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Original Article

Mycoplasma genitalium infection and resistance-associated mutations to macrolides and fluoroquinolones among high-risk patients in Taiwan

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Abstract *Background: Mycoplasma genitalium* is an emerging etiology of sexually transmitted infections (STIs) with increasing resistance to antimicrobials. Surveillance on the epidemiology of *M. genitalium* infection and antimicrobial resistance is warranted.
Methods: Between September 2021 and August 2023, people with HIV (PWH) and people without HIV (PWoH) at risk of STIs were screened for *M. genitalium* infection using a multiplex polymerase-chain-reaction assay of specimens collected from the rectum, urethra, oral cavity, and vagina. The prevalences of resistance-associated mutations (RAMs) of *M. genitalium* to fluoroquinolones, macrolides, and tetracycline were investigated.

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resistance;
Tetracycline;
Macrolides;
Fluoroquinolones

Results: During the 2-year study period, 1021 participants were enrolled, including 531 PWH and 490 PWoH. Overall, 83 (8.1%) and 34 (7.6%) participants had *M. genitalium* infection at baseline and during follow-up, respectively, with the rectum being the most common site of detection (61.5%). With the first course of antimicrobial treatment, 27 of 63 (42.9%) participants with *M. genitalium* infection were cured during follow-up, including 24 of 58 (41.4%) who received doxycycline monotherapy. The prevalence of RAMs to macrolides, fluoroquinolones, and tetracyclines at baseline were 24.3%, 22.4%, and 7.9%, respectively. Though PWH had more *M. genitalium* infection (10.2% vs 5.9%, $p = 0.01$), a higher rate of RAMs to macrolides (41.0% vs 14.7%, $p < 0.01$) was found in PWoH.
Conclusions: Among high-risk populations, the prevalence of *M. genitalium* infection was 8.1%. The overall genotypic resistance of *M. genitalium* to macrolides and fluoroquinolones was moderately high in Taiwan. Detection of *M. genitalium* infection and antimicrobial resistance is warranted to ensure resistance-guided antimicrobial treatments to be administered.

Introduction

Mycoplasma genitalium has emerged as an important sexually-transmitted pathogen of cervicitis in female patients and non-gonococcal urethritis (NGU) in male patients due to increasing trends of antimicrobial resistance to first-line treatments globally.[1] In general population, *M. genitalium* is rarely identified and most people infected with *M. genitalium* have no symptoms.[2] However, in people presenting with non-gonococcal urogenital infection, particularly in men with urethritis, *M. genitalium* has become the leading etiology.[3] Nucleic-acid amplification tests is currently the most commonly used method to detect *M. genitalium*,[4] which may facilitate early diagnosis and guide clinicians to start appropriate treatments timely.

Treatments of *M. genitalium* infection have become challenging because, unlike *Chlamydia trachomatis* infection that responds favorably to either doxycycline or azithromycin, *M. genitalium* infection has a poor response to doxycycline and a high rate of persistence after treatment with azithromycin.[5–8] Moreover, coinfection with *Neisseria gonorrhoeae* or *C. trachomatis* in patients with *M. genitalium* infection is not uncommon, which may further complicate the treatment choice and outcome assessment.[3] Because of the increasing trends of resistance and failure to respond to first-line treatment, resistance-guided sequential therapy has been introduced, in which sequential combination treatment is based on the detection of resistance-associated mutations (RAMs) to macrolides and/or fluoroquinolones.[9–11]

In Taiwan, the prevalence of *M. genitalium* infection and its antimicrobial resistance are rarely investigated. In this 2-year surveillance study, we aimed to examine the prevalence of *M. genitalium* infection and its RAMs to macrolides, fluoroquinolones, and tetracycline among high-risk populations seeking care at a university hospital in Taiwan.

Methods

Study design and study population

From September 1st, 2021 to August 31st, 2023, people with HIV (PWH), people with recent STIs, and people at risk of STIs at the National Taiwan University Hospital (NTUH) were prospectively enrolled. Recent STIs was defined as presence of any urogenital or rectal symptoms, or diagnosis of early syphilis, gonococcal infection, chlamydial infection, or other STIs than *M. genitalium* infection in the past 6 months. In addition, people with acute hepatitis C virus (HCV) infection were also included.[12] People at risk of STIs was defined as sexual partners of people with STIs, people who were receiving pre-exposure prophylaxis (PrEP) for HIV, people who sought voluntary counseling and testing for HIV, and people who had unprotected sexual behaviors. We collected the information on the demographics, and results of plasma HCV RNA, *Treponema pallidum* particle agglutination (TPPA), and rapid plasma reagin (RPR) titer before and after the visit; and the information on plasma HIV RNA, CD4 count, and antiretroviral therapy were also recorded. The study was approved by the Research Ethics Committee of NTUH (registration no. 201811021RINA) and the participants provided written informed consent.

