2016 and SPSS Statistics version.20.0 (SPSS Inc., Chicago, IL, United States), respectively. Abnormal cytology group (≥ASC-US was defined as women who had a diagnosis of the following cytology findings: ASC-US, ASC-H, LSIL, HSIL or AGC). *C.trachomatis* genotypes were divided into two group, genotype B group and non-B genotypes group, genotype D group and non-D genotypes group, genotype E group and non-E genotypes group, and so on. The chi-square test (χ^2), or two-sided Fisher exact test for 2 × 2 contingency table was used to evaluate the associations between different *C.trachomatis* genotypes, sociodemographic characteristics, reproductive history, sexual behavior, and

urogenital symptoms. Variables with a significance level of p <0.2 were enrolled in the multivariate logistic regression model adjusted by potential confounders. Crude odds ratios (OR), adjusted odds ratio (AOR) and corresponding 95% CIs were calculated. A p <0.05 was considered significant.

Ethics statement

The study was approved and overseen by Ethical Committee of the Shenzhen Nanshan Center for Chronic Disease Control (Approved No. LL20170017).

Results

Population characteristics

Socio-demographic and clinical information in patients with *C.trachomatis* infection were shown in Table 1. A total of 69.1% of C.trachomatis positive women were >35 years old; 73.9% of *C.trachomatis* positive women were middle and low-income. More than half of women were unemployed (63.5%). 94.1% of women were married, and 80.3% reported using condoms or other contraceptive methods, including oral contraceptive use and intrauterine devices; 54.7% had no reproductive tract symptoms.

A total of 24% of women had adverse pregnancy outcomes. Cesarean delivery accounted for 71.2%. 90.7% had never had a *C.trachomatis* test previously.

TABLE 1 Sociodemographic and clinical information in patients with *C.trachomatis* infection.

Variables		Number(n = 375)	%
Age	≤35	116	30.9
	>35	259	69.1
Income	<4,000 RMB	220	58.7
	4,000–8,000 RMB	132	35.2
	>8,000 RMB	23	6.1
Employment status	Unemployed	238	63.5
	Employed	137	36.5
Registered residence	Local	146	38.9
	Nonlocal	229	61.1
Marital status	Married	353	94.1
	Unmarried[a]	22	5.9
Ethnicity	Han	353	94.1
	Minority	22	5.9
Oral contraceptive	No	301	80.3
	Yes	74	19.7
Condom use	No	125	33.3
	Yes	250	66.7
APOs[b]	No	285	76.0
	Yes	90	24.0
Delivery modes	Vaginal delivery	88	23.5
	Cesarean delivery	267	71.2
	Never had children	20	5.3
Vaginal discharge	No	205	54.7
	Yes	170	45.3
Gravidity	No	13	3.5
	Yes	362	96.5
Previous *C.trachomatis* test	No	340	90.7
	Yes	22	5.9

[a]Unmarried include single, divorced.
[b]APOs: adverse pregnancy outcomes, refers to spontaneous abortion, stillbirth, preterm labor, low birth weight, infertility, infant death, ectopic pregnancy.

C.trachomatis coinfection with other STIs and Liquid-based cytology test results

As shown in Table 2, the most prevalent coinfection pathogen was HPV, which accounted for 15.47%, particularly high-risk HPV (96.55%, 56/58), followed by *vulvovaginal candidiasis* (10.13%), *candida albicans* (2.67%), *trichomonas vaginitis* (2.4%), and NG (0.8%).

The distribution of C.trachomatis genotypes by cytology status

As shown in Table 3, among 375 *C.trachomatis* positive cervical swabs, 306 were genotyped successfully, and 69 samples containing less *C.trachomatis* DNA were insufficient for genotyping. Out of 306 samples, nine genotypes were identified. The most common genotype was E (25.16%, 77/306), followed by J (22.55%, 69/306), F (17%, 52/306), D (14.4%, 44/306), K (7.2%, 22/306), G (6.9%, 21/306), H (5.2%, 16/306), B (1.0%, 3/306), and Ia (0.7%, 2/306).

22 (7.19%) samples had abnormal cervical cytology; The remaining were negative for intraepithelial lesion or malignancy (NILM) (92.81%, 284/306). We found a positive association between *C.trachomatis* genotype H infection and abnormal cervical cytology [p = 0.001, OR = 7.3 (95% CI: 2.28–23.41)].

Associations between C.trachomatis genotypes and clinical characteristics

As shown in Table 4, after adjusting for potential confounders by multivariate logistic regression analysis, we found that no associations were observed between different *C.trachomatis* genotypes and gravidity, vaginal cleanliness,

adverse pregnancy outcomes or reproductive tract symptoms. However, the association of genotype K with contraception, including condom use, oral contraceptive use, and intrauterine devices was observed ($p = 0.03$, aOR = 0.36, 95% CI: 0.14–0.93). Genotype D was associated with gravidity ($p = 0.02$, aOR = 0.2, 95% CI: 0.05–0.75). Most importantly, compared to other CT genotypes, there was a significant association between genotype H and abnormal cervical cytology ($p = 0.006$, aOR = 8.16, 95% CI: 1.83–36.6).

TABLE 2 *C.trachomatis* coinfection with other STIs.

Coinfecting pathogens	Test results	No.	Prevalence (%, 95% CIs)*
NG[a]	Negative	372	
	Positive	3	0.8 (1–1.7)
Candida albicans	Negative	365	
	Positive	10	2.67 (1.04–4.3)
Trichomonas vaginitis	Negative	366	
	Positive	9	2.4 (0.85–3.95)
Vulvovaginal candidiasis	Negative	337	
	Positive	38	10.13 (7.08–13.19)
HPV	Negative	317	
	Positive	58	15.47 (11.81–19.13)
Multiple HPV infection	No	370	
	Yes	5	1.33 (0.17–2.49)
HR-HPV[b]	Negative	319	
	Positive	56	14.93 (11.33–18.54)
Liquid-based cytology test	Normal	346	
	Abnormal	29	7.73 (5.03–10.44)

[a]NG refers to neisseria gonorrhoeae.
[b]HR-HPV refers to high-risk HPV.
*95% CIs, 95% confidence intervals, $p < 0.05$ was considered significant.

Discussion

In this study, 375 *C.trachomatis* positive cervical swabs were collected from a population-based cross-sectional survey with a relatively larger sample size (n = 9,090) (28). This study extended the existing literature by distribution of *C.trachomatis ompA* genotypes and its associations with abnormal cervical cytology.

In this study, we found that most participants had no *C.trachomatis* test previously, which indicated that they were unaware of the cervical lesions that *C.trachomatis* cause. And almost half reported that they had no vaginal discharge, which aligns with the fact that many infections are asymptomatic, thus delaying diagnosis.

As for coinfection, the most prevalent coinfection pathogen was HPV, especially high-risk HPV. The prevalence of coinfection with NG was relatively low in our study.

In this study, 306 samples were genotyped successfully and 69 samples containing less *C.trachomatis* DNA were insufficient for genotyping. The failure rate of *C.trachomatis ompA* gene sequencing in this study (18.4%, 69/375) was higher than in a study conducted in mainland China (5.83%, 14/240), but lower than other studies (Taiwan, 29.6%, 43/145; mainland China, 20.2%, 33/163) (29–31). The difference may be due to clinical samples (cervical swabs and urine) and different sequence methods performed in different studies.

Out of 306 successfully genotyped samples, nine different *C.trachomatis* genotypes from B and D-K were detected. The

TABLE 3 The prevalence of *C.trachomatis* genotypes by cytology status.

C.trachomatis genotypes	Overall ($n = 306$) *n* (%)	NILM[a] ($n = 284$) *n* (%)	Abnormal cervical cytology ($n = 22$) n (%)	*p*	OR (95% CIs)*
B[b]	3 (0.98)	3 (1.06)	0	-	-
D[c]	44 (14.38)	43 (15.14)	1 (4.55)	0.294	0.27 (0.04–2.04)
E	77 (25.16)	71 (25)	6 (27.27)	0.813	1.13 (0.42–2.99)
F	52 (16.99)	49 (17.25)	3 (13.64)	0.888	0.76 (0.22–2.66)
G	21 (6.86)	19 (6.69)	2 (9.09)	1	1.4 (0.3–6.42)
H	16 (5.23)	11 (3.87)	5 (22.73)	0.001	7.3 (2.28–23.41)
I	2 (0.65)	2 (0.7)	0	-	-
J	69 (22.55)	64 (22.54)	5 (22.73)		1.01 (0.36–2.85)
K	22 (7.19)	22 (7.75)	0	-	-

[a]NILM refers to negative for intraepithelial lesion or malignancy, means normal cervical cytology.
[b,c]genotype B, compare to non-genotype B (the rest genotypes, include D, E, F, G, H, Ia, J, and K); genotype D, compare to non-genotype D (the rest genotypes, include B, E, F, G, H, Ia, J, and K). The rest genotypes can be done in the same manner.
*95%CIs, 95% confidence intervals, $p < 0.05$ was considered significant.

TABLE 4 Associations between *C.trachomatis* genotypes and clinical characteristics.

Genotypes	*n* (%, *n* = 306)	Contraception			Gravidity (≥1)			Abnormal vaginal discharge		
		n (%)	*p*	aOR[a] (95% CI)	*n* (%)	*p*	aOR (95% CI)	*n* (%)	*p*	aOR (95% CI)*
B[b]	3 (1.0)	1 (33.3)	0.99	-	3 (1.0)	1	-	1 (33.3)	0.5	0.43 (0.04–4.93)
D	44 (14.4)	32 (72.7)	0.79	0.89 (0.39–2.01)	39 (88.6)	0.02	0.2 (0.05–0.75)	27 (61.4)	0.19	1.56 (0.81–3.03)
E	77 (25.2)	48 (54.5)	0.18	1.65 (0.80–3.39)	76 (98.7)	0.22	3.68 (0.46–29.6)	36 (46.8)	0.3	0.76 (0.45–1.28)
F	52 (17.0)	35 (67.3)	0.55	1.27 (0.58–2.81)	51 (98.1)	0.64	1.65 (0.20–13.5)	25 (48.1)	0.52	0.82 (0.45–1.50)
G	21 (6.9)	13 (61.9)	0.26	2.38 (0.53–10.66)	21 (1.0)	1	-	12 (57.1)	0.64	1.24 (0.50–3.04)
H	16 (5.2)	13 (81.3)	0.94	1.05 (0.28–3.92)	16 (1.0)	1	-	9 (56.3)	0.64	1.28 (0.45–3.64)
Ia	2 (0.7)	1 (50.0)	0.99	-	2 (1.0)	1	-	1 (50.0)	0.91	1.18 (0.07–19.3)
J	69 (22.5)	41 (59.4)	0.14	0.61 (0.31–1.17)	66 (95.7)	0.93	0.94 (0.24–3.71)	37 (53.6)	0.77	1.08 (0.63–1.86)
K	22 (7.2)	19 (86.3)	0.03	0.36 (0.14–0.93)	20 (90.9)	0.16	0.31 (0.06–1.59)	11 (50.0)	0.85	0.92 (0.38–2.20)

Genotypes	*n* (%, *n* = 306)	Adverse pregnancy outcomes			Reproductive tract symptoms			Abnormal cervical cytology[c]		
		n (%)	*p*	aOR (95% CI)	*n* (%)	*p*	aOR (95% CI)	*n* (%)	*p*	aOR (95% CI)
B	3 (1.0)	0	-	-	1 (33.3)	0.87	1.22 (0.11–13.9)	0	-	-
D	44 (14.4)	7 (15.9)	0.14	0.52 (0.22–1.24)	27 (61.4)	0.17	1.61 (0.82–3.15)	1 (2.30)	0.27	0.30 (0.04–2.57)
E	77 (25.2)	21 (27.3)	0.41	1.28 (0.71–2.33)	22 (28.6)	0.81	0.93 (0.53–1.65)	6 (7.80)	0.73	1.21 (0.42–3.50)
F	52 (17.0)	9 (17.3)	0.32	0.63 (0.31–1.47)	15 (28.8)	0.95	1.02 (0.53–1.98)	3 (5.80)	0.43	0.58 (0.15–2.28)
G	21 (6.90)	7 (33.3)	0.25	1.77 (0.68–4.60)	6 (28.6)	0.92	0.95 (0.36–2.56)	2 (9.50)	0.99	1.01 (0.19–5.37)
H	16 (5.20)	4 (25.0)	0.76	1.2 (0.37–3.90)	3 (18.8)	0.36	0.55 (0.15–1.99)	5 (31.3)	0.006	8.16 (1.83–36.6)
Ia	2 (0.70)	2 (100)	1	-	1 (50.0)	0.59	2.14 (0.13–30.5)	0	-	-
J	69 (22.5)	17 (24.6)	0.87	1.06 (0.56–1.98)	20 (29.0)	0.87	0.95 (0.52–1.72)	5 (7.20)	0.81	1.15 (0.37–3.55)
K	22 (7.20)	5 (22.7)	0.98	1.01 (0.36–2.87)	5 (22.7)	0.52	0.71 (0.25–1.99)	0	-	-

[a]aOR: refers to adjusted odds ratio.

[b]B genotype, compared to non-genotype B (the rest genotypes, include D, E, F, G, H, Ia, J, and K). The rest genotypes can be done in the same manner.

[c]Abnormal cervical cytology refers to ASC (atypical squamous cells), SIL (squamous intraepithelial lesions), ASC-US (atypical squamous cells of undetermined significance), and normal cervical cytology refers to NILM.

*95%CIs, 95% confidence intervals, $p < 0.05$ was considered significant.

10.3389/fpubh.2022.1036264

results in this study were diverse when compared with other studies (31, 32). We speculate that the specific location of the study, Shenzhen, may be linked to the diversity. Because Shenzhen is a modern city with rapid economic development and large unregistered, young, floating migrant population. It has been reported that *C.trachomatis* is the most common STI mainly affecting young individuals (1).Thus, it is reasonable that a diverse array of genotypes existed in our study. And similar to other studies, genotypes E, F, J, and D were the most prevalent in Guangdong (1).

Among nine *C.trachomatis* genotypes, E and J were the predominant genotypes in this study, which is slightly different when compared with two studies carried out in mainland China where F and E, D, and E genotypes were the most common, respectively (28, 31). Genotype E was one of the stable genotypes among the general population despite the existing difference, while G and D were identified as the most prevalent genotypes among MSM (33). Lymphogranuloma venereum genotype L1-L3 was absent in our study, but was also recognized in the MSM (34).

Interestingly, genotype B was detected in cervical swabs in our study, it was found associated with trachoma and neonatal conjunctivitis in other studies previously (5, 32, 35). This results indicated that genotype B may lead to multiple anatomical sites infection, not only caused eye infection, but also reproductive tract infection. To our knowledge, this was the first time that genotype B was found among women in Shenzhen, excluding a previous study conducted in MSM (33).

We observed that clinical manifestations including abnormal vaginal discharge, were not associated with any genotypes. These results are in accordance with some research, but different from others (1, 32, 36). Previous studies have shown that genotype K was associated with abnormal vaginal discharge, and genotype G was related to low abdominal pain (30, 37). Although the association between parity and STIs has been demonstrated, the association between parity and genotypes was not found in this study (17, 38).

The rate of abnormal cervical cytology in this study (7.2%) was higher than in a previous study (2.8%) (39). However, in terms of the relationship between *C.trachomatis* positive and cervical precancerous lesions, our findings varied with the previous literature (17, 18). A case-control study and a meta-analysis showed that *C.trachomatis* infection was a higher risk factor of CC, while other studies found no association between the *C.trachomatis* and CC or abnormal cervical cytology (20, 39, 40). Our result was similar to the latter.

In present study, genotype E and J are the most frequent genotypes, but these two was not significantly related to the abnormal cervical cytology, suggesting that genotype E may be not the pathogenic factor for abnormal cervical cytology. Genotype H was associated with abnormal cervical cytology, indicating that genotype H may be the pathogenic factor for abnormal cervical cytology, which is a little different from the previous study reported by Chen (1). They found that patients with genotype G infection commonly had abnormal cervical cytology ($p = 0.029$, OR = 1.868, 95% CI: 1.124–3.106). However, the association between genotype G and abnormal cervical cytology was not observed in our study (1). Genotypes B, D, G, and I were shown to be related to abnormal cervical cytology in a different case control study, which was also not found in this study (40).

Limitations

Several limitations of our study should be acknowledged. First, the genotyping method used in our study could not identify strains with multiple genotypes infections, despite being sensitive and specific. Second, the failure rate of *C.trachomatis ompA* gene sequencing in this study (18.4%, 69/375) was a little higher, which may influence the study's conclusion. Third, the sample size ($n = 375$) of this study was small, and samples collected in the study were limited to the Nanshan district, Shenzhen, so the representativeness of genotype distribution may be affected. Further research is needed to enroll a population comprising of both males and females and enlarge the target region.

Conclusions

In conclusion, *C.trachomatis* genotype H may increase the risk of cervical intraepithelial lesion in terms of cross-sectional epidemiology, which indicates that H is a higher risk *C.trachomatis* genotype. This provides some evidence for clinical diagnosis and treatment of *C.trachomatis*.

Data availability statement

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

Ethics statement

The study was approved and overseen by Ethical Committee of the Shenzhen Nanshan Center for Chronic Disease Control (Approved No. LL20170017). The patients/participants

provided their written informed consent to participate in this study.

Author contributions

Conceptualization: X-sC, BL, Z-zL, L-lL, SS, LZ, Q-hW, and L-sT. Data curation: L-lL, Q-hW, and LZ. Formal analysis: L-lL, Q-hW, BL, and X-sC. Investigation: L-lL, Q-hW, LZ, and L-sT. Methodology: L-lL, SS, and Q-hW. Project administration and resources: Z-zL, L-lL, and SS. Software: L-lL and SS. Supervision: BL, Z-zL, L-sT, and X-sC. Validation: BL, X-sC, and Z-zL. Visualization: BL and X-sC. Writing—original draft and writing—review & editing: L-lL. All authors contributed to the article and approved the submitted version.

Funding

This study received financial support from the Shenzhen Nanshan Science and Technology Planning Project (SZ010).

Acknowledgments

The authors are grateful for the help received from nurse and doctors in Shenzhen Nanshan Maternity & Child Healthcare Hospital.

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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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 31 October 2022
DOI 10.3389/fpubh.2022.1018724

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases - Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 13 August 2022
ACCEPTED 14 October 2022
PUBLISHED 31 October 2022

CITATION
Shi L, Luo J, Chen Y, Chen L, Hu H, Qiu T, Liu X, Xu X, Chen Y, Zhang Z, Zhou Y, Lu J and Fu G (2022) Prevalence of syphilis and chlamydia trachomatis infection among female sex workers in Jiangsu, China: Results from a multicenter cross-sectional and venue-based study. *Front. Public Health* 10:1018724. doi: 10.3389/fpubh.2022.1018724

Prevalence of syphilis and chlamydia trachomatis infection among female sex workers in Jiangsu, China: Results from a multicenter cross-sectional and venue-based study

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Background: Female sex workers (FSWs) are considered highly vulnerable to sexually transmitted infections (STIs), but available data on the prevalence of STIs among FSWs in China is limited at a provincial level. This study aimed to evaluate the prevalence of STIs and risk factors among FSWs in Jiangsu, China.

Methods: We conducted a multicenter cross-sectional study in seven cities of Jiangsu to investigate the prevalence and risk factors associated with HIV and other STIs. Blood and urine were collected to test for HIV, syphilis, Hepatitis C (HCV), Neisseria gonorrhoeae (NG) and Chlamydia trachomatis (CT) infections.

Results: We enrolled 3,580 FSWs. The overall prevalence of bacterial STIs was 6.2% (5.4%–7.0%). The prevalence of HIV, syphilis infection, HCV, NG and CT were 0.1% (95%CI, 0.0–0.2), 1.8% (95%CI, 1.4–2.3), 0.3% (95%CI, 0.1–0.5), 0.3% (95%CI, 0.2–0.5) and 4.3% (95%CI, 3.6–5.0), respectively. Most FSWs (85.6%) reported consistent condom use with clients in the past month. Only 10.6% of FSWs reported group sex, and 68.3% self–reported HIV testing in the previous year. According to the multivariable model, having group sex in the past year (aOR, 2.521, 95%CI: 1.366–4.651) and HIV infection (aOR, 26.260, 95%CI: 2.432–283.563) were associated with a higher risk of syphilis infection. Migrants (aOR, 1.669, 95%CI: 1.163–2.395), having a history of STIs in the past year (aOR, 4.601, 95%CI: 1.003–21.118), and NG infection (aOR, 38.549, 95%CI: 11.214–132.514) were associated with a higher risk of CT infection. On the contrary, FSWs aged older than 25 were associated with lower risk of syphilis infection (25–34: aOR, 0.339, 95%CI: 0.151–0.763) and CT infection (25–34: aOR, 0.503, 95%CI: 0.316–0.802; ≥35: aOR, 0.578, 95%CI: 0.362–0.925).

Conclusion: This study's prevalence rates of syphilis and CT infections show the need to promote comprehensive STIs control and prevention strategies, including behavioral intervention and STIs screening, especially in younger high–risk populations. With the increasing coverage of HIV testing, integrating other STIs screening with HIV testing may be a reasonable way to implement comprehensive STIs control and prevention.

KEYWORDS

sexually transmitted infections, prevalence, female sex worker, HIV, China

Introduction

According to the World Health Organization, 374 million new infections [including Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), Syphilis, and Trichomoniasis] were estimated nationwide in 2020. Nearly 35% of new infections were CT, one of the world's most commonly reported sexually transmitted infections (STIs) (1). The prevalence of CT and NG were high in key populations [CT: 16.3% and NG: 3.0% among men who have sex with men (MSM), CT: 17.0% and NG: 2.3% among female sex workers (FSWs)] (2, 3) than in the general population (CT: 10.2% and NG: 4.09% among women) (4). The case report of CT was under–estimated nationwide in China, considering the CT was previously not a notifiable reporting infectious diseases according to the Law of the People's Republic of China on the Prevention and Treatment of Infectious Diseases. STIs can cause serious outcomes without treatment in females, such as infertility, pelvic infection, pregnancy complications, and an increased risk of other STIs (especially HIV).

FSWs remain at high risk of HIV and other STIs worldwide. A global meta–analysis, covering 101 countries and 2,103,380 women, reported that the estimated HIV prevalence remained high and stable between 10.4 and 11.8% among FSWs from 2014 to 2018 (5). A cross–sectional study, conducted at Yunnan in 2008, reported prevalence rates of 36.8% for NG, 46.3% for CT, and 22.1% for trichomonas vaginalis among FSWs in China (6). Syphilis infection was estimated at 1.7% among FSWs in China, which was 6.8 times higher than among general population women in 2017 (7). FSWs contribute significantly to STIs transmission networks through their engagement in frequent unprotected sexual activities, multiple commercial sex partners (8–10), and limited asymptomatic STIs awareness (11). An Ecuador study reported that 25.5% of FSWs had unprotected sexual activities at least once in the previous 3 months (12). Another study in Australia observed an inconsistent condom use rate of 24% for vaginal sex with clients in 2021. About fellatio, the rate of inconsistent condom use reached 74% among clients in this population (13). In some instances, FSWs faced risks of reduced payment or intimate partner violence during negotiations for safe sex with routine clients or intimate sexual partners. Offers of higher monetary incentives for condomless sex sometimes derailed condom–use negotiations and further exposed FSWs to STIs (14, 15).

Meanwhile, a 2010 China study found that 39.8% of FSWs experienced challenges in using a condom correctly, such as condom rupture, and only used condoms during ejaculation (16). Most studies have demonstrated that STIs infection facilitates HIV infection. Controlling STIs could contribute to the Aim of "Zero AIDS" not only among FSWs but also in other high–risk sub–populations and the general population (17, 18). So, identifying the prevalence of STIs and risk characteristics of FSWs could contribute to improving strategies for controlling STIs in this population and preventing STIs from their clients and the general population.

Jiangsu, located in eastern China, belongs to the Yangtze–river economic zone, one of the three most developed and richest areas in China. The commercial sex industry flourished alongside China's rapid economic growth since the policy of economic reforms and open policies was initiated in the 1980s (19). With the rapid economic development, residents increased disposable income. Most rural–urban immigrants migrate to this province for more job opportunities and higher salaries. By 2021, the migrant population in Jiangsu reached almost 24 million, accounting for 25% of the whole population in Jiangsu province (20). Compared to indigenous residents, migrants lacked sources of health care, had poorer awareness of STIs–related knowledge, and were more likely to engage in condomless sex and commercial sex, increasing the likelihood of HIV/STIs acquisition in FSWs (21–23). Overlapping these characteristics, FSWs contribute to the worsening epidemic of HIV/STIs in Jiangsu. However, limited data on STIs surveillance among the high–risk population, especially FSWs in Jiangsu,

Abbreviations: CT, Chlamydia trachomatis; NG, Neisseria gonorrhoeae; STIs, Sexually transmitted infections; FSWs, Female sex workers; ELISA, Enzyme-linked immunosorbent assay; TP, Treponema pallidum; TRUST, Toluidine red untreated serum test; NAATs, Nucleic amplification tests; OR, Odds ratio; aOR, adjust Odds ratio; CI, Confidence interval.

have been reported. One study conducted in 2009 reported a high rate of syphilis (4.9%), CT (14.6%), and NG (5.4%) prevalence among FSWs in two Jiangsu province cities (24). Another study in Changzhou demonstrated high rates of CT (17.0%) and NG (2.3%) among FSWs in 2011 (3). This study aimed to investigate the prevalence of STIs and associated factors to evaluate and improve the comprehensive policies to control STIs in a provincial level.

