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Chlamydia trachomatis infection among at-risk populations in Taiwan: Emergence of genovariant L2b and treatment response to antimicrobials

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ABSTRACT

Background: Chlamydia trachomatis infection is one of the most common sexually transmitted infections (STIs). This study investigated the prevalence and genotype distribution of *C. trachomatis*, and treatment response, focusing on the recent emergence of genovariant L2b in Taiwan.

Methods: This prospective study was conducted from August 2021 to September 2023, enrolling 1023 participants, including 492 people who received pre-exposure prophylaxis (PrEP) for HIV and 531 people with HIV (PWH). Multiple-site sampling and genotyping of *C. trachomatis* identified were performed. Participants testing positive for *C. trachomatis* received a 7-day course of doxycycline and test-of-cure (TOC) assessments were conducted post-treatment.

Results: Among the participants, mostly MSM (92.7%), 26.4% tested positive for *C. trachomatis,* with 77.8% identified in the rectum. The prevalent genotypes were J (26.4%), G (24.0%), and B (17.7%). Treatment with a 7-day course of doxycycline resulted in clearance for most cases (91.1%). Genotyping investigations for those repeatedly testing positive (8.9%) showed reinfections with different genotypes. Eighteen cases of asymptomatic rectal carriage of genovariant L2b were detected, predominantly in PWH (88.9%). The L2b genovariant was cleared with 7 days of doxycycline on TOC assessments. The prevalence of L2b increased over time, with multi-locus sequence typing showing ST53 as the predominant strain.

Conclusions: C. trachomatis was prevalent among PWH and PrEP users and the prevalence of L2b genovariant was increasing in Taiwan. A 7-day course of doxycycline was effective in clearing L2b genovariant in asymptomatic participants. Continued surveillance to monitor the evolving epidemiology of chlamydia in Taiwan is warranted.

Introduction

Chlamydia trachomatis infection is one of the most prevalent bacterial sexually transmitted infections (STIs) worldwide. *C. trachomatis* used to be classified into three serogroups by serotyping of the major outer membrane protein (MOMP) and now can be genotyped by the MOMP-

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encoding gene, *ompA*.[1] The genotypes A, B, Ba, and C can cause trachoma, while the genotypes D through K may result in a wide spectrum of STIs. Of note, the genotypes L1, L2, and L3 will cause lymphogranuloma venereum (LGV), which can manifest as invasive urogenital infections. To curb transmission of *C. trachomatis*, prior studies have conducted multiple-site sampling to improve the diagnostic yield and detect more cases for further treatment.[2]

Rectal LGV, presenting as acute proctitis, has been noted among men who have sex with men (MSM) in Western countries since 2003[3] and was mainly caused by the genovariant L2b.[4] Host factors associated with rectal LGV included HIV infection, recent hepatitis C virus infection, group sex party, and recent use of anal enema.[5–7] If untreated, rectal LGV may cause complications, such as megacolon, anal stricture, or colorectal fistula.[8]

Although rectal LGV has been present in Western countries for decades and the recombinant strain L2b/D-Da has been spreading to Israel,[9] it has not been reported in other Asian countries; until recently, a study in Taiwan reported five symptomatic cases due to *C. trachomatis* L2b.[10] Longitudinal follow-up studies are lacking in Asia focusing on the treatment response of chlamydial infections to antimicrobials. Therefore, the current surveillance study aimed to investigate the prevalence of chlamydia among at-risk populations by multiple-site sampling, to assess the genotype distribution of *C. trachomatis*, to examine the treatment response to antimicrobials, and to evaluate the genetic relatedness of genovariant L2b identified.

Methods

Study setting and population

This prospective study was conducted at the National Taiwan University Hospital, a tertiary center for HIV care in northern Taiwan. From August 31, 2021 to September 1, 2023, adult clients seeking pre-exposure prophylaxix (PrEP) for HIV and people with HIV (PWH) who underwent clinical evaluation and treatment of STIs were enrolled. At screening, medical records were reviewed and an anonymous, self-administered questionnaire interview was performed, along with evaluation by symptoms and physical examinations. Clinical data of PWH were obtained from their electronic medical records and collected in a standardized case record form. The collected details encompassed recent CD4 count, plasma HIV RNA load, ongoing antibiotic therapy, and the history of STIs, such as gonorrhea, genital warts, and syphilis. Cases of syphilis with a rapid plasma reagin (RPR) titer $\geq$4 or existence of related syphilis symptoms at the time of chlamydia testing or within the past 3 months were recorded. Several STI pathogens, including *Treponema pallidum*, *Neisseria gonorrhoeae*, *Mycoplasma genitalium*, *M. hominis*, *Ureaplasma urealyticum*, *U. parvum*, and *Trichomonas vaginalis*, were tested at the same time. Screening tests were performed on the specimens concurrently collected from multiple sites (urethral swab, rectal swab, oral rinse, and vaginal swab).[11] The study was approved by the Research Ethics Committee of National Taiwan University Hospital (registration number, NTUH-201811021RINA) and the participants provided written informed consent before inclusion.