Detection of *M. genitallium* and antimicrobial resistance-associated mutations

All participants were screened for *M. genitalium* with the use of multiplex polymerase-chain-reaction (PCR) assay (Allplex™ STI Essential Assay, Seegene Inc., South Korea), which can detect 7 urogenital pathogens (*M. genitalium, C. trachomatis, N. gonorrhoeae, Trachomatis vaginalis, M. hominis, Ureaplasma urealyticum,* and *U. parvum*).[13,14] The clinical specimens were self-collected from the rectum, urethra, and oral cavity simultaneously. For female participants, additional vaginal specimen was also self-collected.

For participants found to be infected with *M. genitalium*, regardless the sites of positive results or the clinical symptoms, doxycycline 100 mg twice daily was administered for a total of 7 days, unless the doxycycline was contraindicated or there were other concerns. All specimens tested positive for *M. genitalium* were tested for the RAMs to macrolides at A2058 and A2059 in region V of the 23S rRNA gene, to fluoroquinolones at Gly81, Asp82, Ser83, Asp87, and Val103 in *parC* and Met95,

Asp99, and Phe108 in *gyrA* gene, and to tetracycline at G966, C967, and C1192 in 16S rRNA gene by in-house PCR assay.[15—17]

Per the European guidelines, participants with *M. genitalium* infection are advised to undergo the test-of-cure (TOC) assessment no earlier than 3 weeks after completing the full course of treatment.[10,18] TOC testing was performed on the clinical specimens collected from the rectum, urethra, and oral cavity in male participants, and additionally, vagina in female participants, using the same multiplex PCR assay. If *M. genitalium* was detected again, determinations of RAMs were repeated.

To identify sexually-transmitted HCV viremia, pooled-plasma HCV RNA testing was conducted according to the methods described previously.[19] Determinations of TPPA (FTI-SERODIA-TPPA; Fujirebio Taiwan Inc., Taoyuan, Taiwan) and RPR (BD Macro-VueTMRPR Card tests) titer were performed, along with evaluation of clinical presentations for the diagnosis of syphilis.

Follow-up and definitions

Negative results of TOC testing for *M. genitalium* after treatments indicated cure or spontaneous clearance of *M. genitalium* infection, while positive results indicated treatment failure. Participants who tested negative for *M. genitalium* on enrollment but tested positive during the follow-up were defined as being cases of incident infection. Reinfection was defined for participants confirmed to be cured or have spontaneous clearance of *M. genitalium* but subsequently testing positive for *M. genitalium*.

Statistical analysis

All statistical analyses were performed using Stata/MP 14.0 (STATAcorp LLC, Texas, USA). Categorical data were presented as frequencies and percentages. The χ^2 test or Fisher's exact test were used to examine differences between categorical data. All continuous variables were expressed as means or medians with interquartile ranges (IQRs) and compared using the independent t test or Mann—Whitney U test. All tests were two-tailed, and P-values of <0.05 were considered statistically significant.

Results

Clinical characteristics of the participants

During the 2-year study period, 1021 participants contributing to a total of 2260 clinical visits for *M. genitalium* testing were enrolled, including 531 PWH (PWH group) and 490 people without HIV (PWoH group). The clinical characteristics of the participants are shown in Table 1. Participants in PWH group were significantly older than PWoH (38.1 $\pm$ 8.1 vs 31.0 $\pm$ 6.3 years, $p < 0.01$). Most participants in PWH group had achieved plasma HIV-1 RNA <200 copies/ml with antiretroviral therapy before participating in the study (96.0%). All people with HCV viremia were participants in PWH group (2.9% vs 0%, $p < 0.01$) (Table 1).

