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Original Article

Characteristics of rectal chlamydia among men who have sex with men in southern Taiwan, 2020–2022: An emerging threat of rectal lymphogranuloma venereum L2b

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KEYWORDS
Lymphogranuloma venereum L2b;
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Abstract *Background:* The prevalence of rectal chlamydia among men who have sex with men (MSM) without human deficiency virus infection (non-HIV) remains uncertain in Taiwan, and rectal lymphogranuloma venereum (LGV) among MSM has never been reported in the Far East.
Material and methods: From January 2020 to April 2022, MSM coming for anonymous voluntary counseling and testing, for pre-exposure prophylaxis, and for antiretroviral therapy were enrolled. All participants submitted his fecal samples and completed a QR-code questionnaire.

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Gonorrhea;
HIV

Medical records of those who took regular medical visits for HIV were recorded. Multiplex polymerase chain reaction (PCR) was performed for all fecal samples, and *ompA* gene sequencing was therefore performed for each *Chlamydia*-positive fecal sample.
Results: Among 341 MSM during 2020–2022 in southern Taiwan, 21 (6.2%) had rectal chlamydia infection. Risk factors of rectal chlamydia included co-infection with rectal gonorrhea (adjusted odds ratio [AOR] 6.78, 95% confidence interval [CI] 1.44–31.91, $P = 0.015$) and multiple sexual partners (AOR 1.373, 95% CI 1.002–1.882, $P = 0.048$). Further *ompA* gene sequencing from 19 *Chlamydia*-positive fecal samples revealed that the prevalent genotypes or genovariants were Da (26.3%) and L2b (26.3%), followed by B (21.1%), J (14.3%), and G (9.5%). All cases of rectal LGV genovariant L2b presented as acute proctitis with diarrhea, anal pain, or discharge and were treated successfully with prolonged treatment of doxycycline.
Conclusions: Rectal gonorrhea and multiple sexual partners are risk factors for rectal chlamydia. Clinicians in Taiwan should be aware of the emerging threat of rectal LGV among MSM with acute proctitis.

Introduction

Chlamydia trachomatis (*C. trachomatis*) is a Gram-negative, obligate intracellular bacterium. By serotyping of the major outer membrane protein (MOMP), *C. trachomatis* can be classified into three serogroups: B (serovars B, Ba, D, E, L1, and L2), C (serovars A, C, H, I, and J), and the intermediate serogroup (serovars F, G, K, and L3).[1] The serovars A, B, Ba, and C can result in trachoma, the leading cause of congenital blindness worldwide. The serovars D through K may lead to a wide spectrum of sexually transmitted diseases (STDs), including urethritis, epididymitis, cervicitis, and pelvic inflammatory disease. The serovars L1, L2, and L3 cause lymphogranuloma venereum (LGV), which often manifests in invasive urogenital infections with regional lymphadenopathy or ulcerative anorectal infections.[2] With the advancement of molecular diagnosis techniques, the maneuvers of serotyping have been replaced by genotyping of the MOMP-encoding gene, *ompA*.[3] Therefore, the International Committee on Systematics of Prokaryotes (ICSP) recommends that by *ompA* sequencing *C. trachomatis* strains can be classified as different genovariants and genotypes.[4]

Among the male population, *C. trachomatis* can lead to non-gonococcal urethritis or even complication, such as epididymitis. The literature shows rectal chlamydia can present as acute proctitis if infected by non-LGV serovars, but the symptoms are often milder, or even asymptomatic compared with those infected with LGV serovars.[5] Not surprisingly, the reported prevalence rates of rectal chlamydial infections among men who have sex with men (MSM) varied among countries.[6] In the past decade, the prevalence of *C. trachomatis* infection among the male population in Taiwan has only been investigated in two studies conducted in northern Taiwan by the active screening of urine or rectal swabs samples.[7,8] Of note, Lin et al. have found among HIV-infected MSM the detection yield rate of *C. trachomatis* was more common in rectal swabs than in urine samples.[8] However, the epidemiological information on *C. trachomatis* infection, especially rectal chlamydia in southern Taiwan is scarce.