Laboratory investigations of etiologies of STIs

The clinical specimens (oral rinse and urethral, rectal swab, and vaginal swab) were collected with the use of the collection kit (BD MAX UVE Specimen Collection Kit; Becton, Dickinson and Company, Sparks, MD, USA) and tested by a multiplex real-time PCR assay (Allplex™ STI Essential Assay; Seegene Inc, Seoul, Republic of Korea) to identify *C. trachomatis*, *N. gonorrhoeae*, *M. genitalium*, *M. hominis*, *U. urealyticum*, *U. parvum*, and *T. vaginalis*.

Genotyping of C. trachomatis

The genotypes of *C. trachomatis* were investigated through the amplification and sequencing of *ompA* by following the established procedures.[12] In brief, DNA was extracted from *Chlamydia*-positive samples using the QIAamp DNA mini kit (Qiagen, Hilden, Germany), and underwent sequencing via nested PCR targeting four variable domains (VS1-VS4) of *ompA*. The initial 1130-bp outer primer pair, comprising NLO (5′-ATGAAAAAACTCTTGAAATCG-3′) and NRO (5′-CTCAACTGTAACTGCGTATTT-3′), was used, followed by the 584-bp inner primer pair, including MOMP87 (5′-TGAACCAAGCCTTATG ATCGACGGA-3′) and C214 (5′-TCTTCGAYTTTAGGTTTAGATTGA-3′). Electrophoresis results were visualized by the ClearVision DNA stain (Protech Technology Enterprise, Taipei, Taiwan). The *ompA* PCR sequences were compared to those of *C. trachomatis* reference strains (genotype B: accession number AM179410.1, D: JN795440, Da: JN795439, E: JN795438, F: JN795437, H: JN795435, G: JN795436, J: JN795432, K: JN795430, and L2b: JN795427) available in the GenBank (http://www.ncbi.nlm.nih.gov/GenBank).

Multi-locus sequence typing (MLST) of C. trachomatis genovariant L2b

To better disclose the clonal relatedness of *C. trachomatis* genovariant L2b identified in our study, multi-locus sequence typing (MLST) was performed according to the Uppsala scheme available at PubMLST (https://pubmlst.org). Based on the database of PubML, each sequence type (ST) was assigned by analyzing the allelic composition at five highly variable genomic loci (non-housekeeping genes), namely *hctB* (*CT046*), *CT058*, *CT144*, *CT172*, and *pbpB* (*CT682*). To investigate the relevance for common STs within *C. trachomatis* population, a BURST analysis was carried out to categorize strains into distinct groups based on their MLST patterns. The potential ancestral type (AT) was identified by examining the frequency of single-locus variants (SLV), double-locus variants (DLV), and satellites (SAT) among STs within the same group. For major groups, a corresponding diagram was created. In addition, a population snapshot was constructed to visually depict the evolutionary connections between the STs found in our study and those in the database.

Outcomes and follow-up

Patients confirmed to be infected with *C. trachomatis* were treated with a 7-day course of oral doxycycline (100 mg twice daily) according to the treatment guidelines.[13] Test-of-cure (TOC) assessment at one to six months after the completion of antimicrobial treatment was performed during follow-up visits.[13]

Statistical analysis

All statistical analyses were performed using the statistical software IBM SPSS Statistics for Windows, version 22.0 (IBM Corp., USA). The chi-square test or Fisher's exact analysis was used to compare the differences between categorical variables. An independent Student's *t*-test was applied to compare continuous variables in the cases of normal distribution A Mann-Whitney *U* test was performed to compare two independent groups when the dependent variable was either ordinal or continuous without normal distribution. A two-tailed *P* value of less than 0.05 was considered to be statistically significant.