M. genitalium infection and STIs other than *M. genitalium*

At baseline, a total of 83 (8.1%) participants were confirmed with *M. genitalium* infection (Table 1). PWH had more *M. genitalium* infection (10.2% vs 5.9%, $p = 0.01$), more sexually transmitted coinfection other than *M. genitalium* infection (54.1% vs 44.7%, $p < 0.01$), and more likely to have multiple coinfected pathogens (1 (0—2) vs 0 (0—1), $p < 0.01$). For participants in both groups, most *M. genitalium* (61.5%) was identified from the rectal specimens (64.8% in PWH and 55.2% in PWoH group), followed by the urethra (27.7%), vagina (3.6%), and oral cavity (2.4%). Four participants had *M. genitalium* identified from two sites simultaneously (Table 1). Comparisons of the clinical characteristics between the 83 participants with and 938 participants without *M. genitalium* infection at baseline are shown in Table S1. *M. genitalium*-infected participants were more likely to have coinfected pathogens than those without *M. genitalium* infection (69.9% vs 47.8%, $p < 0.01$) (Table S1).

Treatment outcomes of prevalent and incident *M. genitalium* infection

Overall, 39 of 83 (47.0%) *M. genitalium*-infected participants and 490 of 938 (52.2%) participants without *M. genitalium* infection at baseline did not undergo repeat testing or were lost to follow-up (Fig. 1). Of 448 participants who had no *M. genitalium* infection at baseline and had follow-up visits, 34 (7.6%) were subsequently found to be infected with *M. genitalium* after a median of 182 days (interquartile range, 119—301), and 19 (55.9%) of them had TOC testing after their first course of treatment (Table 2). Of all 117 participants found to have *M. genitalium* infection at baseline or during follow-up, 98 (83.8%) received doxycycline (Table 2); one received doxycycline and azithromycin at the same time to treat concomitant *N. gonorrhoeae* infection; one received doxycycline and moxifloxacin at the same time to treat *M. genitalium*; and 17 (14.5%) did not receive any treatment at the discretion of their primary care physicians.

After the first course of treatment, 39 of 83 (47.0%) *M. genitalium*-infected participants at baseline and 15 of 34 (44.1%) participants with incident *M. genitalium* infection were lost to follow-up (Fig. 1 and Table 2). Twenty-seven of the remaining 63 participants had their *M. genitalium* infection cured or cleared spontaneously, with an overall clearance rate of 42.9% (Fig. 1 and Table 2). Cures were defined in 41.4% (24/58) of the participants receiving doxycycline monotherapy and 100% (1/1) in the participant receiving doxycycline plus azithromycin; and 2 of 4 participants who did not receive treatments had spontaneous clearance of *M. genitalium* during follow-up testing (Fig. 2 and Table S2).

After the first course of treatment, 36 participants, in whom 34 received doxycycline and two did not receive treatment at first, had treatment failure and proceeded to receive a second course of treatment (Fig. 2 and Table S3). Six of them received the third course, and one of them received the fourth to sixth courses, which overall

Table 1 Baseline clinical characteristics of all 1021 included participants.