Methods

Study design and participants

From April to June 2021, a cross–sectional study was conducted in seven cities, named Nanjing, Wuxi, Suzhou, Huai'an, Yancheng, Yangzhou, and Zhenjiang, in Jiangsu province. A convenient sampling approach was used to recruit eligible participants. Firstly, we mapped the sex work venues and stratified them into three subgroups, i.e., high, middle, and low titers by minimum charge (3). High–titer venues included hotels and karaoke bars, and low–titer venues included street or other public outdoor sites. Middle–titer venues included barber bars, hair salons, massage parlors, roadside shops, guesthouses, or roadside restaurants (25). Secondly, we selected the potential venues randomly according to titers. Then, we conducted an on–site survey. All eligible venue members were recruited sequentially. Based on previous studies, we restricted the proportion of participants from each titer was high (<50%), middle (>40%), and low (>10%) in each city respectively to recruit a representative sample (26, 27).

Eligible participants were biologically females aged ≥16 years and self–reported providing vaginal, oral, or anal sex for male clients within the previous 12 months.

This study was approved by Ethics Committee of Jiangsu Provincial Center for Disease Prevention and Control in Jiangsu, China. All participants provided written informed consent before this survey.

Procedures and measures

The Jiangsu Center for Disease Control and Prevention designed a structured questionnaire for data collection. The information included socio–economic data, such as age (< 25/25–34/35 and above), level of education (Secondary education or below/ High school and above), marital status (Single/married), migratory status, and monthly income (< 5000/5000–7999/8000 and above, RMB). Participants who did not have official household registration in Jiangsu were deemed immigrants. Behavioral information included sexual behavior, condom use history, way to find male clients, drug history, HIV testing history, and STIs.

Based on the survey map and stratified titer of venues, the center for disease control and prevention staff contacted the house keepers or stakeholders, or managers to conduct an on–site survey. If they supported this survey, three professional staff (one for enrolling, one for screening and questionnaire, one for collecting sample of blood and urine) would take an on–site survey. Eligible participants would register their cellphone number for this survey. There was unique code linked the registration information, informed consent, questionnaire, blood and urine samples for each participant. The interviews remained anonymous throughout the survey. Each enrolled participant finished a face–to–face structured questionnaire interview.

After completing a questionnaire, each FSW donated 5 ml of venous blood with the help of trained nurses for HIV, Hepatitis C (HCV), and syphilis testing. All participants also provided 5 ml first–void urine held for more than 2 h for CT and NG testing. Finally, each participant received 50 RMB (approximately 7.5 US dollars) as participation incentives.

Testing

The enzyme–linked immunosorbent assay (ELISA) kit screened for plasma HIV and HCV antibodies in blood samples (Zhuhai livzon Diagnostics Inc., Zhuhai, China). If the result of HIV or HCV antibodies were positive, the same blood samples were retested by another ELISA reagent (Wantai Bio–pharmacy Enterprise Co., Ltd., Beijing, China). For HCV, both tests with positive results were recorded as HCV positive. For HIV, participants need provide new blood specimens for confirmatory testing by Western Blot (MP Biomedical Asia Pacific Pte. Ltd., Singapore) if both tests with positive results. Tests with positive results from both ELISA and WB were recorded as HIV infection.

Test for treponema pallidum (TP) was conducted using ELISA (TP–ELISA, Wantai Bio–pharmacy Enterprise Co., Ltd., Beijing, China) and subsequently by toluidine red untreated serum test (TRUST, Wantai Bio–pharmacy Enterprise Co., Ltd., Beijing, China) when the antibody for TP was positive. Positive results for both TP-ELISA and TRUST were recorded as syphilis infection. If only TP–ELISA was positive, we defined as syphilis antibody positive.

Pathogens of NG and CT were tested *via* nucleic amplification tests (NAATs) using (RENDU Biotechnology Co., Ltd., Shanghai, China), followed the manufacturer's instructions. A positive NAATs result of CT or NG was defined as CT or NG infection.

If participants received any positive result of STIs, professional staff would contact with positive participants, and referred them to local infectious disease hospital for further diagnose or treatment and care.

Statistical analysis

Descriptive analysis of categorical variables was reported as percentages. A chi–square test was used to assess the difference between subgroups. Where the chi–square test was not applicable, a fisher exact test was conducted to assess the difference between subgroups. A stepwise backward selection method was used for a multivariable logistic regression. Only variables with $P < 0.2$ were entered into the multivariable regression model. Odds ratio (OR) and adjusted Odds ratio (aOR) with a 95% Confidence interval (CI) were used to present the results. All data analyses were performed using IBM SPSS STATISTISCS (version 19.0, SPSS Inc., Chicago, IL, USA).

Results

Socio–demographic characteristics of FSWs

In total, we enrolled 3,580 FSWs from seven cities of Jiangsu province, including 985 (27.5%) from low titer venues, 1,866 (52.1%) from middle titer venues, and 729 (20.4%) from high titer venues. The mean age of FSWs was 35.0 years old (range, 16.0–65.0 years). The majority of FSWs were older than 35 years old (47.1%), with secondary education or below (69.9%), and currently married (72.4%). Among FSW participants, 43.5% were migrants, and 51.6% earned < 5000 RMB monthly (Table 1).

TABLE 1 Socio–demographic characteristics of 3,580 FSWs in a multi–center cross–sectional and venue–based study in Jiangsu, China.

Variables	Number	Percentage (%)
Age (Mean, Range)	35.0 (16.0–65.0)	
<25	487	13.6
25–34	1408	39.3
≥35	1685	47.1
Education level		
Secondary education or below	2503	69.9
High school and above	1077	30.1
Marital status		
Single	988	27.6
Currently married	2592	72.4
Migrant		
Yes	1557	43.5
No	2023	56.5
Monthly income (CNY)		
<5000	1848	51.6
5000–7999	931	26.0
≥8000	801	22.4
Recruitment tier		
Low	985	27.5
Middle	1866	52.1
High	729	20.4

STIs–related behavioral characteristics of FSWs

Most FSWs (82.6%) reported staying in the enrolled cities for commercial sex work for more than 6 months, and 43.1% of FSWs stayed in the same city for their last venue for commercial sex work (Table 2).

Considering the way to find clients, 87.3% of FSWs reported ever in local fixed venues. 30.9% of FSWs found clients using the phone and providing door–to–door service, and 47.3% found clients using the internet. The main way to find clients was fixed venues (62.8%) and the internet (29.1%) (Table 2).

Most FSWs (85.6%) reported consistent condom use with clients in the past month. When they reached the last episode of commercial sex, 96.6% of them reported condom use. On the contrary, 14.4% of FSWs reported unprotected sex with clients in the past month, and 10.6% reported group sex in the previous year. Only 8 (0.2%) of FSWs used illicit drugs in the past year (Table 2).

Testing for HIV and other STIs

Overall, 2,445 (68.3%) FSWs reported receiving HIV testing the previous year, 187 (5.2%) admitted having symptoms of STIs, and 13 (0.4%) reported STIs diagnosis in the previous year (Table 2).

Prevalence of HIV and other STIs

In total 3,580 (100.0%) blood specimens and 3,307 (92.4%) urinary specimens were collected. Only 4 (0.1%, 95%CI, 0.0%−0.2%) case of HIV infection was found. The overall prevalence of bacterial STIs (syphilis, CT or NG) was 6.2% (95%CI, 5.4%−7.0%). The prevalence of syphilis antibody positive was 4.9% (95%CI, 4.2–5.5), and the prevalence of current syphilis infection was 1.8% (95%CI, 1.4–2.3). The prevalence of single CT and NG infections was 4.3% (95%CI, 3.6–5.0) and 0.3% (95%CI, 0.2–0.5), respectively. Only one FSW with current syphilis infection was co–infected with HIV. 4.3% (6/141) of FSWs with CT were co–infected with NG (Table 2), and 11 (0.3%, 95%CI, 0.1–0.5) cases of HCV infections were found (Table 3).

TABLE 2 Risk factors for any sexual transmitted infections (STIs) among FSWs in Jiangsu, China.

Variables	Number	Percentage (%)
Duration time of commercial sex work in enroll city		
<6 months	624	17.4
≥6 months	2956	82.6
The last venue for selling sex		
Other provinces	755	21.1
Other cities in Jiangsu Province	861	24.1
Same city	1543	43.1
None	421	11.8
Condom use with the last client		
Yes	3459	96.6
No	121	3.4
Have unprotected sex with a customer in the past month		
Yes	515	14.4
No	3065	85.6
Group sex in the past year		
Yes	379	10.6
No	3201	89.4
Find clients in fixed venues		
Yes	3124	87.3
No	456	12.7
Find clients by phone		
Yes	1105	30.9
No	2475	69.1
Find clients by internet		
Yes	1692	47.3
No	1888	52.7
The main way to find clients		
Fixed Venues	2248	62.8
Telephone reservation	291	8.1
Internet	1041	29.1
Ever used illicit drugs in the past year		
Yes	8	0.2
No	3572	99.8
History of STIs in the past year		
Yes	13	0.4
No	3567	99.6
History of STIs symptoms in the past year		
Yes	187	5.2
No	3393	94.8
HIV testing in the past year		
Yes	2445	68.3
No	1135	31.7

TABLE 3 STIs prevalence among FSWs in Jiangsu, China.

Variables	Number	Percentage (%)
HIV infection		
Yes	4	0.1
No	3576	99.9
HCV infection		
Yes	11	0.3
No	3569	99.7
Syphilis antibody positive		
Yes	174	4.9
No	3406	95.1
Syphilis infection		
Yes	65	1.8
No	3515	98.2
Co–infection (HIV+Syphilis)		
Yes	1	0.03
No	3579	99.97
Total bacterial STIs infection (Syphilis/CT/NG)		
Yes	221	6.2
No	3359	93.8
NG infection ($N = 3307$)		
Yes	11	0.3
No	3296	99.7
CT infection ($N = 3307$)		
Yes	141	4.3
No	3166	95.7
Co–infection (CT+NG) (N = 141)		
Yes	6	4.3
No	135	95.7

STIs, Sexually transmitted infections; HCV, Hepatitis C; CT, Chlamydia trachomatis; NG, Neisseria gonorrhoeae.

Factors associated with syphilis and CT infection

Chi–square or Fisher exact tests were used to compare syphilis and CT infection differences between subgroups of variables. Syphilis infection was significantly associated with age, migrant status, monthly income, group sex in the past year (all $P < 0.05$). CT infection was significantly associated age, marital status, migrant status, recruitment titer, and NG infection (all $P < 0.05$) (Table 4).

In the multivariable model, FSWs who were aged among 25–34 (aOR, 0.339, 95%CI: 0.151–0.763), migrants (aOR, 0.466, 95%CI: 0.257–0.845), and monthly income of 5000–7999 (aOR, 0.450, 95%CI: 0.205–0.991) were less likely to have syphilis infection. On the contrary, having group sex in the past year (aOR, 2.521, 95%CI: 1.366–4.651)

TABLE 4 Differences in syphilis and CT infection between subgroups of variables among FSWs in Jiangsu, China.

Variables	Syphilis infection ($N = 3580$)			CT infection ($N = 3307$)		
	No. positive (%)	No. negative (%)	*P*–value	No. positive (%)	No. negative (%)	*P*–value
Age group (years)			0.006			0.008
<25	12 (18.5)	475 (13.5)		32 (22.7)	427 (13.5)	
25–34	13 (20.0)	1395 (39.7)		50 (35.5)	1234 (39.0)	
≥35	40 (61.5)	1645 (46.8)		59 (41.8)	1505 (47.5)	
Education			0.486			0.451
Secondary education or below	48 (73.8)	2455 (69.8)		95 (67.4)	2227 (70.3)	
High school and above	17 (26.2)	1060 (30.2)		46 (32.6)	939 (29.7)	
Marital status			0.793			0.037
Single	17 (26.2)	971 (27.6)		49 (34.8)	848 (26.8)	
Currently married	48 (73.8)	2544 (72.4)		92 (65.2)	2318 (73.2)	
Migrant			0.002			0.009
Yes	16 (24.6)	1541 (43.8)		76 (53.9)	1354 (42.8)	
No	49 (75.4)	1974 (56.2)		65 (46.1)	1812 (57.2)	
Monthly income (CNY)			0.040			0.897
<5000	40 (61.5)	1808 (51.4)		72 (51.1)	1680 (53.1)	
5000–7999	8 (12.3)	92 3(26.3)		39 (27.7)	838 (26.5)	
≥8000	17 (26.2)	784 (22.3)		30 (21.3)	648 (20.5)	
Recruitment tier			0.164			0.028
Low	24 (36.9)	961 (27.3)		30 (21.3)	889 (28.1)	
Middle	32 (49.2)	1834 (52.2)		90 (63.8)	1658 (52.4)	
High	9 (13.8)	720 (20.5)		21 (14.9)	619 (19.6)	
Duration time of commercial sex work in enroll city			0.123			0.766
<6 months	16 (24.6)	2907 (82.7)		25 (17.7)	531 (16.8)	
≥6 months	49 (75.4)	608 (17.3)		116 (82.3)	2635 (83.2)	
The last venue for selling sex			0.272			0.676
Other provinces	8 (12.3)	747 (21.3)		28 (19.9)	665 (21.0)	
Other cities in Jiangsu Province	20 (30.8)	841 (23.9)		34 (24.1)	784 (24.8)	
Same city	28 (43.1)	1515 (43.1)		57 (40.4)	1333 (42.1)	
None	9 (13.8)	412 (11.7)		22 (15.6)	384 (12.1)	
Condom use with the last client			0.891			0.584*
Yes	63 (96.9)	3396 (96.6)		137 (97.2)	3071 (97.0)	
No	2 (3.1)	119 (3.4)		4 (2.8)	95 (3.0)	
Have unprotected sex with a customer in the past month			0.556			0.999
Yes	11 (16.9)	504 (14.3)		20 (14.2)	449 (14.2)	
No	54 (83.1)	3011 (85.7)		121 (85.8)	2717 (85.8)	
Group sex in the past year			0.001			0.668
Yes	15 (23.1)	364 (10.4)		14 (9.9)	351 (11.1)	
No	50 (76.9)	3151 (89.6)		127 (90.1)	2815 (88.9)	

(Continued)

TABLE 4 (Continued)

Variables	Syphilis infection ($N = 3580$)			CT infection ($N = 3307$)		
	No. positive (%)	No. negative (%)	*P*–value	No. positive (%)	No. negative (%)	*P*–value
The main way to find clients			0.242			0.418
Fixed Venues	43 (66.2)	1027 (29.2)		92 (65.2)	963 (30.4)	
Telephone reservation	8 (12.3)	283 (8.1)		8 (5.7)	269 (8.5)	
Internet	14 (21.5)	2205 (62.7)		41 (29.1)	1934 (61.1)	
Ever used illicit drugs in the past year			0.864*			0.295*
Yes	0 (0.0)	8 (0.2)		1 (0.7)	7 (0.2)	
No	65 (100.0)	3507 (99.8)		140 (99.3)	3159 (99.8)	
HIV testing in the last year			0.708			0.168
Yes	43 (66.2)	2402 (68.3)		91 (64.5)	2216 (70.0)	
No	22 (33.8)	1113 (31.7)		50 (35.5)	950 (30.0)	
History of STIs in the past year			0.623			0.077*
Yes	0 (0.0)	13 (0.4)		2 (1.4)	9 (0.3)	
No	65 (100.0)	3502 (99.6)		139 (98.6)	3157 (99.7)	
History of STIs symptoms in the past year			0.432			0.388
Yes	2 (3.1)	185 (5.3)		10 (7.7)	171 (5.4)	
No	63 (96.9)	3330 (94.7)		131 (92.9)	2995 (94.6)	
HIV infection			0.071*			0.840*
Yes	1 (1.5)	3 (0.1)		0 (0.0)	4 (0.1)	
No	64 (98.5)	3512 (99.9)		141 (100.0)	3162 (99.9)	
HCV infection			0.817*			0.381
Yes	0 (0.0)	11 (0.3)		1 (0.7)	10 (0.3)	
No	65 (100.0)	3504 (99.7)		140 (99.3)	3156 (99.7)	
Syphilis infection						0.525*
Yes	–	–		2 (1.4)	58 (1.8)	
No	–	–		139 (98.6)	3108 (98.2)	
CT infection			0.524*			
Yes	2 (3.1)	139 (4.0)		–	–	
No	63 (96.9)	3376 (96.0)		–	–	
NG infection			0.183*			<0.001*
Yes	1 (1.5)	10 (0.3)		6 (4.3)	5 (0.2)	
No	64 (98.5)	3505 (99.7)		135 (95.7)	3161 (99.8)	

STIs, Sexually transmitted infections; HCV, Hepatitis C; CT, Chlamydia trachomatis; NG, Neisseria gonorrhoeae; *Fisher exact test; –, Not available.

and HIV infection (aOR, 26.260, 95%CI: 2.432–283.563) were associated with a higher risk of syphilis infection. FSWs who were migrants (aOR, 1.669, 95%CI: 1.163–2.395), having a history of STIs in the past year (aOR, 4.601, 95%CI: 1.003–21.118), and NG infection (aOR, 38.549, 95%CI: 11.214–132.514) were more likely to have CT infection (Table 5).

Discussion

To our knowledge, this study was a large sample size and recruited from various categories of sex venues among FSWs in Jiangsu, China. In this multicenter cross–sectional study, the total prevalence of bacterial STIs was 6.2% for FSWs. The STIs prevalence among FSWs were 0.1% for HIV, 1.8% for

10.3389/fpubh.2022.1018724

TABLE 5 Factors independently associated with syphilis and CT infection among FSWs in Jiangsu, China.

Variables	Syphilis infection		CT infection	
	OR (95%CI)	*P*–value	OR (95%CI)	*P*–value
Age group (years)				
<25	Reference		Reference	
25–34	0.339 (0.151–0.763)	0.009	0.503 (0.316–0.802)	0.004
≥35	0.749 (0.355–1.579)	0.447	0.578 (0.362–0.925)	0.002
Migrant				
Yes	0.466 (0.257–0.845)	0.012	1.669 (1.163–2.395)	0.057
No	Reference		Reference	
Month income (CNY)				
<5000	Reference			
5000–7999	0.450 (0.205–0.991)	0.047		
≥8000	1.461 (0.749–2.848)	0.266		
Group sex in the past year				
Yes	2.521 (1.366–4.651)	0.003		
No	Reference			
HIV infection				
Yes	26.260 (2.432–283.563)	0.007		
No	Reference			
History of STIs in the past year				
Yes			4.601 (1.003–21.118)	0.050
No			Reference	
NG infection				
Yes			38.549 (11.214–132.514)	<0.001
No			Reference	

STIs, Sexually transmitted infections; CT, Chlamydia trachomatis; NG, Neisseria gonorrhoeae.

syphilis infection, 0.3% for HCV, 4.3% for CT, and 0.3% for NG, respectively. Nearly half of FSWs were migrants from other provinces, and almost 100% of FSWs reported condom use with their last client. However, almost one–sixth of FSWs reported unprotective sex with clients in the past month. One in ten FSWs reported group sex in the past year, and 68.3% of FSWs got HIV testing in the past year. In the multiple logistic regression model, having group sex and HIV infection were risk factors for syphilis infection; being a migrant, having a history of STIs, and previous NG infection increased the risks of CT infection.

In our study, the CT and NG prevalence was 4.3 and 0.3%, respectively, lower than previous CT and NG prevalence rates reported among FSWs in Jiangsu by other studies. For instance, one study reported a CT prevalence of 14.61% and an NG prevalence of 5.42% in 2009 (24). Another study found a CT prevalence of 17.0% and an NG prevalence of 2.3% among FSWs in 2011 (3). It is possible that different specimens contribute to variations in sensitivity for CT/NG testing may explain the difference between our findings and other study findings (28). Guo and Tang collected cervical specimens to test CT and NG infection using NAATs in their studies. Our study collected first–void urine specimens to test CT and NG using NAATs. Also, compared with 40.7% of FSWs who reported consistent condom use with clients in the last month in Guo's study, 85.6% of participants reported consistent condom use in our study, which might imply a higher rate of STIs prevention among our sample. Nonetheless, the difference in reported prevalence rates implies that multiple specimens could be collected from different anatomical sites to evaluate the actual prevalence of CT/NG among FSWs in future studies.

Our result called for increased attention to stop STIs transmission among youth who sell sex. We found a high prevalence of CT among FSWs, particularly in < 25 years old participants. Young–aged FSWs accounted for nearly one to five FSWs, suggesting a significant burden of bacterial STIs (syphilis and CT infection) compared with other age groups in our study. This finding corroborated with results from previous studies (11, 25, 29). Meanwhile, the high rate of CT infections indicated continuing high levels of risky

behaviors among FSWs in Jiangsu. In China, there was still no national surveillance plan for CT and NG among the high–risk population. Our study's observed high rate of bacterial STIs infection also underlines the impendence and necessity of implementing bacterial STIs interventions (especially CT and NG) among FSWs.

Condom use can reduce HIV or other STIs transmission at a community level (30, 31). Since 2004, Jiangsu has promoted 100% condom use among FSWs to prevent HIV and other STIs. In our study, nearly 100% of FSWs reported condom use during their last sexual episode, which was higher than the country–level rate of condom use (77%) among FSWs (32). Nine to ten FSWs reported consistent condom use with their clients in the past month, which was higher than the rate (50.5%) in Zhejiang, another province in the Yangtze–river economic zone (33). Since 2010, the HIV prevalence consistently remained below 0.5%, and the syphilis infection prevalence also decreased from 5.32 to 1.72% from 2011 to 2020 among FSWs (unpublish data). The sentinel surveillance showed that condom promotion could effectively prevent HIV and syphilis transmission among the FSWs sub–population.

In this study, we found that 68.3% of FSWs received HIV testing last year, which was higher than the result from a meta–analysis conducted using data from China (34). China adopted regular HIV testing every three to six months among the high–risk population as part of its national programming (35). With this national guidance, 76.7% of FSWs reported taking at least one HIV test in the last year, from 2018 to 2019, in nine cities in China (36). This study's higher rate of HIV testing may be due to comprehensive prevention measures, such as condom promotion, educational campaigns, and HIV testing and counseling implemented over the years (37, 38). However, there were no recommended CT/NG screening strategies in China, even though a high prevalence of CT/NG was observed in multiple studies among the high–risk population in China (39–41). The increasing trend of HIV testing implies that integrating CT/NG testing with HIV testing was a reasonable way to screen for STIs, considering nearly 50% of NG and 75% of CT remain asymptomatic among FSWs (11, 42).

This study is the first–time urine samples have been collected for CT and NG testing in a community in Jiangsu. Based on clinical study protocols, previous studies usually collected cervical specimens to test CT and NG. However, according to a study on model sampling for HPV–based cervical cancer screening in 2019, 79% of participants had positive feelings about urine–based testing rather than cervical–vaginal sampling (18%) (43). According to findings from two studies conducted in Jiangsu, the rate of providing a cervical specimen for CT and NG tests was 28.57% (24) and 71.59% (3), respectively. However, 92.37% of participants in our study provided a urine sample for the CT/NG test, which showed high acceptability and feasibility of using urine samples. Furthermore, Cervical–based sampling needs a professional clinician and appropriate space for the sampling. In a community–based survey, we could not provide enough room for cervical samples, or participants refused to provide cervical swabs. Therefore, using first–void urine samples was more convenient for both researchers and participants. Further study on the sensitivity of CT/NG from different anatomical sites should be undertaken.