Results

Demographic and clinical characteristics of the participants

During the 2-year study period, 1023 participants were enrolled and tested for *C. trachomatis* infection and other STIs. Of the 1023 participants, 492 (48.1%) were PrEP users and 531 (51.9 %) were PWH. Their

median age was 33.5 years (interquartile range, 29–40 years), and 975 (95.3%) were male. Among the 531 PWH, most (97.4%; n = 517) had been receiving antiretroviral therapy with a median CD4 cell count of 625 cells/mm^3 and PVL <200 copies/mL in 96.6% (n = 513) at enrollment. Of 1023 participants, MSM accounted for 92.7% (n = 948), heterosexuals 6.3% (n = 64), and bisexuals 0.9% (n = 10) (Table 1).

Characteristics of participants with C. trachomatis infection

Among the 1023 participants, 270 (26.4%) tested positive for *C. trachomatis*. The overall prevalence of *C. trachomatis* infection was 27.3% (n = 145/531) and 25.4% (n = 125/492) in PWH and PrEP users, respectively. Regardless of the status of chlamydia infection, syphilis was common as shown by 42.2% in those testing positive and 39.4% in those testing negative for *C. trachomatis*. For the 411 cases of syphilis, 33 (8.0%) were primary syphilis, 121 (29.4%) secondary syphilis, 221 (53.8%) early latent syphilis, 3 (0.7%) late latent syphilis, and 33 (8.0%) syphilis with unknown duration (Table 1).

The prevalence of gonorrhea was 21.6% (n = 221) among the participants with a significantly higher prevalence in those testing positive for *C. trachomatis* (30.9%, n = 81/270) than those testing negative (18.6%, n = 140/753) ($P < 0.0001$). *U. urealyticum* was the most prevalent co-pathogen being detected, followed by *M. hominis*, and *M. genitalium*. *U. urealyticum* and *M. hominis* infections were also significantly more prevalent among participants testing positive than those testing negative for *C. trachomatis* (Table 1).

For 270 paricipants testing positive for *C. trachomatis*, most were male (98.9%, n = 267) and MSM (95.2%, n = 257) with a median age of 33.1 years. The most prevalent site tested positive for *C. trachomatis* was the rectum (77.8%, n = 210/270) in PWH (90.3%, n = 131/145) or PrEP users (63.2%, n = 79/125), followed by urethral orifice (22.9%, n = 62/270) and oral cavity (11.1%, n = 30/270). Besides, 25 (9.3 %) participants had more than one site tested positive for *C. trachomatis* (11 % in PWH and 7.2% in PrEP users). As compared to PrEP users, PWH had a higher prevalence of syphilis (71.0% [103/145] *vs.* 8.8% [11/125]; $P < 0.00001$) or gonorrhea (37.3% *vs.* 21.1%; $P = 0.002$). Likewise, *U. urealyticum* coinfection was common among the participants testing positive for *C. trachomatis* (52.2%, n = 141/270), including 83 PWH (57.2%) and 58 PrEP users (46.4%). *M. hominis* and *M. genitalium* coinfection was present in 25.6% and 9.6%, respectively, of the participants testing positive for *C. trachomatis* (Table 2).

Of the 270 participants who tested positive for *C. trachomatis*, 201 (74.4%) were asymptomatic. Among the participants with symptoms (n = 69), presentations included skin rash (62.3%), urethral discharge (13.0%), anal ulcer with bleeding or discharge (8.7%), penile ulcer (7.2%), diarrhea (2.9%), sore throat (2.9%), and oral ulcer (2.9%). Of the 173 participants with rectal chlamydia (210 positive samples), 7 (4.0%) reported rectal symptoms, including anal discharge (n = 3), anal ulcer with bleeding (n = 2), and diarrhea (n = 2); six of these seven participants (85.7%) with rectal symptoms had concurrent rectal STIs: gonorrhea (n = 2), syphilis (n = 1), *M. genitalium* infection (n = 1), gonorrhea plus syphilis (n = 1), *U. urealyticum* infection plus syphilis (n = 1), and gonorrhea plus *M. hominis, U. urealyticum* and *M. genitalium* infection (n = 1). Of the 57 participants with urethral infections, five (8.8%) were symptomatic with urethral discharge (n = 4) and penile ulcer (n = 1); four of them (80.0%) had concurrent urethral STIs: gonorrhea (n = 2), syphilis (n = 1), and syphilis plus *U. urealyticum* infection (n = 1). Of the 29 participants with *C. trachomatis* identified in oral rinse specimens, two (6.9%) were symptomatic with sore throat (n = 1) and oral ulcer (n = 1), with one having concurrent *U. urealyticum*

Table 1
Baseline characteristics of participants tested positive and negative for *Chlamydia trachomatis*.