	PWH (n = 531)	PWoH (n = 490)	Total (n = 1021)	p-value
Male gender, n (%)	531 (100)	438 (89.4)	969 (94.9)	<0.01
Mean age (SD), years	38.1 (8.1)	31.0 (6.3)	34.7 (8.1)	<0.01
Cause for testing, n (%)				
Syphilis	245 (46.1)	17 (3.5)	262 (25.7)	
Urogenital symptoms	149 (28.1)	15 (3.1)	164 (16.1)	
Partner of a patient with STIs	31 (5.8)	25 (5.1)	56 (5.5)	
PrEP user	0	382 (78.0)	382 (37.4)	
VCT for HIV	0	49 (10.0)	49 (4.8)	
Unprotected sex	54 (10.2)	2 (0.4)	56 (5.5)	
PWH without STIs	52 (9.8)	0	52 (5.1)	
Plasma HIV RNA <200 copies/ml, n/N (%)	501/522 (96.0)	NA	501/522 (96.0)	
Concurrent HCV viremia, n/N (%)	11/385 (2.9)	0/278 (0)	11/663 (1.7)	<0.01
MG-positive, n (%)	54 (10.2)	29 (5.9)	83 (8.1)	0.01
Site of MG detected, n (%)				
Rectal only	35 (64.8)	16 (55.2)	51 (61.5)	0.10
Urethra only	17 (31.5)	6 (20.7)	23 (27.7)	
Oral only	1 (1.9)	1 (3.5)	2 (2.4)	
Vagina only	0	3 (10.3)	3 (3.6)	
Rectal + Urethra	0	1 (3.5)	1 (1.2)	
Urethra + Oral	1 (1.9)	1 (3.5)	2 (2.4)	
Urethra + Vagina	0	1 (3.5)	1 (1.2)	
With concurrently detected sexually transmitted pathogen other than *Mycoplasma genitalium*, n (%)	287 (54.1)	219 (44.7)	506 (49.6)	<0.01
Median numbers of sexually transmitted pathogen detected other than *Mycoplasma genitalium*, n (IQR)	1 (0–2)	0 (0–1)	0 (0–1)	<0.01
Chlamydia trachomatis, n (%)	121 (22.8)	75 (15.3)	196 (19.2)	<0.01
Neisseria gonorrhoeae	103 (19.4)	56 (11.4)	159 (15.6)	<0.01
Trachomatis vaginalis	0	0	0	
Mycoplasma hominis	94 (17.7)	50 (10.2)	144 (14.1)	<0.01
Ureaplasma urealyticum	191 (36.0)	130 (26.5)	321 (31.4)	<0.01
Ureaplasma parvum	6 (1.1)	36 (7.4)	42 (4.1)	<0.01
Participants with follow-up test, n (%)	208 (39.2)	284 (58.0)	492 (48.2)	<0.01
Total duration of follow-up, (IQR), days	189 (82–349)	217 (120–429)	201 (91–378)	<0.01
Interval between follow-up visits, (IQR), days	83 (28–153)	87 (81–98)	86 (62–106)	0.02

N = total number of participants tested; n = number of participants with data.
Abbreviations: HCV, hepatitis C virus; IQR, interquartile range; NA, not applicable; PrEP, pre-exposure prophylaxis; PWH, people with HIV; PWoH, people without HIV; SD, standard deviation; STI, sexually transmitted infection; VCT, voluntary counseling and testing.

contributed to twenty cases of cure (Fig. 2). During subsequent follow-up, 10 participants who had been cured subsequently contributed to 12 episodes of reinfection; one of them had three episodes of reinfection after the first cure and the intervals from previous cure to the next episode of reinfection were 84, 49, and 15 days, respectively; and each of the remaining nine participants had one episode of reinfection after a median interval of 91 days (range, 15–372) from previous cure. Two of them had no further results of *M. genitalium* at the end of study (Fig. S1).

Resistance-associated mutations (RAMs) and the treatment success rate with doxycycline

Of all diagnosed cases of *M. genitalium* infection in 117 participants before treatments, 107, 116, and 114 samples were successfully amplified and sequenced for determinations of RAMs to macrolides, fluoroquinolones, and tetracycline, respectively. RAMs to macrolides, fluoroquinolones, and tetracycline at baseline were present in 26 (24.3%), 26 (22.4%), and 9 (7.9%) of the samples, respectively (Table 2). The profiles of resistance and their association with treatment outcomes are shown in Table S2, Table S3, and Fig. S1.

Of the RAMs to macrolides, the prevalence of A2058 mutation in 23S RNA was more common than that of A2059 (61.5% vs 38.5%); and, of the RAMs to fluoroquinolones, *parC* mutation at Ser83 was the most common (80.8%) and *gyrA*-mediated RAM to fluoroquinolones was not found (Table 2). However, one *gyrA*-mediated RAM to fluoroquinolones was found in a participant without HIV (PWoH-1) after the third course of treatment after he had received doxycycline, doxycycline plus moxifloxacin, and doxycycline in his first, second, and third course of treatment (Fig. S1). Nine participants were found to have *M.*