LGV, an STD caused by one of three *C. trachomatis* genotypes, L1, L2, and L3, has been known as an endemic STD in tropical areas, presenting with genital skin lesions and inflammation of regional lymph nodes (so-called buboes).[9] In contrast with typical LGV, rectal LGV can lead to anorectal symptoms, such as anal pain, anal discharge, tenesmus, bloody discharge, diarrhea, or lower abdominal cramps, but rarely inguinal lymphadenopathy. Compared to non-LGV rectal chlamydia, rectal LGV commonly results in acute proctitis, requires longer antibiotic treatment, and may be confronted with complications, such anal stricture or megacolon.[10]

Although endemic in tropical areas, typical LGV had been rarely seen in industrialized countries for decades. Rectal LGV was occasionally reported among MSMs in the 1980s and has re-emerged among MSM since 2003 in Europe, North America, and Australia.[11–16] Of note, these LGV outbreaks were predominantly caused by the genovariant L2b, and the affected MSM were more often to be co-infected with human immunodeficiency virus (HIV) or hepatitis C virus (HCV).[17] Several studies in Asian countries, including China, Thailand, and Taiwan, have focused on the detection and genotyping of *C. trachomatis* in urine samples or rectal swabs from MSM in the past two decades,[7,18–22] but LGV was not found in these published investigations. Therefore, we conducted a cross-sectional surveillance study among MSM with or without HIV in southern Taiwan, to investigate the prevalence, clinical characteristics, and molecular epidemiology of rectal chlamydia.

Material & methods

Study setting and definitions

From January 2020 to April 2022, a cross-sectional surveillance study to detect chlamydia, gonorrhea and other enteric pathogens was conducted. MSM coming for the anonymous voluntary counselling and testing (VCT) for STDs, pre-exposure prophylaxis (PrEP), or antiretroviral therapy against HIV infection in a tertiary medical center in southern Taiwan, were invited. All enrolled participants

have given their written informed consent, if they were PrEP users or HIV-infected patients who were followed up at the outpatient clinics of the study hospital. Those who came for the anonymous VCT did not sign their name on the informed consent but a code consisting of birth year and the initial alphabet and the last four digits of the identification card number. The participants were asked to complete a QR code-based questionnaire for age, sexual behavior, diet habit, gastrointestinal symptoms, substance abuse, current medication, prior STDs, underlying chronic diseases, and family history of irritable bowel disease or psychiatric disorder. For symptomatic participants, the relevant anorectal symptoms, including anal pain, anal discharge, tenesmus, bloody discharge, diarrhea, or lower abdominal cramps, were queried in the QR code-based questionnaire. For HIV-infected participants enrolled from the anonymous VCT or the HIV outpatient clinic, clinical information was retrieved from their electronic medical records and recorded in a standardized case record form, including recent CD4 counts and plasma HIV-1 viral loads, antibiotic therapy, and prior STDs (such as gonorrhea, genital warts, or syphilis), or hepatitis virus infections. Chemsex was defined as the use of illicit drugs to intensify the pleasure of the sexual activity. The presence of diarrhea for at least 14 days was referred to have chronic diarrhea. HCV infection was defined by seropositivity for HCV. The clinical diagnosis of gonorrhea required the isolation of *Neisseria gonorrhoeae* or detection of the nucleic acid of *N. gonorrhoeae* by polymerase chain reaction (PCR) in relevant clinical specimens. The first episode of syphilis was diagnosed by the presence of a reactive titer of the rapid plasma regain/Venereal Disease Research Laboratory (RPR/VDRL) test and a reactive *Treponema pallidum* haemagglutination (TPHA) assay, and recurrent syphilis was defined as the occurrence of an at least 4-fold increase titer of the RPR/VDRL test in the individuals with a prior history of syphilis.

Laboratory investigations

To detect chlamydia, gonorrhea, and other enteric pathogens, fecal samples were collected from the participants. Each fecal sample was submitted for three multiplex PCR tests, *i.e.*, BD Max™ CT/GC Assay, BD Max™ Enteric Parasite Panel, and BD Max™ Enteric Bacterial Panel (BD Diagnostics, Franklin Lakes, U.S.A) to detect *C. trachomatis*, *N. gonorrheae*, *Entamoeba histolytica*, *Cryptosporidium*, *Giardia lamblia*, shiga-toxin producing *Escherichia coli*, *Shigella*, *Salmonella*, and *Campylobacter*, according to the manufacturer's instruction. Fecal specimens were collected unpreserved in a clean container and transported to the microbiology laboratory at room temperature when they were available in the study hospital, or refrigerated if unable to be transported to the laboraotory within 6 h. Before further molecular typing, the residual specimens were stored at −20 °C.