Variable	With *Chlamydia* infection (n = 270)	Without *Chlamydia* infection (n = 753)	Overall (n = 1023)	*P* value
Male gender	267 (98.9)	708 (94.1)	975 (95.3)	0.516
Age, years	33.1 (28–38)	33.8 (29–40)	33.5 (29–40)	0.054
PrEP users	125 (46.4)	367 (48.7)	492 (48.1)	0.707
People with HIV	145 (53.6)	386 (51.3)	531 (51.9)	0.316
Receiving antiretroviral therapy	147 (99.3)	370 (95.9)	517 (97.4)	0.077
Median CD4 count, cells/mm^3	627 (472–814)	622 (486–814)	625 (479–814)	0.661
PVL <200 copies/ml	144 (97.3)	369 (95.6)	513 (96.6)	0.511
MSM	257 (95.2)	691 (91.8)	948 (92.7)	0.064
Heterosexuals	10 (3.7)	54 (7.2)	64 (6.3)	0.044
Bisexuals	3 (1.1)	7 (0.9)	10 (0.9)	0.920
PWID	0	1 (0.13)	1 (0.10)	N/A
Syphilis	114 (42.2)	297 (39.4)	411 (40.1)	0.424
Other etiology of STIs				
Neisseria gonorrhoeae	81 (30.0)	140 (18.6)	221 (21.6)	<0.001
Mycoplasma genitalium	26 (9.6)	92 (12.2)	118 (11.5)	0.253
Mycoplasma hominis	69 (25.6)	128 (16.9)	197 (19.3)	0.002
Ureaplasma urealyticum	141 (52.2)	302 (40.1)	443 (43.3)	<0.001
Ureaplasma parvum	13 (4.8)	56 (7.4)	69 (6.7)	0.141
Trichomonas vaginalis	0	0	0	N/A

Data are expressed as case number (%) or median (interquartile range). **Abbreviations:** MSM, men who have sex with men; N/A, not available; PrEP, pre-exposure prophylaxis; PVL, plasma HIV RNA load; PWID, people who inject drugs; STIs, sexually transmitted infections.

Table 2
Clinical characteristics and the results of test-of-cure assessments among participants seeking pre-exposure prophylaxis and people with HIV who tested positive for *Chlamydia trachomatis*.

Variable	PWH (n = 145)	PrEP users (n = 125)	Overall (n = 270)	*P* value
Male gender	145 (100)	122 (97.6)	267 (98.9)	0.512
Age, years	35.8 (32–41)	29.9 (27–34)	33.1 (28–38)	<0.001
MSM	143 (98.6)	114 (91.2)	257 (95.2)	0.011
Heterosexuals	0	10 (8)	10 (3.7)	0.007
Bisexuals	2 (1.4)	1 (0.8)	3 (1.1)	0.897
Positive sampling site				
Urethral orifice	39 (26.9)	23 (18.4)	62 (22.9)	0.098
Rectum	131 (90.3)	79 (63.2)	210 (77.8)	<0.001
Oral cavity	18 (12.4)	12 (9.6)	30 (11.1)	0.463
Multiple sites (≥2 sites)	16 (11.0)	9 (7.2)	25 (9.3)	0.278
Syphilis	103 (71.0)	11 (8.8)	114 (42.2)	<0.001
Concurrent STIs				
Neisseria gonorrhoeae	54 (37.2)	27 (21.6)	81 (30.0)	0.002
Mycoplasma genitalium	14 (9.7)	12 (9.6)	26 (9.6)	0.987
Mycoplasma hominis	50 (34.5)	19 (15.2)	69 (25.6)	<0.001
Ureaplasma urealyticum	83 (57.2)	58 (46.4)	141 (52.2)	0.075
Ureaplasma parvum	6 (4.1)	7 (5.6)	13 (4.8)	0.575
Trichomonas vaginalis	0	0	0	N/A
Treatment received	145 (100)	105 (84)	250 (92.6)	0.324
Number of treatment course provided	160	125	285	
TOC assessments performed	73	96	169	
Positive results	9 (12.3)	6 (6.3)	15 (8.9)	0.169

Data are expressed as case number (%) or median (interquartile range). **Abbreviations:** MSM, men whohave sex with men; PrEP, pre-exposure prophylaxis; PWH, people with HIV; STIs, sexually transmitted infections; TOC, test-of-cure.

infection and syphilis.