Limitations

Several limitations of this study should be considered for interpreting the results. First, as a hidden and marginalized group, we could not reach this population without the cooperation of stakeholders or venue owners, or managers in potential venues. Participants were not randomly recruited based on a convenient sample in this study. Sample representativeness might be jeopardized by the venue–based sampling method, which overlapped with the increasing proportion of non–venue–based venue–seeking sex activities. Second, we only used the urine samples to test NG and CT among FSWs, not rectal or pharyngeal samples. Even though the rate of urogenital chlamydia was higher than that of anorectal chlamydia, the prevalence of CT might be underestimated without a multiple anatomical site specimens (44). Nearly 10% of participants refused to collect a urine sample for the NG and CT testing. That also might have contributed to an underestimation of the prevalence of NG and CT. Third, recall bias could not be ignored since all data associated with risk behaviors are based on self–report. Finally, we could not ignore the impact of COVID−19 pandemic on the prevalence of STIs among FSW. Regardless, our study has provided representative data to show the current rates of STIs prevalence and associated factors related to STIs infection among FSWs in Jiangsu, which may be used to modify future prevention strategies.

Conclusions

The observed percentages of syphilis and CT infections show the need to promote more comprehensive STIs control and prevention strategies, including behavioral intervention and STIs screening, especially among younger high–risk populations. The increasing coverage of HIV testing implies that integrating other STIs screening with HIV testing may be a reasonable way to implement comprehensive STIs control and prevention.

Data availability statement

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

Ethics statement

The studies involving human participants were reviewed and approved by Ethics Committee of Jiangsu Provincial Center for Disease Prevention and Control. The patients/participants provided their written informed consent to participate in this study.

Author contributions

LS, JuL, and GF had the original idea. LC, HH, XX, ZZ, YZ, and JiL conducted the testing for STIs. YuhC, TQ, XL, and YunC collected the data. LS and JuL analyzed the data. LS wrote the main manuscript text. All authors contributed to manuscript revision and approved the submitted version.

Acknowledgments

The authors acknowledge Center for Disease Control and Prevention staff in Jiangsu. Thanks Gifty Marley for language editing.

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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TYPE Original Research
PUBLISHED 17 November 2022
DOI 10.3389/fpubh.2022.992773

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 13 July 2022
ACCEPTED 03 November 2022
PUBLISHED 17 November 2022

CITATION
Weng R, Ning N, Zhang C, Wen L, Ye J, Wang H, Li J, Chen X and Cai Y (2022) Acceptability of rectal self-sampling in non-clinical venues for chlamydia and gonorrhea testing among men who have sex with men: A cross-sectional study in Shenzhen, China. *Front. Public Health* 10:992773. doi: 10.3389/fpubh.2022.992773

Acceptability of rectal self-sampling in non-clinical venues for chlamydia and gonorrhea testing among men who have sex with men: A cross-sectional study in Shenzhen, China

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Background: Rectal Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infections among men who have sex with men (MSM) have become an increasingly important concern. The study aimed to explore (1) the acceptability of rectal self-sampling for chlamydia and gonorrhea testing among MSM in non-clinical venues in Shenzhen city, China; (2) factors associated with the acceptability of rectal self-sampling; and (3) factors associated with rectal CT and NG infections, respectively.

Methods: This cross-sectional study was conducted in two non-clinical settings in Shenzhen, China, from April 2021 to October 2021. Mixed-effects logistic regression analysis was performed to explore the factors associated with acceptance of rectal self-collection for CT and NG testing.

Results: Of the 306 MSM who were offered to perform rectal self-sampling, 133 (43.46%) accepted, and 96.24% (128/133) of them successfully provided a valid rectal sample. The prevalence of urogenital CT and NG infections among 303 MSM was 4.29 and 0.66%, respectively. The prevalence of rectal CT and NG infections among 128 participants was 31.25 and 9.38%, respectively. Participants having been diagnosed with HIV infection showed a higher acceptance of rectal self-collection for CT and NG testing.

Conclusion: This study reported that rectal self-sampling in non-clinical venues for CT and NG testing among MSM was barely acceptable and feasible in China. Most CT and NG infections would have been missed if urethral screening was offered alone, which implies that the CT and NG screening

should be scaled up in the above setting. Integrating free CT tests into regular STI interventions for MSM could also be considered.

KEYWORDS

Chlamydia trachomatis (CT), Neisseria gonorrhoeae (NG), men who have sex with men—MSM, rectal self-sampling, non-clinical venues

Introduction

Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infections are common curable sexually transmitted infections (STI). It is estimated that, in 2016, the global prevalence of CT and NG in 15–49-year-old men was 2.7 and 0.7%, respectively (1). Untreated CT and NG could lead to serious complications in men including epididymitis, reactive arthritis, mucosal inflammation in the oral and anorectal areas, anorectal pain, discharge, and severe scarring (mostly related to the presence of genotype L of CT) (2, 3), and also increase the risk of the human immunodeficiency virus (HIV) transmission (4). For men, around 50% of CT cases and 10% of NG cases were asymptomatic in urethral samples, which indicates the importance of CT and NG screening (5, 6).

Compared with the general population, a higher prevalence of CT and NG infections among men who have sex with men (MSM) was found in many studies (2, 7). Extragenital CT and NG infections among MSM have become an increasingly important concern as extragenital-only infections are very common. More than 85% of extragenital CT infections and 70% of NG infections would have been missed if only urogenital tests were offered (7). Also, pharyngeal and rectal CT and NG infections are more likely to be asymptomatic compared with genital infections (8). Routine screening of extragenital sites among MSM was recommended in several countries (9, 10).

The self-sampling method has been increasingly used as it is preferable, acceptable (11), highly sensitive, and comparable with clinician collection (12), and because physical distancing was suggested in the pandemic of COVID-19. To reach out to more MSM such as those without being regularly seen by a clinician, self-sampling in non-clinical venues including home or non-government organizations (NGO) may be an option or an intervention strategy. There were no studies reporting self-sampling in non-clinical settings in China, and previous studies were only conducted in clinical settings with clinician-sampling (13, 14). Also, there were no policies or strategies to guide extragenital CT or NG screening among MSM in China. Understanding the acceptability of rectal self-collection among MSM in non-clinical venues in the Chinese context could inform the health authorities in developing related policies or strategies. The current study, as a part of the Shenzhen Gonorrhea and Chlamydia Intervention Programme (SGCIP), aimed to explore (1) the acceptability of rectal self-sampling for chlamydia and gonorrhea testing among MSM in non-clinical venues in Shenzhen city, China; (2) factors associated with the acceptability of rectal self-sampling; and (3) factors associated with rectal CT or NG infections.

Materials and methods

Study setting and sampling

This cross-sectional study was conducted in two non-clinical settings in Shenzhen city, China. One setting (Site 1) is a local NGO serving an urban, districtwide catchment area in Luohu district, which provided free HIV/STIs testing and counseling for MSM in collaboration with Shenzhen Center for Disease Control and Shenzhen Center for Chronic Disease Control. Another setting (Site 2) is a local rainbow counseling center serving an urban, citywide catchment area, which was one of the largest centers in Shenzhen to provide free HIV/STIs testing, counseling, and referral for MSM. MSM who seek an HIV/STI checkup or counseling services are encouraged to access these two settings through both booking and walking in. Partner notification is also used to expand services to more MSM. In the current study, the convenience sampling method was used to recruit MSM. The inclusion criteria were: (1) age $\geq$ 18 years; (2) willing to participate and cooperate in the study and provide informed consent; (3) men who had anal or oral sex with another man in the last 12 months; (4) without presenting any CT/NG related symptom. Individuals were only eligible to enroll once in the study to avoid duplicate individuals in the analysis. To ensure confidentiality, participants' questionnaires, biological samples, and CT and NG test results were anonymized by assigning unique survey identification numbers. From April 2021 to October 2021, after signing a written informed consent, all eligible attendees in the study site were invited to fill out a questionnaire and were offered to provide urine and self-collected rectal swabs regardless of their self-reported exposure site(s). All participants provided written informed consent. This study was approved by the Ethical Review Committee of the Shenzhen Center for Chronic Disease Control (Approval No.20180206).

Sample size calculation

As the current study was a pilot study to implement rectal self-sampling, the lowest acceptance rate in previous studies was selected (15). Setting $P = 0.35$ (self-sampling acceptance rate), $d = 0.2P$ (allowable error), $a = 0.05$, $Z_a = 1.96$, the number of sample size was calculated according to the formula, $N = \frac{Z_a^2 * p(1-p)}{d^2}$, and the result was N1 = 179. However, considering the possibility of incomplete information due to errors from sample or questionnaire collection, we increased this sample size by 20% based on N1 and the final sample size was 215.

Questionnaire

For those who were eligible and provided informed consent, a structured paper-based questionnaire was used to obtain data on sociodemographic characteristics (e.g., age, marital status, and education), sexual behaviors, CT and NG infections, testing history, and opinions on CT testing and partner notification. Education was divided into two levels: (1) High school and below, including without any education, primary school, middle school, and high school, and (2) Junior college or higher, including junior college, bachelor's degree, master's degree, and doctoral degree. Information about sexual behaviors was collected referring to a standardized measure of the STI surveillance questionnaire in China.

Specimen collection and laboratory testing

All respondents were provided a diagram and oral instructions by trained staff in each study site about how to perform rectal self-collection and all specimens were collected at visit. DNA was extracted and purified from the specimens by automated magnetism nucleic acid isolation method using the MagNA Pure 96 System (Roche, Switzerland) according to the manufacturer's instructions. Polymerase chain reaction (PCR) of Cobas 4800® System (Roche, Switzerland) was used with Cobas® 4800 CT/NG Amplification/Detection Kit to determine CT and NG infection. Participants were informed of positive laboratory results by phone for further intervention as soon as the results were available.

Statistical analysis

All data from the questionnaires and laboratory results were double entered into computer using Epi Data software (Epi Data for Windows; The Epi Data Association Odense, Denmark) to establish a dataset. Frequencies (%) were used in categorical variables and mean ± SD was used in continuous variables. The χ^2 test was used to explore the differences of acceptance of rectal self-collection in the categorical variables. Also, the χ^2 test was used to explore the differences in the categorical variables between respondents with anorectal infection of CT/NG and respondents without anorectal infection of CT/NG. The acceptance of rectal self-collection was defined as a dependent variable for a mixed-effects logistic regression model, while age, marital status, Shenzhen residency, length of residency, education, ways to find sexual partners, previous HIV infection, and CT/NG testing history were included as fixed effects (Inclusion criteria: $P < 0.10$ in the χ^2 test) and study site was included as a random effect. The rectal NG infection was defined as a dependent variable for a mixed-effects logistic regression model, while Shenzhen residency and condom use during last anal sex were included as fixed effects (Inclusion criteria: $P < 0.10$ in the χ^2 test), and study site was included as a random effect. All data analysis was performed with the R program (Version 4.1.1). All tests were two-tailed, and $P < 0.05$ was defined as statistical significance.

Results

Acceptance of rectal self-sampling and baseline characteristics

The number of total samples was 306, and 183 MSM and 123 MSM were enrolled in site 1 and site 2, respectively. As shown in Table 1, around half of the participants were younger than 30 years old (48.37%, 148/306) and about one-fifth (21.57%, 66/306) were married. Around three-fifths (63.07%, 193/306) received Junior college or higher education, and 76.80% (235/306) had lived in Shenzhen for 2 years or more. Around one-fifth (19.05%, 56/294) of participants had been diagnosed with HIV infection.

All participants provided a urine sample with three invalid samples (3/306) due to an insufficient amount of urine. Around two-fifths (43.46%, 133/306) accepted to perform rectal self-sampling. Only 3.00% (4/133) of participants failed to collect and provide a rectal sample, and the reasons for failure to rectal self-sampling were rectal bleeding (2 participants) and difficulty in the collection (2 participants). Among those who refused to perform rectal self-sampling, only 8.67% (15/173) of them reported the reasons for the refusal including perceived no risk of rectal infection (13 participants) and feeling uncomfortable (2 participants).

CT/NG infections and testing history of participants

The prevalence of urogenital CT and NG infections in 303 samples was 4.29 and 0.66%, respectively. There were no

TABLE 1 Characteristics, sexual behaviors, and CT/NG-related information of 306 men who have sex with men according to rectal self-collection acceptance among two non-government organizations in Shenzhen.

Variables	Overall N	Accepted	(%)	Refused	(%)	χ^2	*P*	AOR (95%CI)	*P*
Total	306	133	(43.46)	173	(56.54)				
Age groups ($n = 306$)						3.69	0.05*		
≥ 30	158	77	(48.73)	81	(51.27)			1	
< 30	148	56	(37.84)	92	(62.16)			1.59 (0.75–3.36)	0.23
Marital status ($n = 306$)						8.36	<0.01*		
Married	66	39	(59.09)	27	(40.91)			1	
Single/Divorced/Widowed	240	94	(39.17)	146	(60.83)			0.98 (0.41–2.34)	0.97
Registered residence in Shenzhen ($n = 306$)						4.25	0.04*		
Yes	154	58	(37.66)	96	(62.34)			1	
No	152	75	(49.34)	77	(50.66)			1.00 (0.50–2.00)	0.99
Length of residency ($n = 306$)						4.61	0.03*		
<2year	71	23	(32.39)	48	(67.61)			1	
≥2year	235	110	(46.81)	125	(53.19)			1.29 (0.58–2.87)	0.54
Education level ($n = 306$)						18.27	<0.01*		
High school or below	113	67	(59.29)	46	(40.71)			1.90 (0.91–3.99)	0.09
Junior college or higher	193	66	(34.20)	127	(65.80)			1	
Sexual orientation ($n = 306$)						0.09	0.76		
Heterosexuality	87	39	(44.83)	48	(55.17)				
Homosexuality/bisexuality	219	94	(42.92)	125	(57.08)				
Ways to find sexual partners ($n = 301$)						11.63	<0.01*		
Others	32	23	(71.88)	9	(28.13)			1	
Bars/ parks	14	7	(50.00)	7	(50.00)			1.50 (0.22–10.39)	0.68
Hookup websites or geosocial networking applications	255	103	(40.39)	152	(59.61)			1.11 (0.35–3.53)	0.87
Rectal sexual behaviors in the past six months ($n = 306$)						0.86	0.35		
Yes	253	113	(44.66)	140	(55.34)				
No	53	20	(37.74)	33	(62.26)				
Condom use during last anal sex ($n = 253$)						4.03	0.04*		
Yes	190	78	(41.05)	112	(58.95)			1	
No	63	35	(55.56)	28	(44.44)			1.88 (0.93–3.83)	0.08
Condom use during anal sex in the past 6 months ($n = 253$)						0.10	0.75		
Inconsistent	117	51	(43.59)	66	(56.41)				
Consistent	136	62	(45.59)	74	(54.41)				
Having casual sexual partners in the past six months ($n = 277$)						0.09	0.76		
Yes	155	74	(47.74)	81	(52.26)				
No	122	56	(45.90)	66	(54.10)				
Previous HIV infection ($n = 294$)						21.61	<0.01*		
No	191	78	(40.84)	113	(59.16)			1	
Yes	56	37	(66.07)	19	(33.93)			3.01 (1.32–6.88)	<0.01*
Never tested	47	10	(21.28)	37	(78.72)			0.76 (0.27–2.15)	0.60
Ever CT/NG tested ($n = 306$)						12.25	<0.01*		
No	254	99	(38.98)	155	(61.02)			1	
Yes	52	34	(65.38)	18	(34.62)			1.46 (0.62–3.43)	0.39
Urine CT ($n = 303$)						0.13	0.72		
Positive	13	5	(38.46)	8	(61.53)				
Negative	290	126	(43.45)	164	(56.55)				
Urine NG ($n = 303$)									
Positive	2	1	(50.00)	1	(50.00)	0.04	0.85		
Negative	301	130	(43.19)	171	(56.81)				

A mixed–effects logistic regression model with study site as a random effect was used for estimating the parameters.

*$P < 0.05$.

AOR, adjusted odds ratio; CI, confidence interval; CT, chlamydia trachomatis; NG, Neisseria gonorrhoeae.

differences in the prevalence of CT and NG in urine samples between participants who accepted rectal self-sampling and those who did not accept (CT: $\chi^2 = 0.13$, $P = 0.72$; NG: $\chi^2 < 0.01$, $P = 1.00$). Among those who provided rectal self-sampling, almost all samples (99.22%, 128/129) were valid, except for one invalid sample with fecal contamination. The prevalence of rectal CT and NG infections among those who accepted rectal self-sampling was 31.25 and 9.38%, respectively. Both CT and NG infections of participants from rectal samples were significantly higher than those from urine samples (CT: $\chi^2 = 41.23$, $P < 0.01$; NG: $\chi^2 = 19.06$, $P < 0.01$). There were no differences in all variables between respondents with rectal infection of CT and respondents without rectal infection of CT (Table 2). Results from the mixed-effects logistic regression model suggested that there were no factors associated with rectal NG infection (Table 2). About CT/NG-related information (Table 1), only 16.99% (52/306) had been tested for CT or NG.

Factors associated with acceptance of CT and NG testing

As shown in Table 1, results from the mixed-effects logistic regression model suggested that participants having been diagnosed with HIV infection (AOR = 3.01, 95% CI = 1.32–6.88) were more likely to perform rectal self-collection for CT and NG testing ($P < 0.05$).

Opinions to CT testing and partner notification

As shown in Table 3, more than half of the participants (58.88%) selected the routine CT test as the most appropriate time. Around one-third (35.53%) of respondents were willing to undertake a CT test if it is free, and half of them were willing to undertake a CT test without any condition. Most of them were willing to be engaged in patient-based partner referral if they were diagnosed with CT infection (Table 3).

Discussion

To our knowledge, this is the first study in China to explore the acceptability of rectal self-sampling for chlamydia and gonorrhea testing among MSM in non-clinical venues. This study demonstrated that rectal self-sampling in non-clinical venues for chlamydia and gonorrhea testing among MSM was barely acceptable and feasible, with 43.46% (133/306) of MSM accepting rectal self-collection and 96.24% (128/133) of MSM successfully providing valid rectal samples in this pilot implementation. A previous study reported a similar acceptance rate (34.99%) of rectal self-sampling in community venues in Vancouver (15). A higher acceptance rate to perform self-collected rectal swabs was found in MSM attending STI clinics in The Netherlands (59.95%) and Ireland (91.55%) (16, 17). The potential reason why the acceptance rate in the study in Ireland was much higher than that in other studies is that the recruited population was HIV-positive MSM. The current study also showed that respondents who had been diagnosed with HIV infection were more likely to accept rectal self-collection, which was consistent with a previous study (15). Sanchez et al. found that HIV-positive MSM were more likely to report STI testing (18). Also, MSM living with HIV were more likely to have accessed NGO services, which further highlights the significance of promoting rectal self-sampling in NGO services (19). Besides, among those who refused rectal self-collection, a large proportion of them (86.67%, 13/15) perceived that they have no risk of rectal infection, which was consistent with the previous finding (20). The reasons for the refusal we found may provide a starting point for improving rectal self-sampling strategies among MSM in China. Information on the high prevalence of rectal CT/NG infection among MSM should be highlighted and delivered to all MSM for increasing the acceptance rate of rectal self-sampling.

The current study reached very high-risk MSM with a high prevalence of rectal CT and NG infections (31.25 and 9.38%, respectively), which was much higher than that (provider collection) in previous studies in China (CT: 11.23–15.57%, NG: 5.01–6.05%) (13, 14). Also, rectal CT infection in the current study was much higher than those MSM performing rectal self-collection in other studies (7.59–14.14%), which was not the case in the rectal NG infection (4.20–10.24%) (7, 8, 16, 21). In our study, among those who performed rectal self-collected swabs, 90.48% (38/42) of CT infection would have been missed and 92.31% (12/13) of NG infection would have been missed if urethral screening was offered alone, which highlighted the importance of rectal CT and NG screening outreach. In addition, there were no factors associated with the rectal CT and NG infection, which implies that all MSM should be screened with this high prevalence of rectal CT and NG infections. Future studies with a larger sample size could be considered to further support these findings.

The current study also found that just a small proportion of participants (16.99%) had had CT/NG test before, which was much lower than that in Australia (57.1%) (22). One important reason was that the first chlamydia screening program was launched in 2017 in China and a huge proportion of MSM was not tested before. Increasing the uptake of screening belongs to one of the major challenges to strengthen the continuum of STI prevention, diagnosis, treatment, and care (23). Previous studies reported an increase in acceptance rate over time after introducing self-taken extra-genital swabs (24, 25). Therefore, this acceptable rectal self-sampling strategy in non-clinical settings could help expand the uptake of CT/NG screening and detect more positive cases.

TABLE 2 Characteristics, sexual behaviors, and CT/NG–related information of 128 men who have sex with men according to rectal CT and NG prevalence among two non–government organizations in Shenzhen.

Variables	Rectal CT N	(%)	χ^2 values	*p*	Rectal NG N	(%)	χ^2 values	*p*	AOR (95%CI)	*p*
Age groups ($n = 128$)			0.49	0.49			<0.01	0.92		
≥30	21	(28.77)			7	(9.59)				
<30	19	(34.55)			5	(9.09)				
Marital status ($n = 128$)			2.84	0.09			0.04	0.85		
Married	7	(20.00)			3	(8.57)				
Single/Divorced/Widowed	33	(35.48)			9	(9.68)				
Registered residence in Shenzhen ($n = 128$)			0.48	0.49			4.16	0.04*		
Yes	16	(28.07)			2	(3.51)			1	
No	24	(33.80)			10	(14.08)			4.44 (0.83–23.69)	0.08
Length of residency ($n = 128$)			0.81	0.37			0.44	0.51		
< 2 year	9	(39.13)			3	(13.04)				
≥ 2 year	31	(29.52)			9	(8.57)				
Education level ($n = 128$)			1.98	0.16			1.34	0.25		
High school or below	24	(36.92)			8	(12.31)				
Junior college or higher	16	(25.40)			4	(6.35)				
Sexual orientation ($n = 128$)			1.16	0.28			0.10	0.75		
Heterosexuality	9	(24.32)			3	(8.11)				
Homosexuality/bisexuality	31	(34.07)			9	(9.89)				
Ways to find sexual partners ($n = 128$)			0.99	0.61			0.86	0.65		
Bars/ parks	1	(14.29)			1	(14.29)				
Hookup websites or geosocial networking applications	32	(32.32)			10	(10.10)				
Others	7	(31.82)			1	(4.55)				
Rectal sexual behaviors in the past six months ($n = 128$)			0.79	0.37			0.07	0.79		
Yes	36	(32.73)			10	(9.09)				
No	4	(22.22)			2	(11.11)				
Condom use during anal sex in the past 6 months ($n = 110$)			0.88	0.35			0.06	0.81		
Consistent	18	(37.50)			6	(9.68)				
Inconsistent	18	(29.03)			4	(8.33)				
Condom use during last anal sex ($n = 110$)			0.06	0.81			4.51	0.03*		
Yes	25	(32.05)			10	(12.82)			1	
No	11	(34.38)			0	(0.00)			<0.001 (<0.001–>999.999)	0.97
Having casual sexual partners in the past six months ($n = 125$)			0.35	0.55			0.47	0.50		
Yes	24	(27.45)			6	(8.11)				
No	14	(32.43)			6	(11.76)				
HIV infection ($n = 120$)			2.58	0.27			0.06	0.97		
No	19	(25.68)			7	(9.46)				
Yes	15	(40.54)			4	(10.81)				
Never tested	3	(33.33)			1	(11.11)				
Ever CT/NG tested ($n = 128$)			0.33	0.57			<0.01	0.95		
No	31	(32.63)			9	(9.47)				
Yes	9	(27.27)			3	(9.09)				

A mixed–effects logistic regression model with study site as a random effect was used for estimating the parameters.
*$P < 0.05$.
%, Constituent ratio.
AOR, adjusted odds ratio; CI, confidence interval; CT, chlamydia trachomatis; NG, Neisseria gonorrhoeae.

TABLE 3 Opinion on CT testing and partner notification.

Variables	*N*	(%)
The most appropriate time to undertake a CT test ($n = 304$)		
Having CT–related symptoms	29	(9.54)
Routine CT screening (annual)	179	(58.88)
After risky sexual behaviors	41	(13.49)
Suggestion from doctors	55	(18.09)
Routine CT screening willingness ($n = 304$)		
Unwilling	15	(4.93)
Willing (If it is free)	108	(35.53)
Willing (If the participant has CT–related symptoms)	36	(11.84)
Willing (Without any condition)	145	(47.70)
Willingness to notify their partner(s) if they were diagnosed with CT infection ($n = 303$)		
No	25	(8.25)
Yes (provider referral)	49	(16.17)
Yes (patient–based partner referral)	210	(69.31)
Yes (expedited partner therapy)	19	(6.27)

%, Constituent ratio.
CT, chlamydia trachomatis.

Our study also suggested that more than half of respondents believed that routine CT screening was the most appropriate to undertake CT test and most of them were willing to undertake routine CT screening. A previous study found that CT screening had great acceptance in this population (26). However, a third of participants were willing to undertake routine CT screening if the test is free, which suggested that offering free CT tests could expand the uptake of screening. Rectal CT/NG screening of MSM was proved to be a cost-effective, scalable intervention (27), which also supports the strategy of free CT tests for MSM. These findings implied that integrating free CT tests into regular STI interventions for MSM could be considered.