For *Chlamydia*-positive fecal samples, further genotyping study of *C. trachomatis* was performed by amplification and sequencing of *ompA*, as described previously.[7] In brief, DNA was extracted from the *Chlamydia*-positive fecal samples by the QIAamp PowerFecal DNA kit (Qiagen, Hilden, Germany), and extracted DNA was sequenced by a nested PCR across four variable domains (VS1-VS4) of *ompA* by the 1130-bp outer primer pair NLO/NRO and the 584-bp inner primer pair MOMP87/C214. Electrophoresis result was demonstrated by staining with ClearVision DNA stain (Protech Technology Enterprise, Taipei, Taiwan), and the PCR sequences of *ompA* were compared to those of reference *C. trachomatis* strains, including the genotype B (accession number JN795441), Da (JN795439), E (JN795438), G (JN795436), J (JN795432), and L2b (JN795427) registered in the genetic sequence database, GenBank (http://www.ncbi.nlm.nih.gov/GenBank).

Statistical analysis

All statistical analyses were performed by the statistical software IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., USA). The chi-square or Fisher's exact test was used to compare categorical variables, and an independent Student *t*-test to compare continuous variables between groups. The Mann—Whitney U test was performed to compare two independent groups, when the dependent variable was either ordinal or continuous, without normal distribution. Multivariable logistic regression, including age as a continuous variable, was performed to find out the risk factor of rectal chlamydia. A two-tailed *P* value of less than 0.05 was considered to be statistically significant.

Ethics statement

The study was approved by the Institutional Review Board of National Cheng Kung University Hospital (B-BR-108-068).

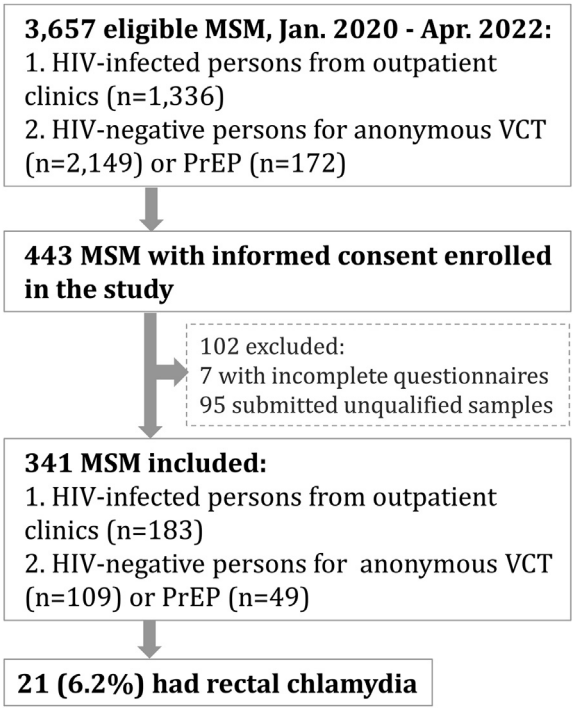

Figure 1. The flowchart of the present surveillance among men who have sex with men (MSM).
Footnote: HIV, human immunodeficiency virus. VCT, voluntary counseling and testing. PrEP, pre-exposure prophylaxis.

This study was conducted according to the principles expressed in the Declaration of Helsinki.

Results

During the study period, from January 2020 to April 2022, a total of 3657 MSM coming for antiretroviral agents in the HIV clinic, anonymous VCT, or PrEP in there study hospital, were eligible for participating the study. There were 443 (12.1%) MSM providing their written informed consent. However, 95 (21.4%) of 443 participants were excluded from this study due to the submission of unqualified stool sample, and 7 (1.6%) excluded due to incomplete questionnaires. Overall 341 (77.0%) completed the questionnaire and also provided their fecal samples included in the present study (Fig. 1). The median age of all participants was 32.0 years (interquartile range, 27.5–37.3), and 183 (53.7%) were people living with HIV (PLWH). None of them was commercial sex workers. There were 56 (16.4%) tested positive for at least one pathogen by the combination of three multiplex PCR assays for the detection of nine pathogens. Of note, twenty-one (6.2%) of all participants had rectal chlamydia due to the detection of *C. trachomatis* in fecal samples, more than a half (12, 57.1%) were PLWH, and ten (47.6%) had a prior history of syphilis. Eleven (52.4%) cases of rectal chlamydia were asymptomatic; four (19%) had co-infection with rectal gonorrhea (Table 1). Compared to MSM without rectal chlamydia, the percentage of rectal gonorrhea among MSM

Table 1 Characteristics of men who have sex with men with and without rectal chlamydia.