Treatment and follow up of participants testing positive for C. trachomatis

Among the 270 participants testing positive for *C. trachomatis*, 250 (92.6%) participants received oral doxycycline at a twice daily dose of 100 mg for 7 days, with 30 (12.0%) participants receiving more than one course of treatment. A total of 285 treatment courses, including 160 for PWH and 125 for PrEP users, were administered during the study period. TOC assessment was performed in the participants who had follow-up visits after the completion of antimicrobial treatment and a total of 169 TOC visits were completed, with 73 TOC assessments completed among PWH and 96 TOC assessments among PrEP users. Forty-four (26.0%) TOC assessments occurred within one month after treatment, 79 (46.7%) between one to three months, and 46 (27.2%) between three to six months. For the 169 TOC assessments, 91.1% (n = 154) showed negative results for *C. trachomatis* and 15 assessments with a positive result occurred in 9 PWH and 6 PrEP users. PWH tended to have a higher rate of *C. trachomatis* infection at TOC assessment than PrEP users (12.3% *vs.* 6.3%; $P = 0.17$) (Table 2 and Fig. 1).

Molecular epidemiology of Chlamydia trachomatis among participants

Among 379 samples tested positive for *C. trachomatis*, genotyping was successfully performed in 88.1% (334 samples, including those collected from the rectum [77.8%], urethra [22.9%], and oral cavity [11.1%]). The prevalent genotypes included genotypes J (26.4%, n = 88/334), G (24.0%, n = 80/334), B (17.7%, n = 59/334), Da (14.1%; n = 47/334), and E (9.0%; n = 30/334) (Fig. 2A). Seven participants, 5 PWH and 2 PrEP users, had discordant *C. trachomatis* genotypes at different sampling sites. Besides, for the participants who failed to achieve clearance on TOC assessments, different *C. trachomatis* genotypes were detected, suggestive of reinfection rather than treatment failure. The most prevalent site of *C. trachomatis* infection on TOC assessments was the rectum (73.3%), especially among PWH (88.9%; n = 8/9).

The genovariant L2b was detected exclusively in the rectal swab samples of 18 participants, among which one harboured L2b in all three collection samples. Of 18 cases of rectal L2b carriage occurred in 16 (88.9%) PWH. Moreover, the detection rate of L2b increased by time, from 0% in August 2021–December 2021 to 14.0% in May 2023–August 2023 ($P = 0.012$) (Fig. 2B). All participants with rectal L2b carriage were asymptomatic and achieved clearance of *C. trachomatis* with a 7-day course of doxycycline treatment.

Multi-locus sequence typing (MLST) of C. trachomatis genovariant L2b

Of the 24 samples with L2b detected, 17 (70.8%) were successfully sequenced by MLST. Fourteen were assigned as ST53, one as ST58, and two as novel STs (ST39 and ST63, respectively). Using the latest released data (October 30, 2023) for BURST analysis, four groups (Groups 1, 2, 3, and 4) and ungrouped singletons were identified (Fig. 3A). Due to the abundance of data, a representative ST was selected from each group for subsequent sequence analyses. Four STs found in the present study (*i.e.,* ST-39, ST-53, ST-58, and ST-63) with the limited inter-ST variations (SLV or DLV), formed a cluster belonging to the Group 2 (Fig. 3B). Using a population snapshot to illustrate the evolutionary relationships at the entire *C. trachomatis* population level, ST-39/-53/-58/-63 were positioned within a distinct evolutionary branch, suggestive of a high degree of genetic relatedness among them and the presence of microevolution within the discovered L2b strains (Fig. 3C).

Discussion

To our knowledge, this is the first study in Taiwan to demonstrate the molecular epidemiology of *C. trachomatis* infection at different anatomical sites and to longitudinally follow the participants with chlamydia after oral doxycycline therapy in Asia.

In the current study, >90% of the participants were male, >90% of the cases of *C. trachomatis* infection occurred in MSM, and >70% of *C. trachomatis* were detected in the rectal site. As the screening tests were not performed based on clinical symptoms, more than 70% of the participants testing positive for *C. trachomatis* did not report any symptoms suggestive of STIs. Due to the high prevalence of concomitant STIs, the contribution of each pathogen to the symptoms reported could not be precisely assessed.

While *C. trachomatis* genotypes D to K are well known to cause a spectrum of STI, the genotype distribution in prior investigations across different periods varied greatly.[14–20] Notably, genotype B, which was more likely to be associated with trachoma than STI, constituted nearly 20% of our genotyped samples. Such a finding was compatible with the findings from a previous study in southern Taiwan,[10] but differed from the studies conducted before 2020.[14–21] Despite the fact that Taiwan has been declared as being trachoma-free for decades, genotype B could be identified at all three body sites among at-risk populations in this study. Whether there was genetic recombination among the indigenous genotype B strains, as previously noted in Spain, another trachoma-free country,[20] further investigations are warranted.