As a pilot implementation of rectal self-sampling in non-clinical settings, it indicated that rectal self-sampling was barely acceptable and the burden of rectal CT and NG among MSM was great, which suggested that integrating rectal self-sampling in non-clinical settings to STI services could be considered to expand surveillance efforts and reach populations who may be more resistant to clinic-based screening (20). Also, conducting self-sampling in non-clinical settings would not increase the work burden on healthcare providers (17). Future implementation should focus more on how to raise the acceptance rates of rectal self-sampling. Many reasons were reported such as fear of taking the swab incorrectly, finding instructions unclear, being unaware of their level of susceptibility to rectal infections, and believing urine testing would identify rectal infections (16, 20). Our findings also provided reasons for the refusal such as perceived no risk of rectal infection and feeling uncomfortable, which should be considered in the future implementation of this rectal self-sampling screening strategy. Novel methods such as postal self-sampling or Internet-based self-sampling could also be considered in the future.

Several limitations should be concerned. First, the convenience sampling method was used to recruit MSM in two non-clinical settings in Shenzhen city, China, which limit the representativeness to the MSM population in China and also may limit the generalizability of the results to other cities. However, over 70% of Shenzhen city's population are temporary migrants (28) and around half of the participants (49.67%, 152/306) belonged to the migrant population without registered residence in Shenzhen, which may represent the MSM population in China to some extent. Besides, MSM are usually hard to reach for research and two large non-clinical settings were included in the study to increase the possibility of generalizability. Second, information related to sexual behaviors was self-reported, which may lead to social desirability bias. Third, information related to NG testing and partner notification was not collected, which could be considered in the future implementation of the study.

In summary, this study found that rectal self-sampling in non-clinical venues for chlamydia and gonorrhea testing among MSM was barely acceptable and feasible. Most CT and NG infections would have been missed if urethral screening was offered alone, which implies that the CT screening should be scaled up in the above setting. HIV status should be taken into account to promote rectal screening.

Data availability statement

The datasets presented in this article are not readily available because the dataset generated and analyzed during the current study could be available from the corresponding author on reasonable request. Requests to access the datasets should be directed to 64165469@qq.com.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Review Committee of the Shenzhen Center for Chronic Disease Control. The patients/participants provided their written informed consent to participate in this study.

Author contributions

RW and YC conceived and designed the study. NN, CZ, LW, JY, HW, and JL supervised the data collection. YC, XC, RW, CZ, LW, JY, HW, JL, and NN performed the research. RW, NN, and YC analyzed and interpreted the results and were the major contributors in writing the manuscript. XC, CZ, LW, JY, HW, and JL revised the manuscript critically. All authors read and approved the final manuscript.

Funding

This work was supported by the Sanming project of Medicine in Shenzhen [grant number SZSM201611077].

Acknowledgments

The authors thank the staff at the study site, Mr. Xuan Xuan, for his assistance in recruiting participants for the study.

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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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 17 November 2022
DOI 10.3389/fpubh.2022.1005481

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases – Surveillance, Prevention and Treatment, a section of the journal Frontiers in Public Health

RECEIVED 28 July 2022
ACCEPTED 17 October 2022
PUBLISHED 17 November 2022

CITATION
Ning N, Weng R, Zhang C, Wen L, Wang H, Ye J, Li J, Chen X and Cai Y (2022) Cluster analysis for symptomatic management of *Neisseria gonorrhoea* and *Chlamydia trachomatis* in sexually transmitted infections related clinics in China. *Front. Public Health* 10:1005481. doi: 10.3389/fpubh.2022.1005481

Cluster analysis for symptomatic management of *Neisseria gonorrhoea* and *Chlamydia trachomatis* in sexually transmitted infections related clinics in China

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Objective: This study aimed to perform a cluster analysis of symptoms linked with *Neisseria gonorrhoeae* (NG) and *Chlamydia trachomatis* (CT) and to identify which cluster of symptoms was associated with a higher risk of NG and CT.

Study design: From 15 April to 16 May 2018, a cross-sectional study was conducted, and patients attending sexually transmitted infections (STI) related clinics were recruited from 22 medical institutions in six districts of Shenzhen city.

Methods: A structured questionnaire was used to collect social-demographic information as well as STI symptoms, and urine samples were collected for nucleic acid detection. Cluster analysis and logistic regression were applied.

Results: Among 8,207 participants, the prevalence of CT and NG infection was 9.04% (742/8,207) and 2.36% (194/8,207), respectively. Among male outpatients, four clusters with distinct symptomatic patterns were identified. Unmarried, having casual sexual partners in the past 6 months, cluster 2 (OR = 6.70, 95% CI = 3.36–13.35) and cluster 4 (OR = 24.53, 95% CI = 12.96–46.44) were risk factors associated with NG infection. Unmarried, cluster 2 (OR = 2.54, 95% CI = 1.83–3.53) and cluster 4 (OR = 3.31, 95% CI = 2.37–4.61) were risk factors associated with CT infection. Among female outpatients, five clusters with distinct symptomatic patterns were identified. Aged 24 years or below and cluster 3 (OR = 3.68, 95% CI = 1.61–8.39) were risk factors associated with NG infection. Aged 24 years or below, unmarried, having a high school/secondary technical school education, and having junior high school or below education were risk factors associated with CT infection.

Conclusion: The cluster of symptoms integrated into risk assessment for CT and NG infections suggests a new strategy of symptomatic management.

Healthcare providers in STI clinics and resource-limited places may use this strategy to identify more potential patients and deliver adequate, acceptable, and equitable STI care for outpatients with a high risk of STI.

KEYWORDS

sexually transmitted infections, genital chlamydia trachomatis infection, *Neisseria gonorrhoeae*, symptomatic management, associated factors

Introduction

Chlamydia trachomatis (CT) and *Neisseria gonorrhoeae* (NG) are the most common sexually transmitted infections (STIs) worldwide. In 2016, the WHO estimated that there were 376.4 million new global cases of four common STIs: chlamydia (127.2 million cases), gonorrhoea (86.9 million cases), trichomoniasis (156 million cases), and syphilis (6.3 million cases) (1). Untreated NG and CT could lead to serious complications in women, including pelvic inflammatory disease, tubal infertility, and obstetric complications, and in men, including epididymitis and reactive arthritis (2). With this great burden of NG and CT, efforts are needed to find and promote appropriate strategies.

Symptomatic management of STI has been widely used and is a useful strategy to assess the risk of STI, with three main processes including identifying consistent groups of symptoms and signs, using the available flowcharts to provide treatment, and taking care of the most serious organisms responsible for producing the syndrome (3). As symptomatic management is a simple and practical strategy, it is still the standard of treatment when laboratory diagnosis is unavailable or when it takes a few days to obtain results in most resource-limited settings (4). Previous studies showed that symptomatic management can provide better treatment for patients with STIs at a significantly lower cost and significantly reduce the prevalence of syphilis, chancroid, gonorrhoeae, and bacterial vaginosis infection (5, 6). Symptomatic management for urethral discharge and genital ulcer disease in men had high sensitivity and accuracy, while in women, symptomatic management for abnormal vaginal discharge, genital ulcers, and genital warts was controversial (4, 7, 8). It may also lead to overtreatment or drug resistance in some subjects. In many diseases, syndrome cluster analysis was used to combine symptoms and identify those clusters associated with a higher risk of infection (9, 10).

Although the prevalence of CT infection in China was high (11, 12), CT infection is currently one of the STIs being monitored in China and is not included in notifiable communicable diseases. Aside from a pilot screening program in Shenzhen (13), the rate of routine screening is low because of the presence of asymptomatic patients, laboratory capability (e.g., primary care medical institutions with limited resources), and cost constraints, especially in rural areas with poor conditions. In China, 36.11% of people live in rural areas (14), and medical resources are limited in most rural areas (15). Therefore, symptomatic management could be a good strategy to find more infected people and provide timely treatment. Recently, an updated version of guidelines for the symptomatic management of symptomatic sexually transmitted infections was published by WHO (16), and there is a need to explore the use of symptomatic management from guidelines for the management of symptomatic sexually transmitted infections in the Chinese context. To better identify patients, a cluster analysis of symptoms linked with NG and CT was used in this study to identify which clusters of symptoms were associated with a higher risk of NG and CT and provide new strategies for symptomatic management in China.

Materials and methods

Sampling methods and recruitment

The multi-stage stratified sampling method was used to select study units. Six administrative districts in the city were randomly selected. In each district, the top four hospitals with the most reported cases of STI infection in 2017 were selected, except for one district with only two hospitals. A total of 22 medical institutions from Nanshan, Luohu, Bao'an, Longgang, Yantian, and Longhua districts were selected. From 15 April to 16 May 2018, the first 15 eligible attendees were recruited daily in the departments of dermatology, urology, and other STI-related departments of each medical institution (such as MSM counseling clinics). This study's inclusion criteria were as follows: (1) aged between 18 and 49 years; (2) having engaged in sexual behavior; and (3) not using antibiotics in the previous 2 weeks. All eligible patients were only recruited. All participants provided informed consent before the study. This study has been approved by the Ethical Review Committee of the Shenzhen Center for Chronic Disease Control (Approval No. 20180206).

Patients recruitment and management

Patients were investigated by medical staff who had received professional training. The questionnaire included age, marital status, education, history of STI testing, casual sex partners in the last 3 months, and NG/CT-related symptoms. After laboratory testing, patients who were positive for CT and/or NG infections were informed of the testing results to ensure timely treatment. Positive cases were suggested to be retested for the infection 3 months after treatment, and partner notification and following treatment of their positive sexual partners were also recommended.

Specimen collection and laboratory testing

A labeled Roche Cobas ® urine collection tube was used to collect 15–30 ml of urine, which was transferred to each hospital's laboratory for preservation. At the central laboratory, DNA was extracted and purified from the urine specimens by the automated magnetism nucleic acid isolation method using the MagNA Pure 96 System (Roche, Switzerland). The extracted DNA was further evaluated for CT and NG based on polymerase chain reaction (PCR) of the Cobas 48001 System (Roche, Switzerland) using the Cobas1 4800 CT/NG Amplification/Detection Kit. Laboratory performance was run according to standard operating procedures (SOPs). A CT or NG infection was defined as having a positive PCR for CT or NG accordingly.

Statistical analyses

Data were double-blind entered, and Epidata 3.0 was used to create the database. The investigator deleted duplicate data by assigning unique study numbers to each participant. First, cluster analysis was conducted on 10 symptoms of men and women, respectively. Second, we adopted a multivariate logistic regression model with forward selection method, defining NG or CT infection as the dependent variable and age, marital status, education, history of STI testing, casual sexual partners in the last 3 months, and NG/CT-related symptom clusters as independent variables. All data analysis was performed on SAS 8.0. All tests were two-tailed, and $P < 0.05$ was considered statistically significant.

Result

A total of 8,207 participants completed the questionnaire and provided a urine sample, with 2,564 (31.01%) men and 5,703 (68.99%) women. Of all participants, 85.70% were aged 24 years or below, 65.50% were married, 31.80% were in junior high school or below, 29.09% were in high school/secondary technical school, 35.91% had casual sexual partners in the last 3 months, 89.50% had no history of STI testing, and 40.68% was asymptomatic. Totally, 2.36% (194/8,207) and 9.04% (742/8,207) of the participants were diagnosed with gonorrhea and chlamydia infections, respectively.

Men

Cluster analysis in men

Among male attendees, four clusters with distinct symptomatic patterns and one group of asymptomatic patients were identified. The frequency of gonorrhea and chlamydia in each cluster is shown in Table 1. In this group, 5.88% (149/2,536) and 10.69% (271/2,536) of the participants were diagnosed with gonorrhea and chlamydia, respectively. The asymptomatic group was the largest, accounting for 50.12% (1271/2,536) of the total cohort. Cluster 1 consisted of 3.35% (85/2,536) of the entire cohort, including (1) scrotum swelling or pain and (2) epididymis pain/swelling/tenderness, and 2.35 and 8.24% of the participants were diagnosed with gonorrhea and chlamydia. Cluster 2 consisted of 23.54% (597/2,536) of the entire cohort, including (1) frequent urination/urgency/urodynia/itching, (2) balanitis, and (3) inguinal lymph node enlargement, and 5.70 and 14.41% of the participants were diagnosed with gonorrhea and chlamydia, respectively. Cluster 3 consisted of 3.55% (90/2,536) of the entire cohort, including (1) genital/perianal watery blisters or pustules and (2) genital/perianal ulcers, and 3.33 and 7.78% of the participants were diagnosed with gonorrhea and chlamydia, respectively. Cluster 4 consisted of 19.44% (493/2,536) of the entire cohort, including (1) urethral serous discharge, (2) urethral purulent discharge, and (3) genital/perianal warts, and 19.88 and 18.86% of the participants were diagnosed with gonorrhea and chlamydia, respectively. Besides, the symptom clusters of the MSM were also obtained, which showed a similar pattern.

Factors associated with NG/CT infection in men

In univariate analyses, six variables were associated with NG/CT infection in men at $P < 0.05$ (shown in Tables 2, 3). Results from the multivariate logistic regression model suggested that the following factors were significantly associated with NG infection: unmarried (AOR = 1.89, 95% CI = 1.32–2.70), having casual sexual partners in the last 3 months (AOR = 2.03, 95% CI = 1.37–2.99), cluster 2 (AOR = 6.70, 95% CI = 3.36–13.35), and cluster 4 (AOR = 24.53, 95% CI = 12.96–46.44). Using cluster 2 and cluster 4 as an indicator of NG can find 88.59% (132/149) NG positive cases, but the number of tests accounted for only 42.98% (1,090/2,536) of total male participants. Results from the multivariate logistic regression

10.3389/fpubh.2022.1005481

TABLE 1 Symptom clusters of the respondents in the men cohort ($N = 2,536$).

Symptoms	Asymptomatic $n = 1,271$	Cluster 1 $n = 85$	Cluster 2 $n = 597$	Cluster 3 $n = 90$	Cluster 4 $n = 493$
Urethral serous discharge					192
Urethral purulent discharge					164
Frequent urination/urgency/ urodynia/itching			535		
Scrotum swelling or pain		58			
Epididymis pain, swelling and/or tenderness		32			
Genital/Perianal watery blisters or pustules				57	
Genital/Perianal ulcers				49	
Genital/Perianal warts					166
Balanitis			65		
Inguinal lymph node enlargement			2		
NG positive	12	2	34	3	98
CT positive	78	7	86	7	93

TABLE 2 Univariate and multivariate logistic regression analyses of factors associated with NG in men.

Variables	N (%)	UnivariateOR (95% CI)	*P*	MultivariateAOR (95% CI)	*P*
Age group	2,564 (100.00)				
>24	344 (13.42)	1			
≤24	2,220 (86.58)	2.75 (1.88–4.00)	<0.01		
Marital status	2,563 (100.00)				
Married	891 (34.76)	1		1	
Unmarried	1672 (65.24)	2.57 (1.84–3.60)	<0.01	1.89 (1.32–2.70)	<0.01
Education	2540 (100.00)				
Bachelor's degree or above	549 (21.57)	1			
Junior college	518 (20.39)	1.06 (0.62–1.82)	0.83		
High school/secondary technical school	782 (30.79)	1.43 (0.90–2.29)	0.13		
Junior high school or below	692 (27.24)	1.02 (0.61–1.69)	0.94		
Having casual sexual partners in thelast 3 months	2,540 (100.00)				
No	1,186 (46.69)	1		1	
Yes	1,354 (53.31)	2.34 (1.62–3.37)	<0.01	2.03 (1.37–2.99)	<0.01
STI test history	2,521 (100.00)				
Yes	235 (9.32)	1			
No	2,286 (90.68)	1.16 (0.63–2.12)	0.64		
Cluster model	2,536 (100.00)				
Asymptomatic	1,271 (50.12)	1		1	
Cluster 1	85 (3.35)	2.53 (0.56–11.48)	0.23	2.97 (0.65–13.70)	0.16
Cluster 2	597 (23.54)	6.34 (3.26–12.33)	<0.01	6.70 (3.36–13.35)	<0.01
Cluster 3	90 (3.55)	3.62 (1.00–13.06)	0.05	3.27 (0.89–12.02)	0.07
Cluster 4	493 (19.44)	26.03 (14.14–47.91)	<0.01	24.53 (12.96–46.44)	<0.01

model suggested that the following factors were significantly associated with CT infection: unmarried (AOR = 1.33, 95% CI = 1.02–1.74), cluster 2 (AOR = 2.54, 95% CI = 1.83–3.53), and cluster 4 (AOR = 3.31, 95% CI = 2.37–4.61). Using cluster 2

TABLE 3 Univariate and multivariate logistic regression analyses of factors associated with CT in men.

Variables	N (%)	UnivariateOR (95% CI)	*P*	MultivariateAOR (95% CI)	*P*
Age group	2,564 (100.00)				
>24	344 (13.42)	1			
≤24	2,220 (86.58)	1.55 (1.12–2.15)	<0.01		
Marital status	2,563 (100.00)				
Married	891 (34.76)	1		1	
Unmarried	1,672 (65.24)	1.45 (1.12–1.87)	<0.01	1.33 (1.02–1.74)	0.04
Education	2,540 (100.00)				
Bachelor's degree or above	549 (21.57)	1			
Junior college	518 (20.39)	1.38 (0.92–2.08)	0.12		
High school/secondary technical school	782 (30.79)	1.42 (0.97–2.07)	0.07		
Junior high school or below	692 (27.24)	1.52 (1.04–2.23)	0.03		
Having casual sexual partners in the last 3 months	2,540 (100.00)				
No	1,186 (46.69)	1			
Yes	1,354 (53.31)	1.22 (0.95–1.58)	0.12		
STI test history	2,521 (100.00)				
Yes	235 (9.32)	1			
No	2,286 (90.68)	1.23 (0.77–1.97)	0.38		
Cluster model	2,536 (100.00)				
Asymptomatic	1,271 (50.12)	1		1	
Cluster 1	85 (3.35)	1.37 (0.61–3.08)	0.44	1.29 (0.54–3.06)	0.57
Cluster 2	597 (23.54)	2.57 (1.86–3.56)	<0.01	2.54 (1.83–3.53)	<0.01
Cluster 3	90 (3.55)	1.29 (0.58–2.88)	0.53	1.26 (0.56–2.82)	0.58
Cluster 4	493 (19.44)	3.56 (2.58–4.91)	<0.01	3.31 (2.37–4.61)	<0.01

and cluster 4 as an indicator of CT can find 66.05% (179/271) CT positive cases, but the number of tests accounted for only 42.98% (1,090/2,536) of total male participants. Also, there was no difference between symptomatic and asymptomatic MSM.

Women

Cluster analysis in women

Among female attendees, five clusters with distinct symptomatic patterns and a group of asymptomatic patients were identified, and 0.79% (45/5,671) and 8.31% (471/5,671) of the participants were diagnosed with gonorrhea and chlamydia, respectively. The frequency of gonorrhea and chlamydia in each cluster is shown in Table 4. The asymptomatic group accounted for 36.47% (2,068/5,671) of the total cohort, and 0.34 and 7.64% of the participants were diagnosed with gonorrhea and chlamydia, respectively. Cluster 1 consisted of 5.40% (306/5,671) of the entire cohort, including (1) urgency/urodynia/urethral swelling, redness or pus overflow, and (2) lower abdominal pain, and 0.33 and 7.84% of the participants were diagnosed with gonorrhea and chlamydia, respectively. Cluster 2 consisted of 1.64% (93/5,671) of the entire cohort, including (1) cervical congestion/mucus or purulent secretions, and 10.75% of the participants were diagnosed with chlamydia. Cluster 3 consisted of 41.10% (2,331/5,671) of the entire cohort, including (1) vaginal secretion increase or odors and (2) vaginal itching, and 1.33 and 8.67% of the participants were diagnosed with gonorrhea and chlamydia, respectively. Cluster 4 consisted of 1.68% (95/5,671) of the entire cohort, including (1) genital/perianal watery blisters or pustules, (2) genital/perianal ulcers, and (3) genital/perianal warts, and 1.05 and 8.42% of the participants were diagnosed with gonorrhea and chlamydia, respectively. Cluster 5 consisted of 13.72% (778/5,671) of the entire cohort, including (1) abnormal leucorrhoea and (2) inguinal lymph node enlargement, and 0.64 and 8.87% of the participants were diagnosed with gonorrhea and chlamydia, respectively.

Factors associated with NG/CT infection in women

In univariate analyses, four variables were associated with NG/CT infection in women at $P < 0.05$ (shown in Tables 5, 6). Results from the multivariate logistic regression model suggested that the following factors were significantly associated

10.3389/fpubh.2022.1005481

TABLE 4 Symptom clusters of the respondents in the women cohort ($N = 5,671$).

Symptoms present	Asymptomatic $n = 2,068$	Cluster 1 $n = 306$	Cluster 2 $n = 93$	Cluster 3 $n = 2331$	Cluster 4 $n = 95$	Cluster 5 $n = 778$
Vaginal secretion increase or odors				2,258		
Urgency/ urodynia/urethral swelling and redness or pus overflow		136				
Abnormal leucorrhoea						776
Cervical congestion/ mucus or purulent secretions			93			
Lower abdominal pain		188				
Genital / Perianal watery blisters or pustules					27	
Genital / Perianal ulcers					22	
Genital / Perianal warts					69	
Vaginal itching				80		
Inguinal lymph node enlargement						3
NG positive	7	1	0	31	1	5
CT positive	158	24	10	202	8	69

TABLE 5 Univariate and multivariate logistic regression analyses of factors associated with NG in women.

Variables	N (%)	UnivariateOR (95% CI)	*P*	MultivariateAOR (95% CI)	*P*
Age group	5,703 (100.00)				
>24	838 (14.69)	1		1	
≤24	4,865 (85.31)	7.09 (3.95–12.73)	<0.01	6.27 (3.46–11.34)	<0.01
Marital status	5,692 (100.00)				
Married	4,524 (79.48)	1			
Unmarried	1,168 (20.52)	3.93 (2.20–7.03)	<0.01		
Education	5,668 (100.00)				
Bachelor's degree or above	905 (15.97)	1			
Junior college	1,202 (21.21)	1.89 (0.59–6.04)	0.28		
High school/secondary technical school	1,623 (28.63)	2.53 (0.85–7.49)	0.09		
Junior high school or below	1,938 (34.19)	1.64 (0.54–4.99)	0.38		
Having casual sexual partners inthe last 3 months	5,651 (100.00)				
No	4,036 (71.42)	1			
Yes	1,615 (28.58)	1.47 (0.81–2.68)	0.21		
STI test history	5,548 (100.00)				
Yes	435 (7.84)	1			
No	5,113 (92.16)	1.88 (0.45–7.78)	0.38		
Cluster model	5,671 (100.00)				
Asymptomatic	2,068 (36.47)	1		1	
Cluster 1	306 (5.40)	0.97 (0.12–7.87)	0.97	1.04 (1.13–8.52)	0.97
Cluster 2	93 (1.64)	<0.01 (<0.01–>999.99)	0.99	<0.01 (<0.01–>999.99)	0.98
Cluster 3	2,331 (41.10)	3.97 (1.74–9.03)	<0.01	3.68 (1.61–8.39)	<0.01
Cluster 4	95 (1.68)	3.13 (0.38–25.72)	0.29	2.40 (0.29–19.97)	0.42
Cluster 5	778 (13.72)	1.90 (0.60–6.02)	0.27	1.90 (0.60–6.03)	0.28

10.3389/fpubh.2022.1005481

TABLE 6 Univariate and multivariate logistic regression analyses of factors associated with CT in women.