Clinical variables	With rectal chlamydia (n = 21)	Without rectal chlamydia (n = 320)	*P* value
Age (median, interquartile range)	30.80 (24.60–34.70)	32.00 (27.60–34.38)	0.138
Anorectal symptoms	47.6 (10/21)	40.9 (131/320)	0.547
Sexual activity in the past year	95 (19/20)	77.2 (247/320)	0.154
Sexual partners in the past year			0.019
0	5 (1/20)	16.8 (52/310)	
1	35 (7/20)	23.2 (72/310)	
2–5	30 (6/20)	41.3 (128/310)	
6–10	10 (2/20)	11.6 (36/310)	
11–20	5 (1/20)	4.8 (15/310)	
21–30	0 (0/20)	0.3 (1/310)	
>30	15 (3/20)	1.9 (6/310)	
Sexual behaviors			
Condomless sex	65 (13/20)	69.1 (221/320)	0.548
Oroanal sex	15 (3/20)	22.6 (70/310)	0.429
Receptive anal sex	56.3 (9/16)	52.4 (108/206)	0.768
Insertive anal sex	37.5 (6/16)	33.5 (69/206)	0.744
Chemsex	4.8 (1/21)	9.7 (30/310)	0.454
Underlying disease or co-infection			
Human immunodeficiency virus infection	57.1 (12/21)	53.4 (171/320)	0.742
Prior syphilis	43.8 (7/16)	28.8 (69/240)	0.204
Chronic diarrhea	25.0 (5/20)	15.2 (47/309)	0.245
Co-infection with rectal gonorrhea	19.0 (4/21)	2.2 (7/320)	<0.001
Family history of irritable bowel syndrome	5.0 (1/20)	10.6 (33/310)	0.421
Psychiatric disease	5.0 (1/20)	4.5 (14/310)	0.920
Medication in the past three months			
Antibiotics	15 (3/20)	21.0 (65/310)	0.522
Pre-exposure prophylaxis	9.5 (2/21)	14.7 (47/320)	0.513
Proton pump inhibitors	0 (0/20)	2.6 (8/309)	0.466
Detection of enteric pathogens other than chlamydia and gonorrhea by multiplex PCR	19.0 (4/21)	9.4 (30/320)	0.145
Genotypes of *Chlamydia trachomatis* (n = 19)[a]			
B	4 (21.1)		
Da	5 (26.3)		
G	2 (9.5)		
J	3 (14.3)		
L2b	5 (26.3)		

[a] The number of *Chlamydia*-positive stool samples was sequenced and genotyped.
Data are expressed as case number (%) or median (interquartile range).

Table 2 Risk factors of rectal chlamydia among men who have sex with men by multivariable logistic regression.

Variables	Adjusted odds ratio	95% confidence interval	*P* value
Coinfection of rectal gonorrhea	6.78	1.44–31.91	0.015
Coinfection of human immunodeficiency virus	2.91	0.82–10.32	0.098
Prior history of syphilis	2.30	0.76–6.97	0.140
Multiple sexual partners	1.37	1.00–1.88	0.048
Age	0.91	0.82–1.01	0.913

with rectal chlamydia was significantly higher (19.0% *vs.* 2.2%, $P < 0.001$), and had more sexual partners in the past year ($P = 0.019$). There was no difference in the prevalence rate of rectal chlamydia among MSM with and without HIV infection (6.6% *vs.* 5.7%, $P = 0.82$). No significant difference was present between groups, in terms of age, anorectal symptoms, underlying medical diseases, recent medications, risky sexual behaviors including condomless sex or oroanal sex, or detection of enteric pathogens other than chlamydia and gonorrhea by three multiplex PCR assays in our study (Table 1). Age-adjusted multivariable logistic regression analysis found two independent risk factors of rectal chlamydia, *i.e.*, co-infection with rectal gonorrhea (adjusted odds ratio [AOR] 6.59, 95% confidence interval [CI] 1.39–31.31, $P = 0.018$) and multiple sexual partners (AOR 1.48, 95% CI 1.00–1.88, $P = 0.048$), as shown in Table 2.