While no LGV-related genotypes were identified among MSM from previous studies conducted in China, Thailand, Japan, and

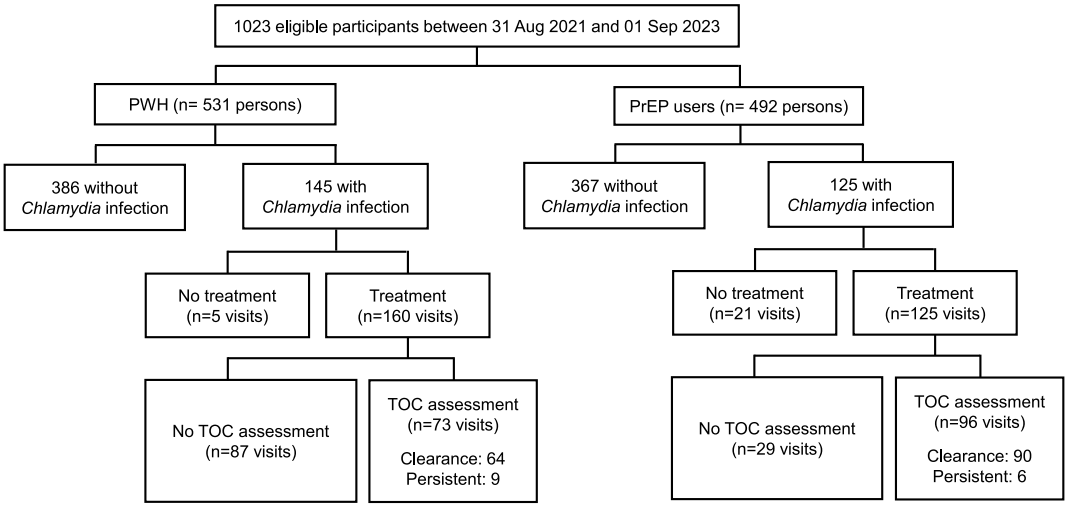


Fig. 1. Flowchart of the surveillance of *Chlamydia trachomatis* infection among at-risk populations, results, treatment and follow-up.

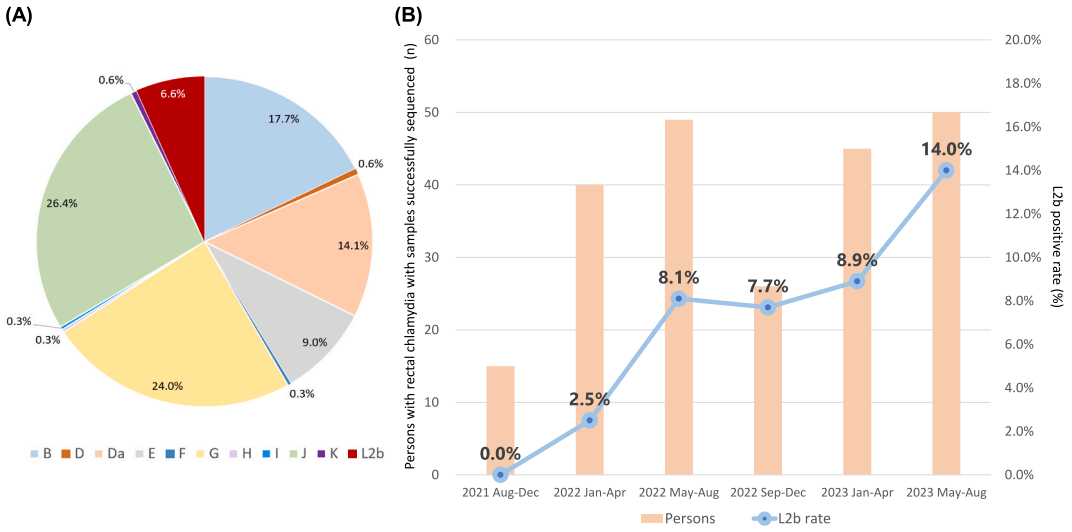
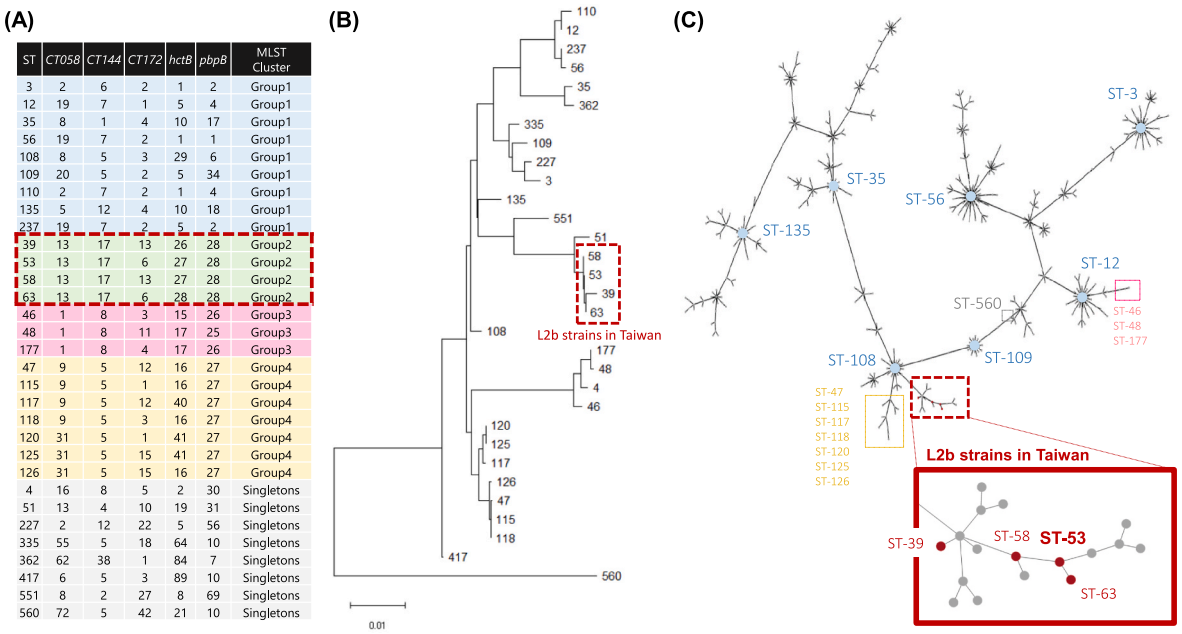