Variables	*n* (%)	Univariate OR (95% CI)	P	Multivariate AOR (95% CI)	P
Age group	5,703 (100.00)				
>24	838 (14.69)	1		1	
≤24	4,865 (85.31)	2.44 (1.97–3.04)	<0.01	1.53 (1.16–2.01)	<0.01
Marital status	5,692 (100.00)				
Married	4,524 (79.48)	1		1	
Unmarried	1,168 (20.52)	2.51 (2.06–3.06)	<0.01	2.08 (1.62–2.68)	<0.01
Education	5,668 (100.00)				
Bachelor's degree or above	905 (15.97)	1		1	
Junior college	1,202 (21.21)	1.40 (0.99–2.00)	0.06	1.32 (0.92–1.89)	0.14
High school/secondary technical school	1,623 (28.63)	1.57 (1.13–2.19)	<0.01	1.52 (1.08–2.14)	<0.01
Junior high school or below	1,938 (34.19)	1.76 (1.28–2.42)	<0.01	1.82 (1.31–2.54)	<0.01
Having casual sexual partners in the last 3 months	5,651 (100.00)				
No	4,036 (71.42)	1			
Yes	1,615 (28.58)	1.11 (0.90–1.36)	0.34		
STI test history	5,548 (100.00)				
Yes	435 (7.84)	1		1	
No	5,113 (92.16)	1.69 (1.10–2.60)	0.02	1.55 (1.00–2.39)	0.05
Cluster model	5,673 (100.00)				
Asymptomatic	2,068 (36.47)	1			
Cluster 1	306 (5.40)	1.03 (0.66–1.61)	0.90		
Cluster 2	93 (1.64)	1.46 (0.74–2.86)	0.27		
Cluster 3	2,331 (41.10)	1.15 (0.92–1.43)	0.22		
Cluster 4	95 (1.68)	1.11 (0.53–2.34)	0.78		
Cluster 5	778 (13.72)	1.18 (0.88–1.58)	0.28		

with NG infection: aged 24 years or below (AOR = 6.27, 95% CI = 3.46–11.34) and cluster 3 (AOR = 3.68, 95% CI = 1.61–8.39). Using cluster 3 as an indicator of NG can find 63.27% (31/49) NG positive cases, but the number of tests accounted for only 41.10% (2,331/5,671) of total female participants. Results from the multivariate logistic regression model suggested that the following factors were significantly associated with CT infection: aged 24 years or below (AOR = 1.53, 95% CI = 1.16–2.01), unmarried (AOR = 2.08, 95% CI = 1.62–2.68), high school/secondary technical school (AOR = 1.52, 95% CI = 1.08–2.14), and junior high school or below (AOR = 1.82, 95% CI = 1.31–2.54).

Discussion

This is the first study in China to explore the use of different symptom clusters as an indicator to identify NG and CT infection in STI-related clinics, providing new insight into symptomatic management. Symptomatic management and screening for both NG and CT based on symptom clusters were recommended for men presenting symptom clusters 2 and 4, including urethral syndrome (e.g., serous or purulent discharge and frequent urination/urgency/urodynia/itching), genital/perianal warts, balanitis, and inguinal lymph node enlargement, which was consistent with findings from previous studies and the WHO guideline (16–19). Using these two clusters as an indicator of testing can identify 88.59% of NG-positive cases and 66.05% of CT-positive cases, respectively. For women, we found that those with increased vaginal discharge, odor, and itching (cluster 3) had a higher risk of NG infection and were recommended to be tested for gonorrhea infection. Using this cluster as an indicator of testing can identify 63.27% of NG-positive cases. Similar results about vaginal discharge syndrome (e.g., vaginal discharge increases or odor and itching) were reported in a previous study (20). However, we suggested that symptomatic management was not suitable for both NG and CT infection in women in the Chinese context, which was inconsistent with the WHO guideline (16), but it still provided thoughts about NG/CT case finding in women, such as promoting opportunistic screening based on symptoms, age, and risk behaviors. We also identified several non-symptom risk factors, and the inclusion of risk assessment in symptomatic management was beneficial in reducing over-treatment rates and increasing correct treatment rates and was cost-effective (21).

According to this study, if laboratory testing is available, men with urethral discharge syndromes, genital/perianal warts, balanitis, and inguinal lymph node enlargement are recommended to take the NAAT test for NG/CT infection. If laboratory tests are not available, symptomatic management could be used for men with the above symptoms for NG and CT to ensure same-day treatment. Risk factors of NG (e.g., unmarried and having casual sexual partners in the last 3 months) and CT (e.g., unmarried) in men found in this study could also be considered in symptomatic management or screening with higher accuracy. Clusters including abnormal discharge and frequent urination/urgency/urodynia/itching were found to be an indicator of NG/CT infection, and these clusters belong to urethral discharge syndromes, which was consistent with the WHO guideline and a previous study (16, 19). Similarly, the guidelines revealed that the odds of NG or CT infection among men with urethral discharge is 10 times the odds among men with no urethral discharge (16). Other studies also reported that urethral discharge syndromes can be a diagnostic symptom for the NG and CT in men, which was suggested as a cost-effective strategy (5, 22). Similarly, the performance of urethral discharge syndromes for NG and CT infections has been adequate in other studies as well (23, 24). The guidelines also recommended that if the urethral discharge is present but tests are negative, treatment for non-gonococcal and non-chlamydial urethritis (such as *Mycoplasma genitalium* or *Trichomonas vaginalis*) should be considered (16). Also, similar findings about other important but relatively unusual symptoms were shown in previous studies. A study showed that among those with a confirmed NG or CT visit, balanitis was observed in 24% of cases and genital warts in 12% of cases (25). Lymphogranuloma venereum (LGV) is caused by CT, and 22.5% of infections had symptoms of inguinal lymph node enlargement (26). Therefore, it would be useful to link these symptoms with NG and CT when offering symptomatic management. NAAT is relatively expensive and not available everywhere, especially in some resource-limited places or rural areas, so primary care settings with many patients with urethral discharge syndromes may face challenges. Antigen detection and point-of-care-test (POCT) are cheap and convenient. For NG and CT, the sensitivity and specificity of the POCT assay were more than 90%, and it can facilitate testing and reduce missed patients, which is recommended in resource-limited places (27).

We suggest that symptomatic management based on vaginal discharge syndrome is not highly effective in detecting NG in women. Meanwhile, NG is highly resistant and should not be treated for symptoms alone (28), and NAAT or other accurate tests are needed to confirm the diagnosis. Therefore, vaginal discharge syndrome along with risk factors for NG (e.g., aged 24 years or below) could be considered an indicator of NG screening. The results of this study showed that diagnosis based on vaginal discharge syndrome (vaginal secretion increase or odors and vaginal itching) could detect 63.27% of NG cases in women, but with low sensitivity. Studies have shown that cervicovaginal cytokine concentrations did not differ between women with asymptomatic STIs and those with symptomatic STIs (29). Symptomatic treatment based on vaginal discharge is more accurate in the population with a high prevalence of NG and CT (female sexual workers, etc.), with a positive predictive value of more than 27% but less accurate in the general population (30, 31). Most vaginal discharge abnormalities are due to vaginal infections caused by *Trichomonas vaginalis* and bacterial vaginosis rather than cervical infections caused by NG and CT (32). In Morocco, a study showed that the prevalence of NG was 0.37% and CT was 3.8% among those with vaginal discharge syndrome (20). Therefore, we do not recommend treatment of NG in the general population of women based on symptoms alone.

Symptomatic management is not suitable for CT infection in women in the Chinese context as none of the symptoms were associated with CT infection, which was inconsistent with the WHO guideline (16). The results of this study showed that women infected with CT have no specific symptoms. One possible reason for the above situation was that more than 85% of CT infections in women are asymptomatic, and asymptomatic infections can last for months (33). Risk factors for CT (e.g., aged 24 years or below, unmarried, high school/secondary technical school, and junior high school or below) in women found in this study could be considered in CT screening with higher accuracy. For health authorities, it may be better to focus on the improvement of the laboratory capability in China rather than promote symptomatic management based on STI symptoms. During the COVID-19 pandemic in China, any patients with a fever seeking healthcare in secondary and tertiary hospitals needed to be screened by polymerase chain reaction (PCR) testing (34), which improved the capability of laboratories in these hospitals, so how to integrate NAAT testing for NG and CT infection into these hospitals should also be considered.

The prevalence of NG and CT was 5.23 and 13.21%, respectively. A surprising finding in this study was that 9.79% (19/194) of NG and 31.81% (236/742) of CT infections were asymptomatic, which would have been missed if using symptomatic management. Although the proportion of asymptomatic patients in this study was lower than in other studies due to the recruitment of clinical patients, all patients should be screened if accurate testing for NG/CT is available. Asymptomatic infection is a burden in the control of sexually transmitted diseases because it can serve as a bridge population in the transmission of NG/CT. Similar to symptomatic infection, asymptomatic infection also leads to adverse outcomes and affects people's reproductive health, which calls for an urgent need for interventions for this population. The result of this study indicated that having casual sexual partners in the last 3 months, aged 24 years or below, and having lower education were sociodemographic and behavioral factors of NG/CT infections, which could be considered in the expansion of routine screening in the general population. Promoting

health literacy and sexually transmitted infection education is important because most people lacked an understanding of NG and CT infections, and having a correct understanding of the outcome of CT infection was associated with high screening willingness (35).

There were some limitations to the study. First, the follow-up data of positive cases was not collected, which should be considered in future studies. Second, the sample size of MSM in this study was not large enough for analyses, and more research is needed to further explore the differences between symptomatic and asymptomatic MSM. Third, information related to sexual behaviors was self-reported, which may lead to social desirability bias.

Conclusion

This study found that NG and CT symptomatic management was recommended for men but not for women. Sociodemographic and behavioral factors of NG/CT infections, including having had casual sexual partners in the last 3 months, being aged 24 years or below, and having lower education, could be integrated into symptomatic management and considered in the expansion of routine screening.

Data availability statement

The datasets presented in this article are not readily available because of patient privacy. Requests to access the datasets should be directed to the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by Ethical Review Committee of the Shenzhen Center for Chronic Disease Control (Approval No. 20180206). The patients/participants provided their written informed consent to participate in this study.

Author contributions

NN and YC conceived and designed the study. NN, RW, CZ, LW, JY, HW, and JL supervised the data collection. YC, XC, RW, CZ, LW, JY, HW, JL, and NN performed the research. NN, RW, and YC analyzed and interpreted the results and were the major contributors in writing the manuscript. XC, CZ, LW, JY, HW, and JL revised the manuscript critically. All authors read and approved the final manuscript.

Funding

This study was supported by Sanming project of Medicine in Shenzhen [Grant No. SZSM201611077].

Acknowledgments

We are indebted to all participants in this study for their cooperation. We thank the staff at all participating Center for chronic diseases control and Center for chronic diseases control.

Conflict of interest

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

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 23 November 2022
DOI 10.3389/fpubh.2022.1005334

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 28 July 2022
ACCEPTED 08 November 2022
PUBLISHED 23 November 2022

CITATION
Wang H, Weng R, Zhang C, Ye J, Wen L, Li J, Lu Y, Ning N, Hong F, Chen X and Cai Y (2022) High chlamydia infection and its associated factors among patients seeking clinic-based STI services in Southern China: A preliminary cross-sectional study. *Front. Public Health* 10:1005334. doi: 10.3389/fpubh.2022.1005334

High chlamydia infection and its associated factors among patients seeking clinic-based STI services in Southern China: A preliminary cross-sectional study

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Objective: *Chlamydia trachomatis* (CT) infection is one of the most common sexually transmitted infections (STIs) worldwide. This study aimed to provide prevalence and associated factors data among patients seeking clinic-based STI services for estimating the disease burden of CT.

Study design and method: A cross-sectional survey was conducted among patients attending clinics for STI services. Patients' social-demographic and behavioral information was collected and CT infection was determined by nucleic acid amplification test (NAAT) with self-collected urine specimens. Associated factors were identified using logistic regression.

Results: Among the 8,324 participants, the overall prevalence was 9.0% with 10.7% for males and 8.3% for females respectively. Multivariate analysis showed that aged < 24 [adjusted odds ratio (aOR) = 1.27, 95% confidence interval (CI) = 1.01–1.59], being unmarried (aOR = 1.64, 95%CI = 1.35–2.00), having junior high school or below education level (aOR = 1.47, 95%CI = 1.13–1.91), having no access to health insurance (aOR = 1.27, 95%CI = 1.07–1.51), and being positive for *Neisseria gonorrhoeae* (NG, aOR = 4.49, 95%CI = 3.25–6.21) were significantly associated with CT infection.

Conclusion: We found that CT infection is prevalent among patients seeking clinic-based STI services in Southern China. Targeted interventions could be implemented for patients with a higher risk of CT infection including those aged < 24, being unmarried, having junior high school or below education level, having no access to health insurance, and being positive for NG. In addition, routine CT screening could be considered a public health strategy by the government.

KEYWORDS

chlamydia infection (CT), STI services, patients, Southern China, associated factors

Introductions

Chlamydia caused by *Chlamydia trachomatis* (CT) is the most common sexually transmitted infection (STI) (1) which can cause significant morbidity, particularly in women. Due to the reason that up to 85% of cases in women and men are asymptomatic (2), CT infection often remains undiagnosed and untreated. Untreated CT infection can lead to pelvic inflammatory disease (PID), chronic pelvic pain, ectopic pregnancy, and tubal factor infertility in women (3), urethritis, epididymitis, and other complications including infertility in men (4). CT infection has become a main global public health concern. Based on the estimation of the World Health Organization (WHO) in 2016, there were an estimated 127.2 million new cases of CT infection per year (5). Data from surveillance programmes in the United States (6), the United Kingdom (UK) (7), and Canada (8) indicated an increasing trend of CT infection in recent years. Based on the national case-reporting system in China, the reported incidence of CT infection has increased from 35.8/100,000 in 2011 to 37.1/100,000 in 2015 (9). However, the burden of CT infection in many parts of China is unknown because of the significant under-reporting of the infections, particularly asymptomatic infections, in health facilities. To provide baseline data for estimating the disease burden, developing intervention programmes, and planning for resource allocations, we carried out a preliminary cross-sectional survey to estimate the prevalence of the infection and explored the factors associated with the infection among patients attending clinics for STI services in Southern China.

Materials

Study setting and population

The survey was a cross-sectional study in Shenzhen, a "special economic zone" located in south coastal China and adjacent to Hong Kong. The city has witnessed an alarming increase in its economy, migration of population, and the spread of syphilis and other STIs (10). The survey was conducted using a stratified sampling strategy to recruit potential participants. There are 10 administrative districts in Shenzhen, and 6 of them were randomly selected to do the survey. Four hospitals that reported a high number of STI cases in the previous year were selected in each district and except for one district with only two hospitals. During the period of the survey (from April 15 to May 16, 2018), the first 15 patients attending clinics at departments of dermatology, gynecology, urology, and andrology for seeking STI services were invited to participate in the study according to the eligibility criteria. The criteria included being a patient aged 18–49 years old, seeking STI-related services, and having not any antibiotic use in the last 2 weeks. Written informed consent should be obtained before the survey can be conducted.

Questionnaire interview and specimen collection

An interview with a structured questionnaire was conducted by the physician to collect demographic and behavioral data as well as clinical findings. After completing the interview, participants were asked to provide a self-administered 15–30 mL first-catch urine specimen. A research nurse was assigned to check the integrity of questionnaire information and instruct participants on specimen collection. Urine specimens were collected using the Cobas1 urine specimen collection kit (Roche P/N 05170486190) according to the manufacturer's instructions. The specimens were temporarily stored at 4°C at the local laboratory for 10 days maximum before being transported to a central laboratory for testing.

Laboratory assays

At the central laboratory, DNA was extracted and purified from the urine specimens by an automated magnetism nucleic acid isolation method using the MagNA Pure 96 System (Roche, Switzerland) according to the manufacturer's instructions. The extracted DNA was further evaluated for CT and *Neisseria gonorrhoeae* (NG) based on polymerase chain reaction (PCR) of the Cobas® 4800 System (Roche, Switzerland) using Cobas® 4800 CT/NG Amplification/Detection Kit. Diagnosis reagents and supplies were preserved under the requested condition. Laboratory performance was run according to standard operating procedures (SOPs). CT or NG infection was defined as having a positive PCR for CT or NG.

Statistical analyses

All data from questionnaires and laboratory tests were double-entered into the computer to establish a database using Epidata software (V.3.1, Denmark). The Epidata 3.1 dataset was subsequently transferred to the IBM SPSS Statistics for Windows Version 23.0 (IBM Corp., Armonk, NY) for statistical analyses. Univariate analysis was used to determine the association between variables and CT infection. Crude odds ratio (cOR), adjusted odds ratio (aOR), and 95% confidence interval (CI) were calculated. To adjust for potential confounders, all factors associated with the infection at $P < 0.2$ in univariate analysis were included in multivariable logistic regression analysis using a backward stepwise procedure. Variables significant at $P < 0.05$ were considered the factors independently associated with the infections.

Patient and public involvement statement

Participation in this study was voluntary and the questionnaire was anonymous. Confidentiality of the study data can protect the privacies of the participants. Participants who tested positive for CT and/or NG were contacted privately by the research team members for further diagnosis, treatment, and other interventions at the STI clinic in Shenzhen Center for Chronic Disease Control. Partner notification was conducted according to the routine process in the clinic.

Results

Participant characteristics

Out of the 8,444 patients who provided urine specimens, 120 did not participate in a questionnaire interview. Therefore, a total of 8,324 (98.6%) participants were included in the final analyses. The average age was 32.1 years old (standard deviation [SD] 7.3 years), and 14.3% (1183/8281) of them were younger than 24 years old. About one-third of the participants were males (30.9%, 2567/8309). Most of the participants were married (75.1%, 6207/8269), heterosexuals (98.2%, 8039/8189), migrants (73.7%, 6022/8173), and living in Shenzhen for more than 2 years (77.3%, 6331/8189). 38.8% (3232/8324) of the participants had a monthly income above 5,000 CNY (China Yuan), most of whom were workers (25.4%, 2099/8264) and government employees (25.6%, 2119/8264). Less than one-fifth (17.7%, 1457/8223) finished their education in college or above. 37.6% (3096/8229) had no hold of health insurance and 36.2% (2973/8203) had casual sex in the last 3 months. 21.7% (1787/8231) knew about CT. 2.4% (196/8324) had positive *Neisseria gonorrhoeae* detection. More details are shown in Table 1.

Prevalence and associated factors of CT infection

Among the 8,324 participants, 751 people were identified as CT positive, giving an overall prevalence of 9.0% with 10.7% for males and 8.3% for females respectively. The highest prevalence was detected among people aged younger than 24 (15.4%). CT infection of participants (positive and negative) was regarded as the dependent variable and the other factors were used as the independent variables in the logistic regression model. In the univariate analyses, government employees (cOR = 0.75, 95%CI = 0.57–0.97) were significantly associated with a decreased risk of CT infection ($P < 0.05$). Meanwhile, male (cOR = 1.33, 95%CI = 1.14–1.56), aged < 24 (cOR = 2.10, 95%CI = 1.76–2.52), being unmarried (cOR = 2.10, 95%CI = 1.80—-.46), migrants (cOR = 1.40, 95%CI = 1.16–1.68), residing in Shenzhen for < 2 years (cOR = 1.44, 95%CI = 1.22–1.71), entertainment service providers (cOR = 1.73, 95%CI = 1.03–2.91), having junior college or below education level, having no access to health insurance (cOR = 1.59, 95%CI = 1.37–1.85), having casual sex in the last 3 months (cOR = 1.21, 95%CI = 1.04–1.41), having no knowledge about CT (cOR = 1.23, 95%CI = 1.01–1.49) and positive for NG (cOR = 5.52, 95%CI = 4.06–7.49) were significantly associated with an increased risk of CT infection ($P < 0.05$). Sexual orientation and monthly income were not significantly associated with CT infection ($P > 0.05$).

In the univariate analyses, 11 variables were associated with CT infection at $P < 0.20$ (Table 2). In the multivariate analyses using these 11 variables as independent variables and potential interactions between these variables, the following factors were found to be significantly associated with CT infection: aged < 24 (aOR = 1.27, 95%CI = 1.01–1.59), being unmarried (aOR = 1.64, 95%CI = 1.35–2.00), having junior high school or below education level (aOR = 1.47, 95%CI = 1.13–1.91), having no access to health insurance (aOR = 1.27, 95%CI = 1.07–1.51), and being positive for NG (aOR = 4.49, 95%CI = 3.25–6.21). More details are shown in Table 2.

Discussions

The findings from our study indicated a high prevalence of CT infection among both male and female patients attending clinics in departments of dermatology, gynecology, urology, and andrology in Shenzhen and highlighted the risk factors associated with the infection in this population. STI surveillance plays an important role in measuring the magnitude of the STI burden in the general and target populations to assist in programme planning, monitoring trends over time and identifying emerging infections and outbreaks, providing data to advocate for mobilization of resources, and assisting in evaluating the effectiveness of the response (11). Prevalence assessment is one of the core components of WHO–recommended STI surveillance programming.

Our study was a cross–sectional study focusing on genital CT infection with the largest sample size of patients seeking clinic–based STI services in China. Among 8,324 patients, the total prevalence of CT infections was 9.0% with 10.7% for males and 8.3% for females respectively, which was higher than the general population of 2.1% for males and 2.6% for females in China (1999–2000) (12). This CT prevalence was also higher than that reported in many high–income countries (13), such as 1.7% in the US (14), 1.5% in the UK (15), and 1.7% in France (16) among the general

TABLE 1 Socio–demographic, and behavioral data of survey participants at baseline ($n = 8324$).

Characteristic	Frequency* (%)	Characteristic	Frequency* (%)
Sex		**Occupation**	
Male	2567 (30.9)	Workers	2099 (25.4)
Female	5742 (69.1)	Entertainment services	129 (1.6)
Age in years		Businessman/Catering services	1606 (19.4)
≤ 24	1183 (14.3)	Government employees	2119 (25.6)
>24	7098 (85.7)	Household/Unemployed	1267 (15.3)
Marital status		Others	1044 (12.6)
Single and others	2062 (24.9)	**Education level**	
Married	6207 (75.1)	Junior high school or below	2636 (32.1)
Sexual orientation		Senior high school	2407 (29.3)
Heterosexuality	8039 (98.2)	Junior college	1723 (21.0)
Homo–or bi–sexuality	150 (1.8)	College or above	1457 (17.7)
Residence		**Hold of health insurance**	
Shenzhen	2151 (26.3)	Yes	5133 (62.4)
Other places	6022 (73.7)	No	3096 (37.6)
Duration in Shenzhen		**Casual sex in the last 3 months**	
<2 years	1858 (22.7)	Yes	2973 (36.2)
≥2 years	6331 (77.3)	No	5230 (63.8)
Monthly income (CNY)		**Knowledge about CT**	
<3000	1672 (20.1)	Unknown	6444 (78.3)
3001–5000	2775 (33.3)	Known	1787 (21.7)
5001–10000	2666 (32.0)	***Neisseria gonorrhoeae* detection**	
>10000	566 (6.8)	Negative	8128(97.6)
Not quite clear	645 (7.7)	Positive	196(2.4)

*The sum of participants in some items may be less than the total number of 8324 because some participants did not respond to these items. CNY, China Yuan, the minimum wage in Shenzhen, China in 2018 was 2200 CNY. CT, *Chlamydia trachomatis*; %, Constituent ratio.

population used by Urine NAAT. Compare with high–risk populations, such as 8.5% among cross–border truck drivers in Hong Kong (17), and 6.5% among MSM in Jiangsu Province (18), this CT prevalence remained at a high level. The relatively high prevalence of CT infection further emphasized the importance of urgently implementing comprehensive interventions among patients seeking clinic–based STI services.

The findings in our study showed the highest prevalence (15.4%) was detected among patients seeking clinic–based STI services aged younger than 24. After controlling for confounding factors, aged younger than 24 was still significantly associated with an increased risk of CT infection. This finding was similar to the findings of the US CDC, which was reported in 2013. The highest incidence of CT in the US was among people aged between 14 and 24 years (19). This result may indicate that patients of this age group had more exposure to new infections due to greater sexual activity and lower education on STI prevention. Opportunistic screening for CT among young sexually active adults had been recommended in many high–income countries including the USA, the UK, Australia, Sweden, Denmark, and Norway (20–22). The UK had run a nationwide program called the National Chlamydia Screening Programme (NCSP) (23), which targeted all sexually active men and women under 25 years of age for annual chlamydia screening through various clinical and non–clinical settings. It would be a feasible measure to develop a CT screening strategy for young people in China, which could be started in economically developed regions such as Shenzhen city.

In addition, participants that were single/divorced/widowed were almost 50% more likely to have a CT infection than those who were married. This was consistent with the results of Wong WC et al. (24) and Walsh MS et al. (25) studies. It could be seen that maintaining loyal marital status was an effective protective factor for CT infection after excluding age, not having casual sex in the last 3 months, and other confounding factors. Moreover, our results showed that a positive for NG was significantly associated with an increased

TABLE 2 Prevalence and associated factors of chlamydial infection among patients seeking clinic–based STI services in Shenzhen, China.