Among 19 of 21 cases of rectal chlamydia, *ompA* could be amplified and sequenced from their specimens. In addition to L2b (5 cases, 26.3%), the identified genotypes or genovariant of *C. trachomatis* included Da (5, 26.3%), B (4, 21.1%), J (3, 14.3%), and G (2, 9.5%). Of 14 participants with non-LGV rectal chlamydia, those with genotype B, G, or J, as well as three of the five cases with genotype Da were all asymptomatic. Compared to non-LGV rectal chlamydia, the cases of rectal LGV were often symptomatic (100% *vs.* 35.7%, $P = 0.033$). Moreover, four (80%) cases of rectal LGV had HIV coinfection or a prior history of syphilis, and two (40%) had recent shigellosis caused by ciprofloxacin-resistant *Shigella flexneri* within one year and had received antibiotic treatment before this surveillance

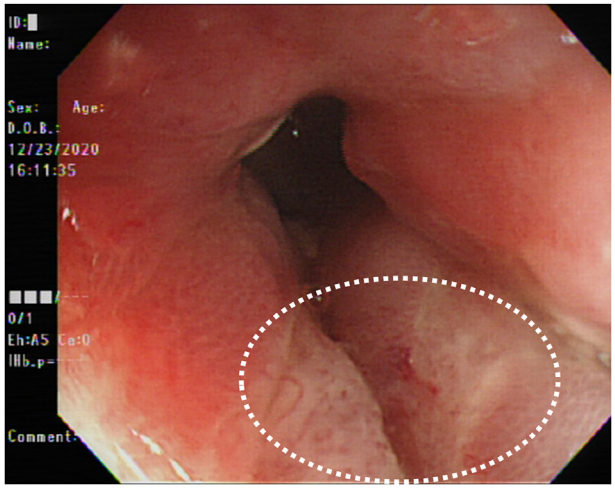

Figure 2. Endoscopic findings of rectal lymphogranuloma venereum: small ulcers and swelling mucosa surrounding the anus (dotted white circle).

(Table 3). Only one had chronic hepatitis C infection. One case with recent shigellosis (case 1 in Table 3) underwent colonoscopy because diarrhea did not resolve after the initiation of antimicrobial therapy for shigellosis. Multiple anal ulcers and perianal swelling were noted (Fig. 2). All rectal LGV cases received oral doxycycline, 100 mg twice daily, for at least 14 days, and their anorectal symptoms resolved uneventfully (Table 3).

Discussion

To our knowledge, this is the first report describing the epidemiology and clinical characteristics of rectal chlamydia in southern Taiwan among MSM with and without HIV infection, but also the first to report rectal LGV due to the genovariant L2b in the Far East. Most of our cases of rectal chlamydia were asymptomatic or mildly symptomatic, but those with rectal LGV had bothersome anorectal symptoms. An earlier study in northern Taiwan showed that 22.1% of HIV-infected patients had rectal chlamydia, detected by the BD MAX™ CT/GC/TV triplex assay.[8] Our study conducted in southern Taiwan noted that the corresponding result was 6.2%. Such a discrepancy may be related to

Table 3 Characteristics of five men who have sex with men with rectal lymphogranuloma venereum due to genovariant L2b.

Case	Age, year	Clinical symptoms	HIV	CD4 lymphocyte count (/mm^3)	Plasma HIV viral load (copies/ml)	Hepatitis C virus	Prior history of syphilis	Co-infections	Oral doxycycline, duration
1	35.4	Chronic diarrhea, abdominal cramps	+	764.3	Not detected	–	+	Shigellosis	21 days
2	32.0	Diarrhea	+	545	60.9	–	+	Shigellosis, gonorrhea	14 days
3	42.0	Diarrhea, tenesmus	+	1000	Not detected	+	+		14 days
4	20.0	Diarrhea	+	836	<20	–	–		14 days
5	28.9	Loose stool, anal pain, anal discharge	–	Not available	Not available	–	+	Anal condyloma	28 days

HIV, human immunodeficiency virus.

different samples collected for multiplex PCR test (feces versus rectal swab), behavioral changes during the COVID-19 pandemic, or geographical variation.