Fig. 2. Genotyping of *Chlamydia*-positive samples in the present study: (A) the overall distribution of genotypes (B) the trend of genovariant L2b at rectum among at-risk population.



ST	CT058	CT144	CT172	hctB	pbpB	MLST Cluster
3	2	6	2	1	2	Group1
12	19	7	1	5	4	Group1
35	8	1	4	10	17	Group1
56	19	7	2	1	1	Group1
108	8	5	3	29	6	Group1
109	20	5	2	5	34	Group1
110	2	7	2	1	4	Group1
135	5	12	4	10	18	Group1
237	19	7	2	5	2	Group1
39	13	17	13	26	28	Group2
53	13	17	6	27	28	Group2
58	13	17	13	27	28	Group2
63	13	17	6	28	28	Group2
46	1	8	3	15	26	Group3
48	1	8	11	17	25	Group3
177	1	8	4	17	26	Group3
47	9	5	12	16	27	Group4
115	9	5	1	16	27	Group4
117	9	5	12	40	27	Group4
118	9	5	3	16	27	Group4
120	31	5	1	41	27	Group4
125	31	5	15	41	27	Group4
126	31	5	15	16	27	Group4
4	16	8	5	2	30	Singletons
51	13	4	10	19	31	Singletons
227	2	12	22	5	56	Singletons
335	55	5	18	64	10	Singletons
362	62	38	1	84	7	Singletons
417	6	5	3	89	10	Singletons
551	8	2	27	8	69	Singletons
560	72	5	42	21	10	Singletons

Fig. 3. Multi-locus sequence typing (MLST) of *C.* trachomatis genovariant L2b strains (A) grouping by BURST analysis and (B) phylogenetic relatedness by the neighbor-joining method (C) population snapshot of L2b sequence types (STs) in the present study with other representative STs.in MLST database.