Associated factors	Prevalence of CT (%)	Univariate		Multivariate	
		cOR (95% CI)	*P*-Value	aOR (95% CI)	*P*-Value
Sex					
Male	10.7	1.33(1.14–1.56)	0.000*	–	
Female	8.3	1.00		1.00	
Age in years					
≤ 24	15.4	2.10(1.76–2.52)	0.000*	1.27(1.01–1.59)	0.042*
>24	8.0	1.00		1.00	
Marital status					
Single or others	14.3	2.10(1.80–2.46)	0.000*	1.64(1.35–2.00)	0.000*
Married	7.3	1.00		1.00	
Residence					
Shenzhen	7.2	1.00		1.00	
Other places	9.8	1.40 (1.16–1.68)	0.000*	–	
Duration in Shenzhen					
<2 years	11.2	1.44(1.22–1.71)	0.000*		
≥2 years	8.3	1.00		1.00	
Occupation					
Workers	9.9	1.03(0.80–1.33)	0.801	–	
Entertainment services	15.5	1.73(1.03–2.91)	0.038*	–	
Businessman/Catering services	10.3	1.08(0.83–1.40)	0.560	–	
Government employees	7.3	0.75(0.57–0.97)	0.028*	–	
Household/Unemployed	7.8	0.80(0.60–1.07)	0.133	–	
Others	9.6	1.00		1.00	
Education level					
Junior high school or below	10.1	1.58(1.24–2.01)	0.000*	1.47(1.13–1.91)	0.004*
Senior high school / technical secondary school	9.5	1.47(1.15–1.88)	0.002*	1.26(0.97–1.64)	0.089
Junior college	8.7	1.34(1.03–1.74)	0.032*	1.25(0.95–1.66)	0.111
College or above	6.7	1.00		1.00	
Hold of health insurance					
Yes	7.5	1.00		1.00	
No	11.5	1.59(1.37–1.85)	0.000*	1.27(1.07–1.51)	0.007*
Casual sex in the last 3 months					
Yes	10.1	1.21(1.04–1.41)	0.015*	–	
No	8.5	1.00		1.00	
Knowledge about CT					
Unknown	9.4	1.23(1.01–1.49)	0.036*	1.23(1.00–1.51)	0.054
Known	7.8	1.00		1.00	
***Neisseria gonorrhoeae* detection**					
Negative	8.4	1.00		1.00	
Positive	33.7	5.52(4.06–7.49)	0.000*	4.49(3.25–6.21)	0.000*

*P < 0.05. cOR, crude odds ratio; aOR, adjusted odds ratio; CI, confidence interval; CT, *Chlamydia trachomatis*.

risk of CT infection. This was consistent with the findings of Guangdong Province (26). Therefore, it would be better to suggest patients have a CT infection test when they need to have an NG detection.

Regarding the educational level, we found that participants with lower educational levels were at higher risk of incident CT infection, and this association remained after adjusting for age and occupation. Similar results were obtained in other studies (27–29). In addition, we found a higher prevalence in patients who had no hold of health insurance. Poor healthcare–seeking behavior associated with higher infection rates, lower partner referral, or inadequate care had been reported for people with lower socioeconomic status in many countries (30–32). A German study showed that the higher prevalence in groups with low or medium social status could have a lower chance of testing CT and lower healthcare use because a quarterly fee had to be paid by persons, which will pose a possible barrier for people with lower income (33–35). It might be useful to reduce the CT infection of this population by strengthening the publicity and education of CT among the low–education population and reducing the cost of CT screening and treatment.

However, our study had some limitations. First, the study did not investigate the number of sexual partners and condom use of STD patients in the last month. As a result, the CT infection situation cannot be analyzed in depth of the patient's sexual behaviors. Second, due to the lower number of CT–reported cases in private hospitals, it cannot be compared with public hospitals and other private hospitals, which have a higher number of CT–reported cases. Third, Shenzhen has a relatively open–minded sexual attitude, and it is also an economically developed area with a relatively higher young population. Thus, the result of the study cannot represent the health situation of chlamydial infection in the whole country, and cannot be compared with other cities with a lower socioeconomic status.

In conclusion, CT infection is prevalent among patients seeking clinic–based STI services, particularly those aged younger than 24 in Shenzhen, China. Furthermore, patients that were single/divorced/widowed, positive for NG, with low educational level, and having no hold of health insurance were significantly associated with an increased risk of CT infection. It is strongly recommended that clinicians perform key screening and interventions for CT in these populations. At the same time, these findings suggest that CT–integrated prevention and control projects could be considered in routine public health services by the government. However, the high prevalence of CT is not enough for the screening strategy. Efforts are needed in future studies on several areas such as the cost–effectiveness of screening strategies, the burden of disease estimates, the capacity of CT screening in hospitals, the willingness of being screened of patients, and the knowledge of CT in patients.

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

HW, YC, and XC conceived and designed the study. FH, CZ, LW, JY, and RW supervised the data and samples collection. HW, YC, XC, CZ, LW, JY, JL, YL, and NN performed the research. HW, RW, YL, YC, and XC analyzed, interpreted the results, and were the major contributors in writing the manuscript. All authors read and approved the final manuscript.

Funding

This work was supported by the Sanming project of Medicine in Shenzhen (grant number SZSM201611077).

Acknowledgments

We appreciated all participants in this study for their cooperation and thank you for the staff /physicians at all participated hospitals, Center for Chronic Diseases Control, and Center for Disease Control and Prevention.

Conflict of interest

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

The reviewer GF declared a shared affiliation with one of the author XC to the handling editor at time of review.

Publisher's note

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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 09 December 2022
DOI 10.3389/fpubh.2022.1038391

OPEN ACCESS

EDITED BY
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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 07 September 2022
ACCEPTED 23 November 2022
PUBLISHED 09 December 2022

CITATION
Sun S, Zhang L, Wu Q, Tian L, Ding Y, Liu L, Ye H, Li B and Luo Z (2022) The association between adverse pregnancy outcomes with genital *Chlamydia Trachomatis* infection among pre-pregnancy couples in Shenzhen, China: A cross-sectional study.
Front. Public Health 10:1038391.
doi: 10.3389/fpubh.2022.1038391

The association between adverse pregnancy outcomes with genital *Chlamydia Trachomatis* infection among pre-pregnancy couples in Shenzhen, China: A cross-sectional study

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Objectives: To investigate the prevalence of adverse pregnancy outcomes (APOs) in women and the impact of pre-pregnancy couples' genital *Chlamydia Trachomatis* (GCT) infection and other infections on APOs.

Study design: Data on genital infections were collected from the Free Pre-pregnancy Health Check (FPHC) in Shenzhen, China. Data on APOs were collected from a 1-year telephone follow-up of pregnancy status and subsequent pregnancy outcomes.

Methods: APO data were used to count adverse outcomes, and logistic regression was conducted to determine the association between APOs and GCT infection.

Results: From December 2018 to December 2019, among 4,429 couples who underwent FPHC; 1,925 were pregnant, and 1,816 couples were tracked for pregnancy outcomes, including 1,471 normal pregnancies and 345 (19.00%) APOs. The rest of 109 pregnant couples did not answer the phone or refused to answer the pregnancy outcome during the follow-up. Among APOs, the number of spontaneous abortions was 122 (35.36%), the number of macrosomia was 85 (24.64%), the number of low birth weight (LBW) & preterm births (PTB) was 39 (11.30%), the number of LBW was 34 (9.86%), and the number of PTB was 31 (8.99%). The prevalence of GCT infection in females and males was 4.24% [95% Confidence Interval, (CI): 3.41–5.27%] and 3.58% (95% CI: 2.79–4.57%), respectively. More than half (52.69%, 49/93) of the couples were GCT-concordant. The prevalence of APOs in couples without GCT infection was 18.74% (332/1,772). The prevalence of APOs in female GCT-discordant was 32.14% (9/28), and the prevalence of APOs in male GCT-discordant was 25% (4/16). The prevalence of APOs in GCT-concordant was 12.24% (6/49). Multivariable analysis indicated that females 30–35 years old [adjusted Odds Ratio (aOR) = 1.08, 95% CI: 1.01–1.17] and over 35 years old (aOR = 1.16, 95% CI: 1.03–1.32) were more likely to experiencing APOs.

Conclusion: Although only women's age was found to be associated with APOs, the prevalence of APOs with GCT-discordant in couples, especially female GCT-discordant, was higher than in those without infection or who were GCT-concordant, suggesting that these groups, especially in older women, should be paid more attention to in follow-ups to improve reproductive health.

KEYWORDS

Chlamydia Trachomatis, GCT, adverse pregnancy outcome, APOs, concordant

Background

Adverse pregnancy outcomes (APOs) are important public health issues, mainly including spontaneous abortion (SA), stillbirth, ectopic pregnancy, preterm births (PTB), low birth weight (LBW), macrosomia, birth defects, etc., (1). APOs are harmful to the health of pregnant women and fetuses and increase the risk of chronic non-communicable diseases such as adult obesity, hypertension, and diabetes in offspring (2–4). APOs seriously affect the economy and spirit of relevant families.

The occurrence of APOs is related to many factors. Genital *Chlamydia Trachomatis* (GCT) infection is one of the world's most common sexually transmitted infections (5). And studies have shown that GCT infection is related to the occurrence and development of many APOs (1). In 2011, Johnson et al. (6) found that GCT infection significantly increased the risk of LBW in newborns (aOR: 2.07, 95% CI: 1.01–4.24) in the United States (US). A case-control study was done in October 2013 through June 2014 in Iran showed the prevalence of GCT infection in pregnant women with a history of SA was significantly higher than that in pregnant women with normal childbirth (7), and *Chlamydia Trachomatis* (CT) DNA was more common in the pregnancy products and placenta of aborted women (8). In 2018, a meta-analysis showed that there was a slight but statistically significant overall association between GCT infection and PTB (OR = 1.27, 95% CI: 1.05–1.54) (9). In 2020, a systematic review showed that mothers with GCT infection were 1.35 times (OR = 1.35, 95% CI: 1.03–1.76) more likely to develop adverse outcomes than non-infected mothers while reducing GCT infection significantly improved pregnancy outcomes (OR = 0.43; 95% CI: 0.27–0.68) (10).

Screening and treatment of GCT infection can help reduce APOs. For instance, early screening and treatment of GCT infection would significantly reduce the risk of PTB in pregnant women (11). However, since about 70% of women and 50% of men with GCT infection are asymptomatic (12), active screening of high-risk groups is an effective way to prevent and treat GCT infection (13). Currently, countries mainly screen women for GCT infection (12, 14, 15). Still, some studies show that the concordant rate of GCT infection between male and female sexual partners is 10–75%, which means that some women may still be threatened by GCT infection if the infections of these women's partners are not diagnosed and treated timely (16). However, there's no study on the impact of the GCT-concordant status on APOs in China. So, this study was conducted to explore the impact of the GCT infection, the GCT-concordant status of pre-pregnancy couples, and other factors on APOs, through long-term follow-up, and to provide ideas for GCT infection and APOs prevention strategies.

Method

Study participants

Study participants were couples participated in the Free Pre-pregnancy Health Check (FPHC) in Nanshan District, Shenzhen from December 2018 to December 2019. Participants were eligible for participation if they met the following inclusion criteria: (1) willing to test GCT by nucleic acid detection method; (2) willing to participate and signed the informed consent. Before the analysis, the records of all couples were anonymous. This study was an observational study, which was beneficial and harmless to the subjects.

Data collection

The medical staff followed up on the pregnancy status of the couples who participated in the FPHC by telephone 1 year after the FPHC. If they were pregnant within 1 year, we would continue to follow up on their pregnancy outcomes by inquiring about couples. If they were not pregnant within 1 year, the follow-up would be terminated. Follow-up pregnancy outcomes included normal pregnancy, SA, induced abortion in the medical department, therapeutic induced labor, stillbirth, PTB, LBW, macrosomia, and ectopic pregnancy.

The APOs in the current study included (17) (1) SA defined as fetal death occurring before 28 weeks of gestation; (2) PTB (delivery at a gestational age between 28 and <37 weeks); (3) macrosomia (newborn birth weight ≧4,000 g); (4) LBW

(newborn birth weight <2,500 g); (5) stillbirth (intrauterine death of the fetus after 20 weeks of pregnancy); (6) induced abortion in the medical department (pregnancy termination by medical methods due to diseases and other reasons within 14 weeks of pregnancy); (7) therapeutic induced labor (pregnancy termination by medical methods due to diseases and other reasons after more than 14 weeks); and (8) ectopic pregnancy (the embryo attaches outside the uterus).

Female vagina swab samples test

After the medical staff inserted the vaginal dilator to dilate the vagina, the medical staff used a sterile cotton swab to collect the secretion in the posterior vault of the vagina. Medical staff then performed a smear or dye microscopic examination for genital *Candida*, trichomonas, clue cells, pH, whiff test, and vaginal cleanliness. According to Amsel's criteria (18) and other research (19), two of three criteria, including clue cells, pH, and whiff test, been presented to confirm bacterial vaginosis (BV) diagnosis.

Female and male urine samples test

The participants held urine for 2 h, collected 10–20 ml of fresh anterior urine, and then staff transferred 3–5 ml urine to a Roche Cobas urine collection tube (Roche/n05170486190). Samples were stored in a 4°C environment and detection of CT and *Neisseria gonorrhoeae* (NG) by Roche cobas 4800 system occurred within 24 h according to the instruction manual (20). The rest of the urine was tested for proteinuria, occult blood, and white blood cells within 1 h.

Wives with GCT infections whose husband did not have a GCT infection were considered female discordant (female GCT-discordant). Conversely, husbands with GCT infections whose wives did not have a GCT infection were considered male discordant (male GCT-discordant). Wives and husbands with GCT infections were considered concordant (GCT-concordant) (16).

Statistical analysis

Two staff members entered all follow-up information into Epidata 3.0 software (Epidata Association from Denmark), and all test data were from their outpatient records. The test data was exported to Excel software through the outpatient system, and then the follow-up data and test data were matched through the medical registration number. The Chi-square or Fisher test was used to compare the categorical variables between groups, and variables with $P < 0.2$ were incorporated into the univariate and multivariable logistic regressions. In the multivariable model, we adjusted for female age, female proteinuria, male age, and GCT-concordant status. We reported odds ratios (*OR*), 95% confidence intervals (CI) and *P*-values. Results are deemed to be statistically significant when $P \leq 0.05$. All analyses were conducted on R software 3.6.1 (R Development Core Team, Vienna, Austria).

Results

Sociodemographic characteristics

Overall, 4,429 couples participated in the Free Pre-pregnancy Health Check (FPHC), of which 1,925 couples were pregnant, and 1,816 couples were followed up for pregnancy outcomes. The average age of females among the 1,816 couples was 28.13 ± 3.16 years old, and the average age of males was 29.5 ± 3.6.

Prevalence of APOs

During the 1-year follow-up period, 1,925 couples were pregnant, 1,816 were followed up on pregnancy outcomes, and 345 (19.00%) cases of APOs were found (Figure 1). Among the APOs, the majority were SA, macrosomia, and PTB combined with LBW, accounting for 35.36% (prevalence 6.72%), 24.64% (4.68%), and 11.30% (2.15%), respectively (Table 1).

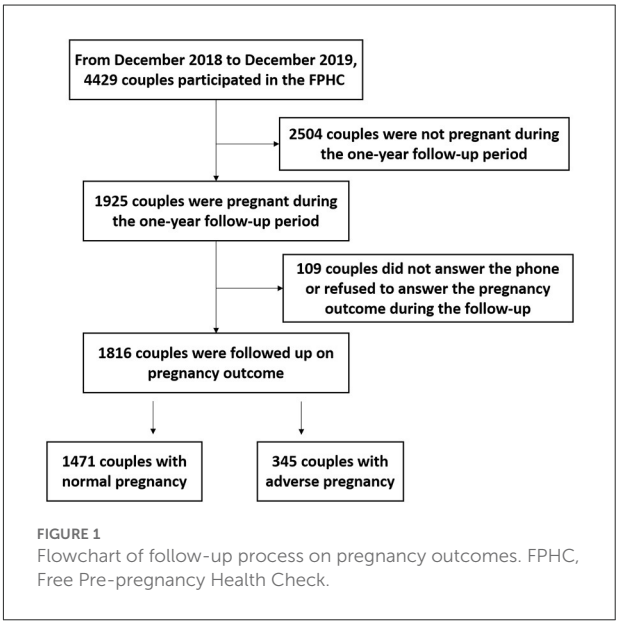

FIGURE 1
Flowchart of follow-up process on pregnancy outcomes. FPHC, Free Pre-pregnancy Health Check.

TABLE 1 APOs and general characteristics of pregnant couples ($n = 1,816$).

Characteristics	Number of cases	Prevalence (%)	Composition ratio (%)
APOs	345	19.00	100
SA	122	6.72	35.36
Macrosomia	85	4.68	24.64
PTB & LBW	39	2.15	11.30
LBW	34	1.87	9.86
PTB	31	1.71	8.99
Stillbirth	10	0.55	2.90
Therapeutic induced labor	9	0.50	2.61
Ectopic pregnancy	8	0.44	2.32
Induced abortion in medicinedepartment	7	0.39	2.03
Female			
Candida	210	11.56	-
BV	53	2.92	-
Vaginal cleanliness III	1,137	62.61	-
Vaginal cleanliness IV	350	19.27	-
Urine occult blood	246	13.55	-
Proteinuria	90	4.96	-
Urine white blood cells	289	15.91	-
GCT	77	4.24	-
Male			
Urine occult blood	139	7.65	-
Proteinuria	114	6.28	-
Urine white blood cells	40	2.20	-
GCT	65	3.58	-
GCT-concordant status			
Female GCT-discordant	28	1.54	-
Male GCT-discordant	16	0.88	-
GCT-concordant	49	2.70	-

Prevalence of GCT and other infections

The detection of female swab samples found that the vaginal cleanliness of most females (81.88%) reached or exceeded grade III, and 11.56% of females had *Candida* infection. Urine samples showed that 13.55% of females had urine occult blood, 4.96% of females had proteinuria, 15.91% of females had urine leukocytes, 7.65% of males had urine occult blood, 6.28% of males had proteinuria, and 2.20% of males had urine leukocytes. The nucleic acid test results of GCT infection in the reproductive tract showed that the prevalence of GCT infection in females and males were 4.24% (95% CI: 3.41–5.27%) and 3.58% (95% CI: 2.79–4.57%), respectively. More than half (52.69%, 49/93) of the couples were GCT-concordant, 31.11% of the couples were female GCT-discordant, and 17.20% of the couples were male GCT-discordant (Table 1). *Trichomonas* and NG were not found in all participants.

Factors correlated with APOs

Chi-square or Fisher test results showed that except for the couple's age, which was related to APOs, no relationship between GCT infection and other symptoms and APOs was observed (Table 2). In the multivariable logistic analysis, female 30–35 years old [adjusted Odds Ratio (aOR) = 1.08, 95% CI: 1.01–1.17] and over 35 years old (aOR = 1.16, 95% CI: 1.03–1.32) were more likely to experiencing APOs (Table 3). Still, the data showed that the prevalence of APOs in female GCT-discordant and male GCT-discordant were higher than the prevalence in non-infected couples (Table 2), even though female GCT-discordant (aOR = 1.13, 95% CI: 0.97–1.30), male GCT-discordant (aOR = 1.05, 95% CI: 0.87–1.28) and GCT-concordant (aOR = 0.95, 95% CI: 0.85–1.06) were not associated with APOs (Table 3).

Discussion

A cross-sectional study conducted in Nanshan, Shenzhen, from December 2018 to December 2019 found that the prevalence of APOs in pre-pregnancy couples in this region was 19.00%, which was similar to the prevalence (15.60%) in other areas of China (17). In this study, the highest of APOs' constituent ratio was with SA (35.36%), which was higher than that in other regions (18.94%) (21). This may be related to the high work pressure of Shenzhen residents, resulting in high psychological stress, and increasing the prevalence of SA (22). The prevalence of LBW (4.02%) was similar to that of the 2010 study in Shaanxi Province (4.4%), while the prevalence of macrosomia (4.68%) was slightly lower (6.3% for macrosomia) than the same 2010 study (23). The prevalence of preterm birth (3.86%) was much lower than that reported by a survey covering 132 cities in China from 2010 to 2013 (24).

It is worth noting that this study found that the prevalence of SA, macrosomia, LBW, and PTB was high in all APOs. In contrast, the prevalence of stillbirth, induced abortion in medicine department, and therapeutic induced labor was low; this situation was similar to other research (25). However, we should be aware that PTB, LBW, and macrosomia are related to chronic diseases in children, such as neurodevelopmental disorders, cardiovascular diseases, and metabolic diseases (26, 27). In addition, PTB-related complications are closely related to neonatal death (27). Therefore, close attention should be paid to these three APOs and effective public health measures should be taken.

This study found that female age was the influencing factor of APOs. With an increase in female age, the incidence of APOs

10.3389/fpubh.2022.1038391

TABLE 2 Chi-square test for APOs and couples' infections or symptoms.

Characteristics	Normal pregnancy	APOs	% of APOs	χ2	*P*
Female					
Age (years)				21.73	<0.001
20–25	160	23	12.57		
26–30	935	199	17.55		
30–35	330	99	23.08		
>35	46	24	34.29		
Candida				<0.01	1.000
No	1,301	305	18.99		
Yes	170	40	19.05		
BV				0.04	0.839
No	1,427	336	19.06		
Yes	44	9	16.98		
Vaginal cleanliness				2.85	0.415
I	235	59	20.07		
II	26	9	25.71		
III	933	204	17.94		
IV	277	73	20.86		
Urine occult blood				0.32	0.572
No	1,268	302	19.24		
Yes	203	43	17.48		
Proteinuria				3.31	0.069
No	1,391	335	19.41		
Yes	80	10	11.11		
Urine white blood cells				<0.01	0.947
No	1,236	291	19.06		
Yes	235	54	18.69		
CT				<0.01	1.000
No	1,409	330	18.98		
Yes	62	15	19.48		
Male					
Age (years)				16.76	0.001
22–25	78	10	11.36		
26–30	768	164	17.6		
30–35	524	127	19.51		
>35	101	44	30.34		
Urine occult blood				<0.01	1.000
No	1,358	319	19.02		
Yes	113	26	18.71		
Proteinuria				<0.01	1.000
No	1,379	323	18.98		
Yes	92	22	19.30		
Urine white blood cells				<0.01	1.000
No	1,439	337	18.98		
Yes	32	8	20.00		
CT				0.35	0.552
No	1,416	335	19.13		
Yes	55	10	15.38		

(Continued)

TABLE 2 (Continued)

Characteristics	Normal pregnancy	APOs	% of APOs	χ2	*P*
CT-concordant status				—	0.165*
Non-infected couples	1,397	326	18.92		
Female GCT-discordant	19	9	32.14		
Male GCT-discordant	12	4	25.00		
GCT-concordant	43	6	12.24		

*Fisher test.

TABLE 3 Univariate and multivariable logistic analysis of factors associated with APOs.

Characteristics	Crude model		Adjusted model*	
	OR (95% CI)	*P*	aOR (95% CI)	*P*
Female				
Age (years)				
20–25	Ref	-	Ref	-
26–30	1.05 (0.99–1.12)	0.109	1.04 (0.97–1.11)	0.286
30–35	1.11 (1.04–1.19)	0.002	1.08 (1.01–1.17)	0.036
>35	1.24 (1.12–1.38)	<0.001	1.16 (1.03–1.32)	0.018
Proteinuria				
No	Ref	-	Ref	-
Yes	0.92 (0.85–1.00)	0.050	0.93 (0.86–1.01)	0.087
Male				
Age (years)				
22–25	Ref	-	Ref	-
26–30	1.06 (0.98–1.16)	0.153	1.05 (0.96–1.14)	0.327
30–35	1.08 (0.99–1.18)	0.067	1.04 (0.95–1.15)	0.360
>35	1.21 (1.09–1.34)	<0.001	1.11 (0.99–1.25)	0.068
GCT-concordant status				
Non-infected couples	Ref	-	Ref	-
Female GCT-discordant	1.14 (0.99–1.32)	0.077	1.13 (0.97–1.30)	0.111
Male GCT-discordant	1.06 (0.88–1.29)	0.537	1.05 (0.87–1.28)	0.592
GCT-concordant	0.94 (0.84–1.05)	0.240	0.95 (0.85–1.06)	0.339

*Female age, female proteinuria, male age, and GCT-concordant status were adjusted for each other.

also increased, which is consistent with Frederiksen et al. (28). A Swiss study involving 2,009,068 pregnant females showed that old age was a significant risk factor for PTB, especially for extreme PTB at 22–31 weeks (29). A multicenter study in the UK showed that the risk of premature delivery among older pregnant females increased by 2.5 times (30). This association likely due to placental dysfunction mediate APOs in advanced maternal age (31).

The prevalence of GCT infection in females (4.24%) in Shenzhen was similar to our previous research (4.12%) (32) and the prevalence in females in the WHO Western Pacific region (4.3%). The prevalence of GCT infection in males (3.58%) in Shenzhen was similar to that of males in the WHO Western Pacific region (3.4%) (5). We did not find an association between male or female GCT infection and APOs, which was expected because all patients were informed of their infection through messages and advised to treat. Vercruysse et al. (33) reported that urogenital CT infection in pregnancy, if adequately treated, has nothing to do with PTB. However, since we could not obtain the treatment data of all patients, we could not accurately answer the association between GCT infection and APOs. Still, meta-analysis showed that in the unadjusted analysis, GCT infection was related to the increased risk of APOs (1).