A study in northern Taiwan reported that two commercial multiplex real-time PCR assays, i.e., BD Max CT/GC/TV assay and Allplex Essential STI Assay (Seegene, South Korea), both had a good performance in detection of pathogens from urine and rectal swab.[25] *C. trachomatis* has well known to be able to colonize the gastrointestinal tract and pose fecal shedding and fecal-oral transmission.[26] Besides, enteric bacterial or parasitic pathogens might be found in fecal samples among asymptomatic[23] and symptomatic[24] MSM. Therefore, our study preferred fecal samples, which are larger in volume than rectal swabs, to investigate the prevalence of chlamydia, gonorrhea, and concurrent enteric pathogens among MSM in southern Taiwan.

Molecular screening of *C. trachomatis* in different anatomical sites among symptomatic or asymptomatic MSM has been conducted in China, Thailand, and Taiwan (Fig. 3). Among *Chlamydia*-positive samples, genotype D was the most prevalent one. However, *C. trachomatis* genotype B, traditionally considered to be associated with trachoma, has been occasionally found in surveillance studies in Taiwan and China (Fig. 3), and also in trachoma-free countries such as Spain.[27] The reason why such a trachoma-causing pathogen could be detected from urogenital or rectal samples remains uncertain. In this study, we found that 4 of 19 cases of rectal chlamydia were caused by genotype B, where trachoma has no longer been a public health issue since the 1980s in Taiwan.[28] The percentage of genotype B seemed higher than in prior investigations. All the rectal chlamydia cases with genotype B in this study were asymptomatic and could be overlooked without active surveillance. A large-scale, nationwide surveillance on different anatomical sites (i.e., urine, pharynx, and rectum) may be needed to elucidate the molecular evolution of *C. trachomatis* strains among MSM.

In many countries including Taiwan, there is an ongoing spread of enteric pathogens among MSM, a healthcare issue of paramount importance requiring the public awareness. Infectious gastroenteritis, including amebiasis, giardiasis, shigellosis, and campylobacteriosis, used to be a public health concern in poor sanitary areas for centuries, but now turns out STDs among MSM in developed countries.[29–32] In our study, anorectal symptoms and chronic diarrhea were self-reported among MSM participants, regardless of HIV infection, and among MSM without rectal chlamydia, 10.9% (35/320) harbored other potential enteric pathogens or gonorrhea detected by a multiplex PCR panel for gastroenteritis pathogens (Table 1). Since 2015, domestic cases of shigellosis in Taiwan have been predominantly noted in MSM or HIV-infected patients, with increasing antimicrobial resistance and changing predominant strains.[32–34] In contrast to no shigellosis recognized among MSM with non-LGV rectal chlamydia, two of five patients with rectal