Vietnam,[17–19,22,23] the present study showed 18 asymptomatic participants testing positive for genovariant L2b that was detected exclusively in the rectal swab samples. Almost all of our asympomatic cases harbouring the genovariant L2b were PWH. In Western countries, PWH has been recognized as a risk group for rectal LGV, which was considered as a severe manifestation of *C. trachomatis* infection and rarely presented as asymptomatic carriage.[5,7] A multicentre, cross-sectional surveillance in the U.K. during 2006–2007 showed 95% of the cases of rectal LGV were symptomatic.[21] In contrast, a universal screening survey of MSM in Australia during 2015–2018 found that LGV accounted for 2.5% of *C. trachomatis* infection and 34.4% were asymptomatic.[24] Our study conducted during 2021–2023 revealed all cases of rectal L2b carriage were asymptomatic. Based on the findings in Australia and Taiwan, it is likely that the prevalence of asymptomatic infection with genotype L may be underestimated, or that the virulence of the genotype L of *C. trachomatis* used to cause LGV has changed over time. More clinical and microbiological investigations are warranted.

The literature consistently suggests that clinical manifestations of LGV may be severe and LGV requires a 21-day course of doxycycline,[13,25,26] since there was delayed microbial cure in the cases of LGV proctitis with persistent detection of LGV RNA for 16 days.[27] However, there is limited research on the optimal treatment duration for asymptomatic rectal carriage of the genotype L. A large randomized, controlled trial in Australia and New Zealand from 2016 to 2019 aimed to compare the efficacy with azithromycin versus doxycycline in treating asymptomatic rectal chlamydia, but LGV cases were excluded.[28] In contrast, another randomized controlled trial in the U.S. showed 7 days of doxycycline was efficacious for all cases of rectal *C. trachomatis* infection, including 4 cases of rectal LGV regardless of symptoms.[29] While the case number of rectal carriage of genovariant L2b remained small in our study, our finding suggested that a 7-day course of doxycycline might be sufficient to eradicate asymptomatic rectal carriage of the genovariant

L2b. Although a few participants with non-LGV chlamydia infection remained positive on the follow-up assessment after the treatment, all of them were found to be infected with different genotypes from the genotypes identified before treatment. In addition, the participants testing positive for *C. trachomatis*, especially PWH, were more often to have concurrent detection of other STI pathogens. Accordingly, these results were suggestive of re-infection, rather than treatment failure, in those with persistent carriage of *C. trachomatis*.

To gain better insights into the transmission chain of *C. trachomatis* in Taiwan, MLST was employed to elucidate the possibility of a specific L2b clone circulating among at-risk populations. Our study revealed that most L2b strains were found to belong to ST53 and one strain ST58. Both STs had been previously reported in Europe.[9,30] It is likely that there is an international spread of the genotype L strains. Moreover, two new ST (ST39 and ST63) were identified, but remained to be genetically similar to the major genotype, ST53. Therefore, our study results highlight the need to continue surveillance for the emerging STI pathogen, *C. trachomatis* genotype L, among both general and at-risk populations in Taiwan.

Our study had several limitations. First, this was a single-center study that was conducted at a university hospital located in an urban area in Taiwan, and most participants were MSM. Therefore, the results may not be generalized to other risk groups or the population in rural or suburban areas. Second, the timing of TOC assessments varied, and not all participants completing antimicrobial treatment had TOC assessments performed due to loss to follow-up. Third, the case number of asymptomatic rectal infection with genovariant L2b remained small, and the effectiveness of the 7-day regimen of oral doxycycline therapy should be interpreted cautiously.

In conclusion, a high prevalence of asymptomatic *C. trachomatis* infection and mixed STIs was observed among PWH and PrEP users. The emergence of asymptomatic rectal carriage of the genovariant L2b, mainly ST53, in northern Taiwan warrants more multi-center studies to understand its extent of spread. Our findings support the continued surveillance using molecular diagnostic tools to better define the evolving epidemiology of chlamydia and the emerging threat of genovariant L2b in high-risk populations in Taiwan.

CRediT authorship contribution statement

Chi-Ying Lin: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Data curation. **Chin-Shiang Tsai:** Writing – review & editing, Writing – original draft, Investigation, Funding acquisition, Formal analysis, Data curation. **Hsin-Yun Sun:** Project administration, Investigation. **Yu-Shan Huang:** Project administration. **Kuan-Yin Lin:** Project administration. **Wang-Da Liu:** Project administration. **Guan-Jhou Chen:** Project administration. **Tzong-Yow Wu:** Project administration. **Li-Hsin Su:** Project administration, Investigation, Data curation. **Hsin-Hui Huang:** Project administration, Data curation. **Sui-Yuan Chang:** Project administration. **Wen-Chien Ko:** Writing – review & editing, Validation, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Chien-Ching Hung:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Ethical approval

The study was approved by the Research Ethics Committee of National Taiwan University Hospital (registration number, NTUH-201811021RINA).

Declarations of competing interest

No potential conflict of interest was reported by the author(s).

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