There were few GCT concordant status studies on GCT infection (16, 34, 35) which may help to understand the extent of sexual partners' infection with the same pathogen. If couples did not test for CT, female or male partners are prone to misdiagnosis, as this omission may lead to GCT infection. In this study, half (52.69%) of the couples with positive GCT infection results were concordant. Similarly, Quinn et al. (35) reported

52% concordant in 101 CT positive samples, Guerra-Infante et al. (36) reported 57% concordant in 14 positive samples, and Schillinger et al. (34) reported 55% concordant in 128 positive samples. Although we did not find an association between GCT-discordant and APOs, the prevalence of APOs in couples with GCT-discordant infection was higher than that in couples without infection or GCT-concordance, which suggests that we may need to pay more attention to the health education of GCT-discordant couples.

Some countries or regions have issued guidelines for CT infection screening (12, 14, 15), however, except for the US, many countries or regions have not carried out universal GCT infection screening for pregnant females. Our data shows that 75.38% of the male patients' partners were positive, meaning that 0.88% of wives would be infected due to male GCT-discordance. In addition, about 70% of females had no apparent symptoms after GCT infection (12). This makes many females unable to get a timely diagnosis and treatment after GCT infection, and it may lead to the occurrence of GCT sequelae and the increase of APOs.

Our study has several limitations. Firstly, we failed to collect other factors related to APOs, such as socioeconomic factors, other infection factors, drug use, etc. These factors would confuse the association between GCT infection and APOs. Secondly, the time between the occurrence of CT detection and the emergence of APOs was at least 10 months. Other events affected APOs, untreated GCT infection resolved spontaneously (37), and GCT reinfection occurred in this period, all of these conditions would affect the results. Thirdly, we did not know the CT genotypes of GCT concordant and discordant couples, as inconsistent genotypes in GCT-concordant meant both husband and wife had multiple sexual partners, it would increase reinfection risk. However, Schillinger et al. showed that 92.6% of concordant couples infected with CT showed the same genotype (34), which may occur in this study.

In conclusion, although only women's age was found to be associated with APOs, the prevalence of APOs in female and male GCT-discordant couples is higher than that in uninfected or GCT-concordant couples, especially in female, which indicates that more attention should be paid to the health education of this group in follow-ups.

Data availability statement

The datasets presented in this article are not readily available because the datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request. Requests to access the datasets should be directed to ZL, paulluo9909@163.com.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethical Committee of the Nanshan Center for Chronic Disease Control (Approved No. LL20170017). The patients/participants provided their written informed consent to participate in this study.

Author contributions

Conceptualization, methodology, project administration, and resources: SS, LZ, QW, LT, YD, LL, HY, BL, and ZL. Data curation: SS, LZ, QW, and LT. Formal analysis: SS, HY, BL, and ZL. Investigation: SS, LZ, QW, LT, YD, LL, and HY. Software: SS and QW. Writing original draft: SS. Writing review and editing: SS, BL, and ZL. All authors contributed to the article and approved the submitted version.

Funding

This study received financial support from the Shenzhen Nanshan Science and Technology Planning Project (NS2022083).

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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frontiers | Frontiers in Public Health

TYPE Original Research
PUBLISHED 13 January 2023
DOI 10.3389/fpubh.2022.1031372

OPEN ACCESS

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SPECIALTY SECTION
This article was submitted to Infectious Diseases: Epidemiology and Prevention, a section of the journal Frontiers in Public Health

RECEIVED 29 August 2022
ACCEPTED 23 December 2022
PUBLISHED 13 January 2023

CITATION
Xu X, Chow EPF, Fairley CK, Chen M, Aguirre I, Goller J, Hocking J, Carvalho N, Zhang L and Ong JJ (2023) Determinants and prediction of *Chlamydia trachomatis* re-testing and re-infection within 1 year among heterosexuals with chlamydia attending a sexual health clinic. *Front. Public Health* 10:1031372. doi: 10.3389/fpubh.2022.1031372

Determinants and prediction of *Chlamydia trachomatis* re-testing and re-infection within 1 year among heterosexuals with chlamydia attending a sexual health clinic

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Background: *Chlamydia trachomatis* (chlamydia) is one of the most common sexually transmitted infections (STI) globally, and re-infections are common. Current Australian guidelines recommend re-testing for chlamydia 3 months after treatment to identify possible re-infection. Patient-delivered partner therapy (PDPT) has been proposed to control chlamydia re-infection among heterosexuals. We aimed to identify determinants and the prediction of chlamydia re-testing and re-infection within 1 year among heterosexuals with chlamydia to identify potential PDPT candidates.

Methods: Our baseline data included 5,806 heterosexuals with chlamydia aged ≥18 years and 2,070 re-tested for chlamydia within 1 year of their chlamydia diagnosis at the Melbourne Sexual Health Center from January 2, 2015, to May 15, 2020. We used routinely collected electronic health record (EHR) variables and machine-learning models to predict chlamydia re-testing and re-infection events. We also used logistic regression to investigate factors associated with chlamydia re-testing and re-infection.

Results: About 2,070 (36%) of 5,806 heterosexuals with chlamydia were re-tested for chlamydia within 1 year. Among those retested, 307 (15%) were re-infected. Multivariable logistic regression analysis showed that older age (≥35 years old), female, living with HIV, being a current sex worker, patient-delivered partner therapy users, and higher numbers of sex partners were associated with an increased chlamydia re-testing within 1 year. Multivariable logistic regression analysis also showed that younger age (18–24 years), male

gender, and living with HIV were associated with an increased chlamydia re-infection within 1 year. The XGBoost model was the best model for predicting chlamydia re-testing and re-infection within 1 year among heterosexuals with chlamydia; however, machine learning approaches and these self-reported answers from clients did not provide a good predictive value (AUC $<$ 60.0%).

Conclusion: The low rate of chlamydia re-testing and high rate of chlamydia re-infection among heterosexuals with chlamydia highlights the need for further interventions. Better targeting of individuals more likely to be re-infected is needed to optimize the provision of PDPT and encourage the test of re-infection at 3 months.

KEYWORDS

***Chlamydia trachomatis*, re-testing, re-infection, heterosexual, predictive model, machine learning, variable importance, risk factors**

Background

Chlamydia trachomatis (chlamydia) is the most common bacterial sexually transmitted infection (STI) globally (1, 2). An estimated 131 million incident cases of chlamydia infections are acquired globally among people aged 15–49 years annually (3). After treatment, chlamydia re-infections are common, occurring in about 10–20% of patients within 12 months (4–8). Identifying and treating chlamydia re-infection promptly among women is vital because re-infection is associated with a higher risk of pelvic inflammatory disease (9). A study in Australia reported that the chlamydia re-infection rate was 22.3 (95%CI: 13.2–37.6) per 100 person-years among women aged 16–25 years (8). Australian guidelines recommend chlamydia re-testing at 3 months after treatment to identify possible re-infection (10, 11). Chlamydia re-testing was relatively low (40%) within 1.5–12 months (12). Therefore, developing innovative measures that increase repeat testing for chlamydia following chlamydia infection is important.

Treatment of partners is necessary for heterosexuals with chlamydia to prevent re-infection and interrupt the chain of chlamydia transmission (13). Patient Delivery Partner Therapy (PDPT) has been proposed as a possible strategy for reducing re-infection and preventing the sequelae of STIs (14). PDPT involves providing antibiotic treatment to the sexual partners of patients diagnosed with a treatable sexually transmitted infection by giving the prescription or medication to the patient's sexual partner(s) *via* the diagnosed patient (15). The purpose of PDPT is to enable sexual partners who may be reluctant to attend a health provider to be treated earlier, thus reducing the risk of re-infecting the diagnosed patient. Two observational studies found that PDPT may lower women's re-infection risk (16, 17). In Australia, PDPT is permissible for heterosexual men and women diagnosed with chlamydia (18). Identifying heterosexuals with chlamydia at high risk of re-infection who could benefit from PDPT is important for implementing PDPT.

Predictive models can assist clinical decision-making (19). Developing a predictive model for predicting chlamydia re-testing and re-infection within 1 year among heterosexuals could be used to identify potential PDPT candidates and prioritize chlamydia re-infection screening and sexual health service planning. Machine learning approaches have some advantages in prediction, including not requiring statistical inferences and assumptions (20), improving accuracy by exploiting complex interactions between risk factors (21), handling a mass of predictors and combining them in a non-linear and highly interactive way (22). Despite these advantages, to our knowledge, only one study used machine learning to predict STI re-infection. Our study used machine learning algorithms to predict the risk of acquiring $\geq$1 or $\geq$2 additional STIs (combinations of gonorrhea, chlamydia, and syphilis) diagnoses within 1 or 2 years after the initial diagnosis among patients (not limited to heterosexuals) in Massachusetts, USA (23). No studies have used machine learning to predict chlamydia re-testing or re-infection among heterosexuals. Our research team has also used machine learning approaches to predict the uptake of HIV/STI testing among men who have sex with men receiving reminders for testing (24).

We aimed to predict chlamydia re-testing and re-infection within 1 year among heterosexuals with chlamydia using machine learning algorithms. We also used univariable and multivariable logistic regression to identify variables associated with chlamydia re-testing and re-infection.

Methods

Our analysis used electronic health records (EHRs) data between January 2, 2015, and May 15, 2020, at the Melbourne

Sexual Health Center (MSHC). We included individuals who were (1) heterosexuals with chlamydia (including heterosexual males and females) and (2) aged 18 years and above.

Predictors

Based on the literature review and expert discussion, we selected potential predictors. Potential predictors were selected from data routinely collected through a computer-assisted self-interviewing system used when patients enter the clinic (25) and from other routinely collected data items in the clinical setting. Self-reported predictors at baseline (i.e., at the time of their diagnosis of chlamydia) were included, such as demographics (age at the consultation, gender, country of birth, access to Medicare, ever sex worker, current sex worker), sexual practices (numbers of sex partners in the last 3 months, sex overseas in the last 12 months, condoms used with sex partners in the last 3 months), self-reported past STI (i.e., chlamydia, genital herpes, genital warts, gonorrhea, syphilis), HIV infection, use of PDPT (i.e., the diagnosed client receiving an extra prescription of antibiotics to give to their sexual partner), injecting drug use in the last 3 months, triage reason as asymptomatic screening, triage reason as STI symptoms, and triage reason as contact of any STI (summarized in Table 1). In the machine learning analysis, we did not include PDPT users.

Outcome

The primary outcome was chlamydia re-infection, defined as the first new chlamydia diagnosis using nucleic acid amplification testing from any anatomical site, including the oropharynx, urethra/urine, or anorectum, at least 30 days after and within 365 days after a positive chlamydia diagnosis. The selection of chlamydia re-infection from 30 to 365 days after a positive chlamydia diagnosis is consistent with a *Chlamydia trachomatis* re-infection study among female adolescents (26). In this study, chlamydia re-testing was defined as those heterosexuals who had new chlamydia diagnosis results (either positive or negative) at least 30 days after and within 365 days after a positive chlamydia diagnosis.

Analysis

Statistical analysis was conducted with R 4.0.3. Descriptive statistics were used to summarize the patient characteristics. We also performed risk factor analysis using univariable and multivariable logistic regression analyses to calculate the unadjusted odds ratio (OR) and adjusted OR. Variables with $p < 0.20$ in the univariable logistic regression analysis were included in the multivariable logistic regression analysis to identify independent risk factors. All statistics were performed using a two-sided test, and statistical significance was considered at $p < 0.05$.

Machine learning algorithms were conducted with Python 3.9.7. We developed 10 commonly utilized machine learning models, including Logistic Regression (LR), K-Nearest Neighbors (KNN), Adaptive Boosting Classifier (AdaBoost), SVM with a Radial Basis Function Kernel (SVM), Gaussian Naive Bayes (GaussianNB), Gradient Boosting Machine (GBM), Light Gradient Boosting Decision Machine (LightGBM), Extreme Gradient Boosting (XGBoost), Random Forest (RF), and Multi-Layer Perceptron (MLP). LR, GBM, AdaBoost, GaussianNB, KNN, SVM, RF, and MLP was built using the *scikit-learn* library. LightGBM was built using the *LightGBM* library. XGBoost was built using the *xgboost* library. We performed a 3 × 10 (3 outer folds, 10 inner folds) nested cross-validation to avoid overfitting and improve generalizability (25, 27). We used the Bayesian optimisation method for tuning the hyperparameters. The performance of machine learning models was measured by the area under the receiver operating characteristics curve (AUC) and accuracy on the independent testing dataset. We also calculated the variable importance of chlamydia re-testing and re-infection within 1 year to investigate the effect of different predictors on prediction (24, 25, 28). First, we obtained the results of variable importance analysis from each fold in the outer loop of nested cross-validation. Then based on the value of AUC, we chose the one closest to the mean AUC and reported the results of this variable importance analysis.

Ethics approval

Ethical approval was granted by the Alfred Hospital Ethics Committee, Australia (project number: 277/20).

Results

Characteristics of the study population

Our study data included 5,806 heterosexual patients diagnosed with chlamydia. Among 5,806 patients, 35.7% (2,070/5,806) were re-tested for chlamydia within 1 year. A total of 14.8% (307/2,070) heterosexuals with chlamydia were re-infected with chlamydia within 1 year. Further details of the sociodemographic characteristics and sexual practices are provided in Tables 1, 2.

TABLE 1 Factors associated with chlamydia re-testing within 1 year among 5,806 heterosexual individuals with chlamydia.

Characteristic	Event rate	Crude OR	95% CI	*p*-value	Adjusted OR*	95% CI	*p*-value
Gender							
Female	1,089/2,752 (39.6%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Male	981/3,054 (32.1%)	0.72	0.65, 0.80	**<0.001**	0.74	0.66, 0.84	**<0.001**
HIV infection							
HIV-negative	2,042/5,762 (35.4%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Living with HIV	28/44 (63.6%)	3.19	1.74, 6.04	**<0.001**	2.27	1.18, 4.50	**0.016**
Triage reason as asymptomatic screening							
No	1,910/5,404 (35.3%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Yes	160/402 (39.8%)	1.21	0.98, 1.49	**0.072**	1.04	0.83, 1.29	0.749
Triage reason as STI symptoms							
No	1,139/3,235 (35.2%)	1 (ref)	1 (ref)				
Yes	931/2,571 (36.2%)	1.04	0.94, 1.16	0.428			
Triage reason as contact of STI							
No	1,703/4,547 (37.5%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Yes	367/1,259 (29.2%)	0.69	0.60, 0.79	**<0.001**	0.79	0.69, 0.91	**0.001**
Past chlamydia							
No	1,663/4,678 (35.5%)	1 (ref)	1 (ref)				
Yes	407/1,128 (36.1%)	1.02	0.89, 1.17	0.738			
Past genital herpes							
No	2,011/5,655 (35.6%)	1 (ref)	1 (ref)				
Yes	59/151 (39.1%)	1.16	0.83, 1.61	0.374			
Past genital warts							
No	1,991/5,610 (35.5%)	1 (ref)	1 (ref)				
Yes	79/196 (40.3%)	1.23	0.92, 1.64	**0.167**			
Past gonorrhea							
No	2,015/5,661 (35.6%)	1 (ref)	1 (ref)				
Yes	55/145 (37.9%)	1.11	0.78, 1.55	0.562			

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TABLE 1 (Continued)

Characteristic	Event rate	Crude OR	95% CI	*p*-value	Adjusted OR*	95% CI	*p*-value
Past syphilis							
No	2,061/5,779 (35.7%)	1 (ref)	1 (ref)				
Yes	9/27 (33.3%)	0.90	0.39, 1.96	0.801			
Current sex worker							
No	1,773/5,255 (33.7%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Unknown/missing	123/280 (43.9%)	1.54	1.21, 1.96	**<0.001**	1.35	0.81, 2.28	0.250
Yes	174/271 (64.2%)	3.52	2.74, 4.56	**<0.001**	2.50	1.88, 3.33	**<0.001**
Sex overseas in the past 12 months							
No	942/2,517 (37.4%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Unknown/missing	188/450 (41.8%)	1.20	0.98, 1.47	**0.080**	1.06	0.78, 1.43	0.703
Yes	940/2,839 (33.1%)	0.83	0.74, 0.93	**<0.001**	0.90	0.79, 1.01	0.077
Use of PDPT							
No	1,947/5,540 (35.1%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Yes	123/266 (46.2%)	1.59	1.24, 2.03	**<0.001**	1.32	1.02, 1.70	**0.033**
Country of birth							
Australia	587/1,610 (36.5%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Not Australia	1,414/3,987 (35.5%)	0.96	0.85, 1.08	0.482	0.92	0.80, 1.06	0.263
Unknown/missing	69/209 (33.0%)	0.86	0.63, 1.16	0.329	0.79	0.57, 1.09	0.152
Access to Medicare							
No	786/1,995 (39.4%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Unknown/missing	155/780 (19.9%)	0.38	0.31, 0.46	**<0.001**	0.41	0.34, 0.50	**<0.001**
Yes	1,129/3,031 (37.2%)	0.91	0.81, 1.03	**0.125**	0.91	0.79, 1.04	0.182
Age							
18–24 years	783/2,331 (33.6%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
25–34 years	1,044/2,895 (36.1%)	1.12	0.99, 1.25	**0.063**	1.13	1.00, 1.27	0.052
35 years old and above	243/580 (41.9%)	1.43	1.18, 1.72	**<0.001**	1.27	1.04, 1.55	**0.019**

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TABLE 1 (Continued)

Characteristic	Event rate	Crude OR	95% CI	*p*-value	Adjusted OR*	95% CI	*p*-value
Injecting drug use in the past 3 months							
No	1,926/5,440 (35.4%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Unknown/missing	132/320 (41.2%)	1.28	1.02, 1.61	**0.034**	0.83	0.52, 1.29	0.404
Yes	12/46 (26.1%)	0.64	0.32, 1.21	**0.191**	0.54	0.26, 1.06	0.085
Numbers of sex partners in the past 3 months							
1 sex partner	497/1,446 (34.4%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
2–5 sex partners	983/2,816 (34.9%)	1.02	0.90, 1.17	0.727	1.13	0.98, 1.30	0.083
5–10 sex partners	265/768 (34.5%)	1.01	0.84, 1.21	0.949	1.20	0.99, 1.46	0.064
More than 10 sex partners	76/196 (38.8%)	1.21	0.89, 1.64	0.225	1.46	1.06, 2.01	**0.020**
Unknown/missing	41/137 (29.9%)	0.82	0.55, 1.19	0.295	0.89	0.57, 1.39	0.626
N/A	443						
Condoms used with sex partners in the last 3 months							
Always use condom	168/395 (42.5%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Not Always	1643/4,793 (34.3%)	0.70	0.57, 0.87	**<0.001**	0.80	0.65, 1.00	0.052
Unknown/missing	1643/4,793 (34.3%)	0.56	0.38, 0.81	**<0.003**	0.65	0.42, 1.00	0.052
N/A	208/443 (47.0%)						

OR, Odds Ratio; CI, Confidence Interval; N/A, no sex in the past 3 months; PDPT, patient delivery partner therapy. * Variables with $p < 0.20$ in the univariable logistic regression analysis were included in the multivariable logistic regression analysis. The bold values indicate variables with $p < 0.20$ in the univariable logistic regression analysis.

10.3389/fpubh.2022.1031372

TABLE 2 Factors associated with chlamydia re-infection within 1 year among 2,070 re-tested heterosexual individuals.

Characteristic	Event rate	Crude OR	95% CI	*p*-value	Adjusted OR*	95% CI	*p*-value
Gender							
Female	132/1,089 (12.1%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Male	175/981 (17.8%)	1.57	1.23, 2.01	**<0.001**	1.55	1.16, 2.08	**0.003**
HIV infection							
HIV-negative	294/2,042 (14.4%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Living with HIV	13/28 (46.4%)	5.15	2.39, 11.0	**<0.001**	4.02	1.64, 9.87	**0.002**
Triage reason as asymptomatic screening							
No	289/1,910 (15.1%)	1 (ref)	1 (ref)				
Yes	18/160 (11.2%)	0.71	0.42, 1.15	**0.187**			
Triage reason as STI symptoms							
No	162/1,139 (14.2%)	1 (ref)	1 (ref)				
Yes	145/931 (15.6%)	1.11	0.87, 1.42	0.390			
Triage reason as contact of STI							
No	251/1,703 (14.7%)	1 (ref)	1 (ref)				
Yes	56/367 (15.3%)	1.04	0.75, 1.42	0.799			
Past chlamydia							
No	247/1,663 (14.9%)	1 (ref)	1 (ref)				
Yes	60/407 (14.7%)	0.99	0.73, 1.34	0.955			
Past genital herpes							
No	303/2,011 (15.1%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Yes	4/59 (6.78%)	0.41	0.12, 1.01	**0.087**	0.40	0.12, 1.00	0.085
Past genital Warts							
No	293/1,991 (14.7%)	1 (ref)	1 (ref)				
Yes	14/79 (17.7%)	1.25	0.66, 2.19	0.462			
Past gonorrhea							
No	295/2,015 (14.6%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Yes	12/55 (21.8%)	1.63	0.81, 3.03	**0.143**	1.65	0.80, 3.18	0.150

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TABLE 2 (Continued)

Characteristic	Event rate	Crude OR	95% CI	*p*-value	Adjusted OR*	95% CI	*p*-value
Past syphilis							
No	306/2,061 (14.8%)	1 (ref)	1 (ref)				
Yes	1/9 (11.1%)	0.72	0.04, 3.93	0.754			
Current sex worker							
No	249/1,773 (14.0%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Unknown/missing	30/123 (24.4%)	1.97	1.26, 3.01	**0.002**	0.55	0.17, 1.81	0.334
Yes	28/174 (16.1%)	1.17	0.75, 1.77	0.461	1.22	0.71, 2.04	0.456
Sex overseas in the past 12 months							
No	137/942 (14.5%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Unknown/missing	37/188 (19.7%)	1.44	0.95, 2.13	**0.076**	0.61	0.28, 1.24	0.199
Yes	133/940 (14.1%)	0.97	0.75, 1.25	0.807	0.91	0.69, 1.20	0.501
Use of PDPT							
No	285/1,947 (14.6%)	1 (ref)	1 (ref)				
Yes	22/123 (17.9%)	1.27	0.77, 2.01	0.327			
Country of birth							
Australia	97/587 (16.5%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Not Australia	199/1,414 (14.1%)	0.83	0.64, 1.08	**0.160**	0.87	0.63, 1.20	0.387
Unknown/missing	11/69 (15.9%)	0.96	0.46, 1.82	0.902	1.11	0.52, 2.18	0.772
Access to Medicare							
No	116/786 (14.8%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Unknown/missing	16/155 (10.3%)	0.66	0.37, 1.13	**0.148**	0.61	0.34, 1.05	0.088
Yes	175/1,129 (15.5%)	1.06	0.82, 1.37	0.656	0.91	0.66, 1.23	0.533
Age							
18–24 years	125/783 (16.0%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
25–34 years	148/1,044 (14.2%)	0.87	0.67, 1.13	0.289	0.78	0.60, 1.02	0.074
35 years old and above	34/243 (14.0%)	0.86	0.56, 1.28	0.458	0.58	0.37, 0.89	**0.016**

(Continued)

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TABLE 2 (Continued)

Characteristic	Event rate	Crude OR	95% CI	*p*-value	Adjusted OR*	95% CI	*p*-value
Injecting drug use in the past 3 months							
No	272/1,926 (14.1%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
Unknown/missing	34/132 (25.8%)	2.11	1.38, 3.15	**<0.001**	2.11	0.73, 5.55	0.144
Yes	1/12 (8.33%)	0.55	0.03, 2.86	0.571	0.30	0.01, 1.95	0.306
Numbers of sex partners in the past 3 months							
1 sex partner	64/497 (12.9%)	1 (ref)	1 (ref)		1 (ref)	1 (ref)	
2–5 sex partners	129/983 (13.1%)	1.02	0.74, 1.42	0.894	1.02	0.73, 1.42	0.927
5–10 sex partners	47/265 (17.7%)	1.46	0.96, 2.19	**0.071**	1.38	0.90, 2.11	0.140
More than 10 sex partners	15/76 (19.7%)	1.66	0.87, 3.04	**0.109**	1.45	0.74, 2.71	0.261
Unknown/missing	3/41 (7.32%)	0.53	0.13, 1.53	0.307	0.51	0.12, 1.51	0.287
N/A	208						
Condoms used with sex partners in the last 3 months							
Always use condom	19/168 (11.3%)	1 (ref)	1 (ref)				
Not Always	230/1,643 (14.0%)	1.28	0.80, 2.16	0.336			
Unknown/missing	9/51 (17.6%)	1.68	0.68, 3.90	0.239			
N/A	208						

OR, Odds Ratio; CI, Confidence Interval; N/A, no sex in the past 3 months; PDPT, patient delivery partner therapy. *Variables with $p < 0.20$ in the univariable logistic regression analysis were included in the multivariable logistic regression analysis. The bold values indicate variables with $p < 0.20$ in the univariable logistic regression analysis.