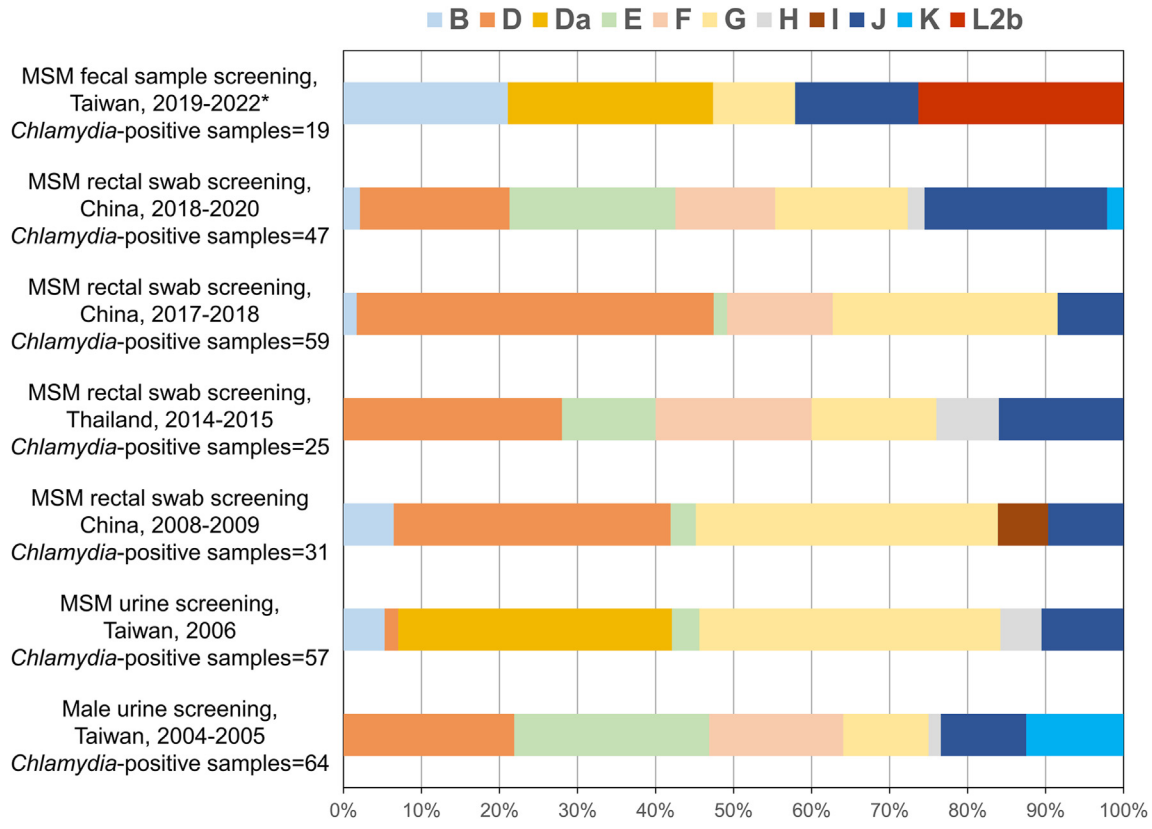

Figure 3. Genotype or genovariant distribution of *Chlamydia trachomatis* among men who have sex with men in Asian countries.
Footnote: The superscripts indicate the reference numbers in the manuscript.
*The present study.

LGV were co-infected with recent shigellosis. Further investigations of the biological or epidemiological interactions between rectal LGV and other gastroenteritis or STD pathogens are necessary.

LGV, an endemic STD in tropical areas, could be seen in southeast Asia during the 1990s.[35] While rectal LGV among MSM has spread throughout western countries since 2003 and changing molecular epidemiology has been reported in several countries in Europe and the Middle East[14,16,36]; however, rectal LGV among MSM has not been reported in the Far East until this study. The differentiation of rectal LGV from rectal chlamydia is essential for early diagnosis and appropriate antimicrobial therapy. For non-LGV rectal chlamydia, seven days of oral doxycycline therapy remains the preferred regimen.[37] In contrast, clinical manifestations of rectal LGV may be more severe than rectal chlamydia, and rectal LGV requires a longer course of doxycycline treatment for cure. To facilitate early diagnosis of rectal LGV among the suspected cases of acute proctitis, real-time multiplex PCR tests can be utilized.[38] Since 2018, universal screening of rectal LGV has been implemented among all rectal chlamydia patients aged 25 years and above in Australia, and the result showed that more than one-third of rectal LGV cases were asymptomatic.[39] Such surveillance strategy can be considered to curb the transmission of the emerging rectal LGV in Taiwan. To achieve a high microbiological cure rate in the treatment of rectal LGV, a meta-analysis preferred 21 days of oral doxycycline therapy at the dose of 100 mg twice daily.[40]

There are some limitations in this research. First, this is a single-center study in southern Taiwan, and more than 80% of the eligible MSM did not participate or were excluded from the surveillance. Therefore, potential selection bias should be considered. Second, our work included three categories of MSM, including those with HIV infection, VCT, or PrEP. They could have or present diverse social and medical behaviors with different acquisition risks of STDs. The overall prevalence of rectal chlamydia may not exactly reflect the true prevalence in these subgroups of MSM. Third, besides the genotyping of *Chlamydia*-positive fecal samples, further multiple-locus variable number tandem repeat analysis or whole-genome sequencing to explore the genetic relatedness of *Chlamydia* isolates to those in other areas, may be considered to track the global transmission network.

In conclusion, the prevalence rate of rectal chlamydia among MSM in southern Taiwan during the era of COVID-19, 2020—2022, was 6.2%. Besides, the worldwide-circulating strain of lymphogranuloma venereum, L2b, was found among symptomatic MSM with rectal chlamydia. Clinicians in Taiwan must be aware of the likelihood of rectal LGV among the risky population with acute proctitis, for whom oral doxycycline therapy for three weeks is warranted.

Acknowledgments

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