10.3389/fpubh.2022.1031372

Factors associated with chlamydia re-testing and chlamydia re-infection within 1 year using univariable and multivariable logistic regression analyses

The potential factors that were included in the multivariable logistic regression analysis of chlamydia re-testing within 1 year among heterosexuals with chlamydia were age, gender, HIV infection, triage reason as asymptomatic screening, triage reason as contact of STI infection, current sex work, use of PDPT, country of birth, access to Medicare, injecting drug use in the past 3 months, had sex with a partner in the past 3 months, numbers of sex partners in the past 3 months, condoms used with sex partners in the last 3 months. Chlamydia re-testing within 1 year among heterosexuals was associated with male gender [adjusted odds ratios (aOR) = 0.74, 95%CI 0.66, 0.84], living with HIV (aOR = 2.27, 95%CI 1.18, 4.50), presentation as contact of STI infection (aOR = 0.79, 95%CI 0.69, 0.91), being a current sex worker (aOR = 2.50, 95%CI 1.88, 3.33), users of patient delivered partner therapy (aOR = 1.32, 95%CI 1.02, 1.70), older age (≥35 years old vs. 18–24 years, aOR = 1.27, 95%CI 1.04, 1.55), unknown/missing on accessing to Medicare (aOR = 0.41, 95%CI 0.34, 0.50), and higher numbers of partners in the last 3 months (>10 vs.1, aOR = 1.46, 95%CI 1.06, 2.01) (Table 1).

The potential factors that were included in the multivariable logistic regression analysis of chlamydia re-infection within 1 year among heterosexuals with chlamydia were age, gender, HIV infection, past genital herpes, past gonorrhea, current sex worker, sex overseas in the past 12 months, country of birth, access to Medicare, injecting drug use in the past 3 months, had sex with a partner in the past 3 months, numbers of sex partners in the past 3 months, and condoms used with sex partners in the past 3 months. Chlamydia re-infection within 1 year among heterosexuals was associated with male gender (aOR = 1.55, 95%CI 1.16, 2.08), living with HIV (aOR = 4.02, 95%CI 1.64, 9.87), and older age (≥35 years old vs. 18–24 years, aOR = 0.58, 95%CI 0.37, 0.89) (Table 2).

Prediction of chlamydia re-testing and chlamydia re-infection within 1 year using machine learning approaches

The best model for predicting chlamydia re-testing within 1 year among heterosexuals with chlamydia was the XGBoost [mean 58.5% (SD 0.1%)]. Adaboost, SVM, LightGBM, and XGBoost performed better than LR in predicting chlamydia re-testing within 1 year among heterosexuals with chlamydia. However, machine learning approaches did not provide a good predictive value for chlamydia re-testing on the testing data (AUC < 60.0%). Further details of model performance are provided in Table 3 and Supplementary Table 1. Variable importance analysis using XGBoost showed that the top 10 identified predictors for chlamydia re-testing within 1 year among heterosexuals with chlamydia included current sex worker, access to Medicare, condoms used with sex partners, age, gender, triage reason as contact of STI infection, injecting drug use, sex overseas, numbers of sex partners, and triage reason as STI symptoms (Figure 1).

The best model for predicting chlamydia re-infection within 1 year among heterosexuals with chlamydia was the XGBoost [mean 58.5% (SD 1.1%)]. Adaboost and XGBoost performed better than LR in predicting chlamydia re-infection within 1 year among heterosexuals with chlamydia. However, machine learning approaches did not provide a good predictive value for chlamydia re-infection on the testing data (all AUC < 60.0%). Further details of model performance are provided in Table 3 and Supplementary Table 1. Variable importance analysis using XGBoost showed that the top 10 identified predictors for chlamydia re-infection within 1 year among heterosexuals with chlamydia included gender, number of sex partners, age, past gonorrhea, past chlamydia, injecting drug use, past genital warts, condoms used with sex partners, sex overseas, and country of birth (Figure 2).

Discussion

To our knowledge, this is the first study using machine learning approaches to predict chlamydia re-testing and re-infection within 1 year among heterosexuals with chlamydia. Our study found a relatively low chlamydia re-testing rate (36%) within 1 year among heterosexuals with chlamydia. Our finding was similar to a previous study reporting that about 40% were re-tested within 12 months in 25 general practice clinics in the Australian Collaboration for Chlamydia Enhanced Sentinel Surveillance system (12). Our study also found a relatively high rate of chlamydia re-infection (15%) within 1 year among heterosexuals with chlamydia. Our result is consistent with previous studies reporting that the chlamydia re-infection rate was about 10–20% of patients within 12 months (4–8). The low rate of chlamydia re-testing and high rate of chlamydia re-infection suggest that further targeted and personalized interventions are warranted among heterosexuals with chlamydia. Our logistic regression analysis showed the determinants of chlamydia re-testing and re-infection within 1 year among heterosexuals with chlamydia. Our machine learning predictive models proposed a new way of using risk prediction models to increase the low chlamydia re-testing rate and reduce the high rate of chlamydia re-infection. Despite the relatively poor prediction of chlamydia re-testing and re-infection among heterosexuals, our findings suggest that researchers could develop a wide range of machine learning algorithms to find the best algorithm for prediction. Our study

TABLE 3 Machine learning model evaluation of chlamydia re-testing and re-infection within1 year among heterosexual individuals with chlamydia on the testing data set (mean/SD).

	Chlamydia re-testing (n = 5,806)		Chlamydia re-infection (n = 2,070)	
	AUC, %	Accuracy, %	AUC, %	Accuracy, %
LR	57.6 (1.3)	65.3 (4.1)	57.3 (1.5)	85.2 (1.6)
Adaboost	57.8 (0.4)	65.4 (3.9)	58.3 (1.7)	85.0 (1.7)
SVM	58.1 (2.4)	65.3 (4.4)	47.8 (0.9)	85.2 (1.6)
XGBoost	58.5 (0.1)	64.9 (3.1)	58.5 (1.1)	85.2 (1.6)
RF	57.0 (1.1)	64.1 (2.6)	56.5 (2.0)	85.2 (1.6)
KNN	53.7 (0.1)	63.0 (3.2)	49.5 (2.5)	84.6 (2.3)
Gaussian NB	56.2 (0.6)	61.1 (2.1)	56.7 (1.6)	59.0 (29.7)
GBM	56.9 (1.5)	64.9 (4.4)	56.6 (3.3)	79.2 (9.9)
LightGBM	58.5 (0.2)	63.3 (2.5)	55.3 (1.7)	85.2 (1.6)
MLP	54.7 (3.8)	64.2 (4.7)	55.8 (1.4)	85.3 (1.3)

SD, standard deviation; AUC, area under the receiver operating characteristics curve; LR, Logistic Regression; KNN, K-Nearest Neighbor; AdaBoost, AdaBoost classifier; SVM, SVM with a Radial Basis Function Kernel; GaussianNB, Gaussian Naive Bayes; GBM, Gradient Boosting Machine; LightGBM, Light Gradient Boosting Machine, XGBoost, Extreme Gradient Boosting; RF, Random Forest; MLP, and multi-layer perceptron.

also provides implications for further data collection in machine learning analysis. In this study, we used routinely collected data from the clinical setting. To improve the performance of learning models in predicting chlamydia re-testing and re-infection, researchers could pay more attention to the quality, comprehensiveness, and accuracy of data collection to avoid "garbage in, garbage out" (29). Our study provides implications for selecting machine learning algorithms and predictors for future machine learning studies in predicting chlamydia re-testing and re-infection among heterosexuals with chlamydia. Better targeting interventions for those at increased risk of re-infection may help reduce their risk or encourage re-testing to diagnose and treat their infection earlier.

We aimed to develop machine learning models to predict chlamydia re-testing behavior within 1 year among heterosexuals with chlamydia using routine questions in the clinical setting. There are already some studies that have utilized machine learning algorithms to predict sexual-health behaviors, including sexual recidivism (30), sexual desire (31), reasons for not using a condom (32), and HIV risk behaviors (33). However, limited studies use machine learning algorithms to predict sexual health service utilization behaviors. We noted a previous study that reported that machine learning approaches could predict HIV/STI re-testing after receiving clinic reminder messages among MSM (24). Another study used machine learning algorithms to predict HIV testing behavior among participants from substance use disorder treatment programs (34). Our study is the first to use machine learning approaches to predict chlamydia re-testing. Therefore, it is difficult to compare our results to others. Despite the poor prediction of chlamydia re-testing behavior, developing good predictive models for this is still necessary and important. Predicting sexual healthcare utilization behaviors (e.g., future chlamydia re-testing) may benefit the management and planning of sexual health services for high-risk populations or focus prevention interventions in advance. We hope our work could encourage more machine learning research to predict chlamydia re-testing behavior among heterosexuals with chlamydia.

We intended to develop predictive models for chlamydia re-infection within 1 year among heterosexuals using machine learning approaches. Predicting future chlamydia re-infection could inform prioritizing interventions. A previous study used machine learning algorithms that could predict chlamydia (AUC = 0.67) acquisition within 1 year among males and females (28). However, our machine learning predictive models indicated that existing self-reported and routinely collected EHRs data could not provide a high predictive value for chlamydia re-infection events within 1 year among heterosexuals with chlamydia (AUC $<$ 0.6). Despite this, our study still provides implications for future machine learning studies focusing on predicting chlamydia re-infection. For example, our results showed that Adaboost, and XGBoost performed better than conventional logistic regression in predicting chlamydia re-infection within 1 year among heterosexuals. Besides, the low predictive performances suggest that existing self-reported and routinely collected EHR data may not include some important predictors for chlamydia re-infection, such as sexual networks and background chlamydia prevalence. Another machine learning study indicated that their models lacked data for specific sexual practices (23). Our models did not include other potential factors because these data were not routinely collected EHRs data in the clinic, such as employment status (8), cervical infection, contact with uncircumcised partners and resuming sex with an untreated partner (35, 36).

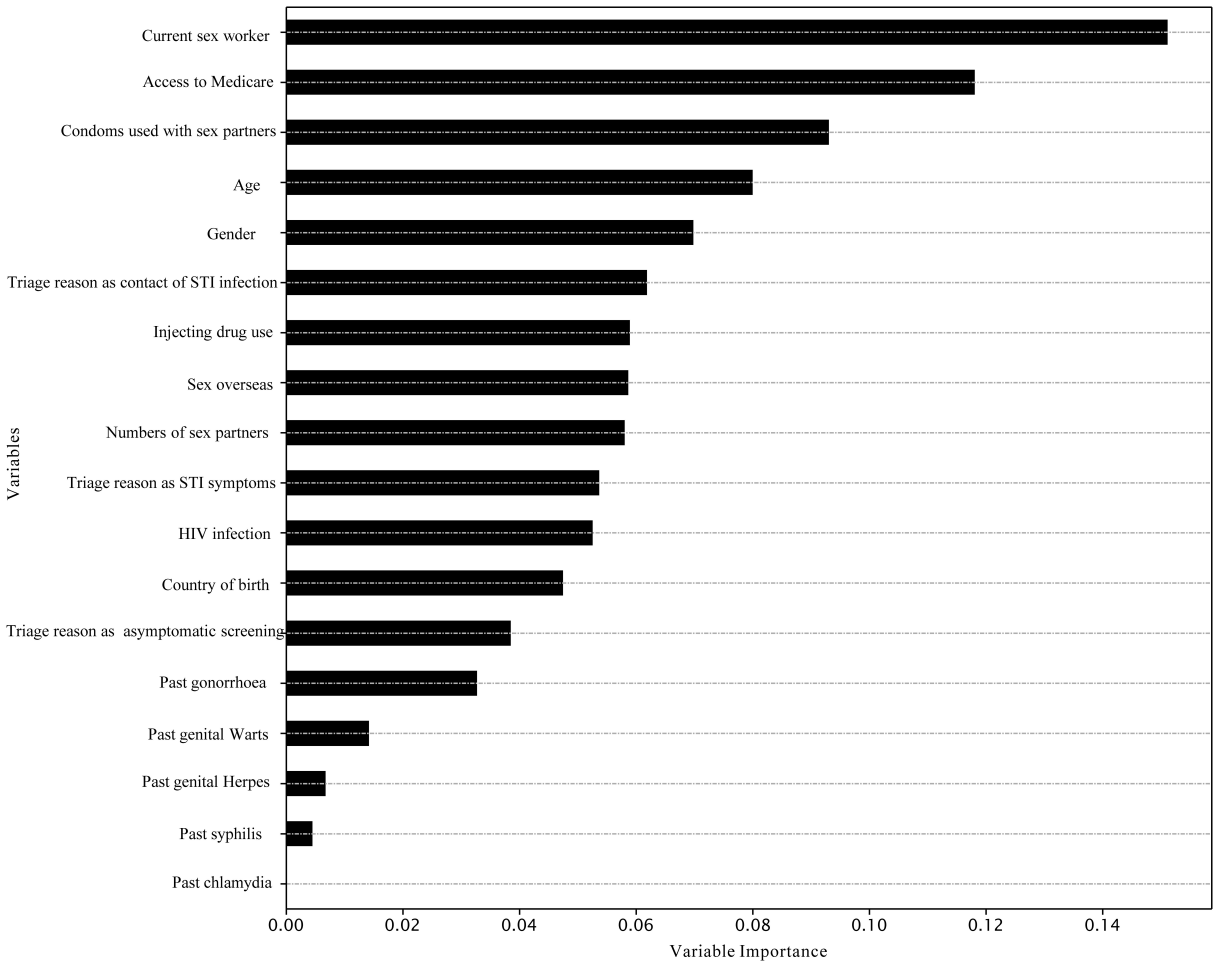

FIGURE 1
Variable importance in the prediction of chlamydia re-testing within 1 year among heterosexuals by XGBoost.

Better targeting of interventions to improve re-testing is needed, especially for those with an increased likelihood of chlamydia re-infection. Our study findings are consistent with previous studies, which also found chlamydia re-testing was associated with higher numbers of sex partners (37) and among heterosexual females (38). We also observed that sex workers showed a higher re-testing rate, probably because they had a legal requirement for 3 monthly testing. Our variable importance analyses suggest other possible predictors for future predictive models of chlamydia re-testing using machine learning approaches. The top identified predictors for chlamydia re-testing were being a current sex worker, access to Medicare, condoms used with sex partners, age, gender, triage reason as contact of STI infection, injecting drug use, sex overseas, number of sex partners, and presentation as symptomatic. We hope our preliminary work encourages more machine learning research to explore the effect of introducing additional predictors on predicting chlamydia re-testing among heterosexuals.

Our study found that the chlamydia re-infection rate was greater among individuals of younger age (18–24 years), male gender, and living with HIV. These factors were consistent with previous studies on risk factors for chlamydia infection. A previous study showed that people living with HIV had more repeat chlamydia infections in the Netherlands (39). Young age at first infection was associated with an increased risk of subsequent chlamydia infection within 1 year among female adolescents in the US (26). A previous study found that being a sex worker was associated with chlamydia (40). These findings may provide clinical and public health researchers and policymakers with a deeper understanding of the drivers of chlamydia re-infection among heterosexuals at the population level. Besides, our variable importance analyses suggest possible important predictors for future predictive models of chlamydia re-infection using machine learning approaches. Consistent with previous studies on risk factors of chlamydia infection, we found that factors such as condom use (41), having sex

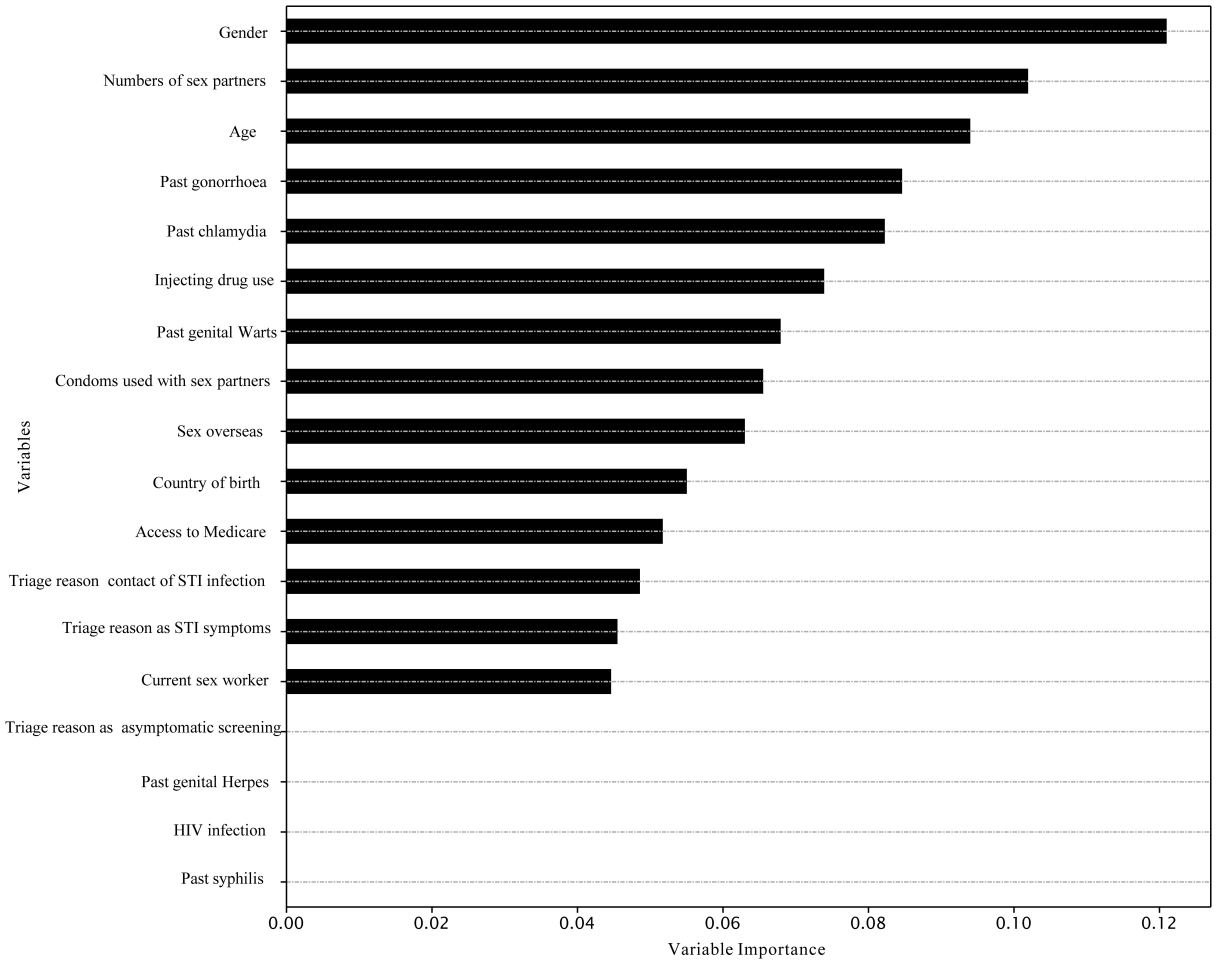

FIGURE 2
Variable importance in the prediction of chlamydia re-infection within 1 year among heterosexuals by XGBoost.

with a female or male partner (42), and sex workers (40) were among the important predictors included in the machine learning models.

Our study has some limitations. First, only about 36% of individuals diagnosed with chlamydia returned for another chlamydia test within 1 year. We did not have information on whether the remaining individuals were tested elsewhere, so this may be an underestimate and may lead to a selection bias in our study. Among those retested, about 15% were re-infected. It is possible that those at higher risk of re-infection may be more likely to be retested. Therefore, the chlamydia re-infection (15%) may be overestimated. Second, our findings were from a single sexual clinic; therefore, studies from different settings (e.g., those attending general practice) would be needed to verify our results. Third, the validity of the predictive factors depends on the accuracy of the self-reported information, subject to participants' recall and non-response bias. There has been substantial work on the CASI system to ensure its validity and accuracy (43). Fourth, we did not include gonorrhea and chlamydia coinfection status in this study. A previous study suggested that gonorrhea and chlamydia coinfection may increase the risk of chlamydia re-infection (36). Last, we included data up to May 2020, which might introduce selection bias due to the COVID-19 lockdown in Melbourne. The first COVID-19 lockdown started on March 30 and ended on May 12, 2020. Thus, our study included a small number of clinic consultations during the COVID-19 lockdown period. A previous study showed that the re-testing patterns and sexual practices might have changed due to COVID-19 (44).

Conclusions

The chlamydia re-testing rate within 1 year was relatively low among heterosexuals with chlamydia; however, the

re-infection rate of chlamydia was relatively high. Our study highlights the need for innovative interventions to increase chlamydia re-testing and reduce re-infection among heterosexuals with chlamydia. Our findings elaborate on the determinants of chlamydia re-testing and re-infection within 1 year among heterosexuals with chlamydia. To our knowledge, it is the first demonstration of machine learning algorithms to identify heterosexuals with chlamydia at high risk of re-infection who could benefit from patient-delivered partner therapy. XGBoost model can improve the prediction of chlamydia re-testing and re-infection compared with traditional logistic regression. Our machine learning predictive models may provide a promising way to develop an innovative method to increase the low chlamydia re-testing rate and reduce the high rate of chlamydia re-infection.

Data availability statement

The data analyzed in this study is subject to the following licenses/restrictions: The data is not publicly available due to privacy or ethical restrictions but will be made available on reasonable request to the corresponding author, with the permission of the Alfred Hospital Ethics Committee. Restrictions apply to the availability of these data, which were used under ethical approval for this study. Requests to access these datasets should be directed to JO, jason.ong@monash.edu.

Ethics statement

The studies involving human participants were reviewed and approved by the Alfred Hospital Ethics Committee, Australia (project number: 277/20). As this was a retrospective study involving minimal risk to the privacy of the study subjects, informed consent was waived by the Alfred Hospital Ethics Committee. All identifying details of the study subjects were removed before any computational analysis.

Author contributions

JO, XX, and LZ conceived and designed the study. XX did data cleaning, established the models and coding, and wrote the first draft and editing. EC, JO, and LZ contributed to data cleaning. EC, CF, MC, IA, JG, JH, NC, LZ, and JO contributed to the interpretation of data and manuscript revision. All authors contributed to the preparation of the manuscript and approved the final manuscript.

Funding

This work was supported by Australian National Health and Medical Research Council Emerging Leadership Investigator Grant (Grant GNT1172873 to EC; GNT1193955 to JO), the Australian National Health and Medical Research Council Leadership Investigator Grant (Grant GNT1172900 to CF), National Natural Science Foundation of China (Grant 81950410639 to LZ), Outstanding Young Scholars Funding (Grant 3111500001 to LZ), Xi'an Jiaotong University Basic Research and Profession Grant (Grant xtr022019003 to LZ, Grant xzy032020032 to LZ), Epidemiology modeling and risk assessment (Grant 20200344 to LZ), and Xi'an Jiaotong University Young Talent Support Grant (Grant YX6J004 to LZ).

Acknowledgments

The authors would like to acknowledge Afrizal Afrizal from Melbourne Sexual Health Center for data extraction. The authors would also like to thank Zhen Yu and Zongyuan Ge at Monash University for their insightful discussion of machine learning techniques.

Conflict of interest

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

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fpubh.2022.1031372/full#supplementary-material

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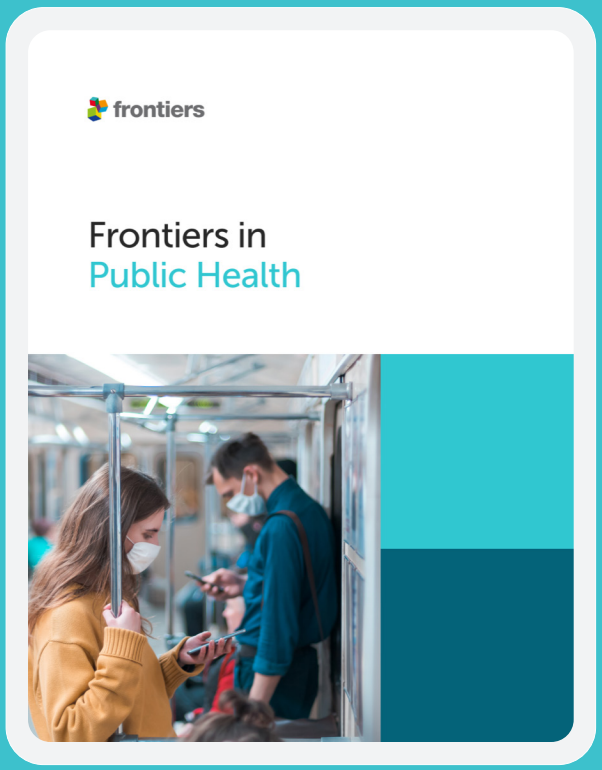

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