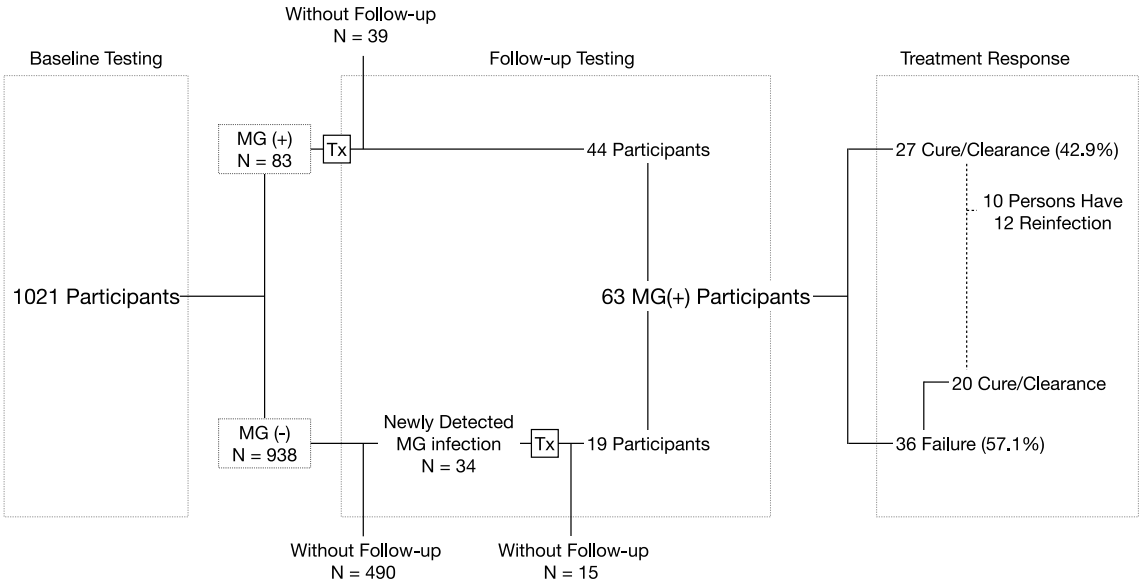

Figure 1. Study flow. (MG, *Mycoplasma genitalium*).

genitalium with RAMs to tetracycline and the most common sites of mutation was at C967 (77.8%) (Table 2).

Of 117 participants with *M. genitalium* infection, 17 (14.5%) were found to have *M. genitalium* harboring combined RAMs before treatments, including 14 (82.4%) with combined RAMs to macrolides and fluoroquinolones, one (5.9%) with RAMs to fluoroquinolones and tetracycline, and two (11.8%) with combined RAMs to macrolides and tetracycline (Table 2). Pre-existent RAMs to macrolides and combined RAMs were associated with failure to the first course of treatment, which was mostly with doxycycline monotherapy (both $p < 0.01$), and the same trend was also noted in their second course of treatment (Table 3). However, the association of preexistent RAMs to tetracycline or fluoroquinolone with treatment outcome was not obvious, neither in the first nor second course of treatment (Table 3, Table S2, and Table S3).

Prevalence of RAMs and response of treatment in PWH vs PWoH

Compared with participants in PWH group, *M. genitalium* in PWoH were had a higher prevalence of RAMs to macrolides before their first course of treatments (41.0% vs 14.7%, $p < 0.01$), but not to fluoroquinolones or tetracycline (Table 3). After excluding those participants who did not receive antimicrobial therapy for *M. genitalium* (a total of 17 participants), PWoH had a lower cure rate of *M. genitalium* infection than PWH (9/25 [36.0%] vs 16/34 [47.1%]). Simiar findings were observed for a higher rate of macrolides resistance and a lower cure rate was still noted in PWoH in their second course of treatment (Table 3).

Discussion

Our study revealed that the baseline prevalence of *M. genitalium* infection in high-risk groups in northern Taiwan was 8.1% (83/1021), which was higher in PWH than PWoH (10.2 % vs 5.9%), and the rectum was the most common site of detection (61.5% at baseline and 79.4% in follow-up). The baseline prevalence of RAMs to macrolides, fluoroquinolones, and tetracycline was 24.3%, 22.4%, and 7.9%, respectively and that of combined RAMs was as high as 14.5%. With doxycycline monotherapy, the clearance rate of *M. genitalium* infection was 41.4%.

The prevalence of *M. genitalium* infection varied among the published studies using different testing kits in different populations and from different countries. In Japan, the prevalence of *M. genitalium* infection ranged from 2.8% in the urinary tract of asymptomatic female students to 6.1% in the urinary tract or rectum of asymptomatic men who have sex with men (MSM), in whom 49.2% were PWH, and up to 14.1% in the vagina of female sex workers.[20—22] In Australia, the prevalence ranged from 1.8% in the urinary tract of backpackers to 9.5% in the urinary tract or rectum of asymptomatic MSM.[23,24] Our study was the first large study to comprehensively evaluate *M. genitalium* infection in at-risk populations in Taiwan and the overall prevalence (8.1%) was similar to those found in other Asia-Pacific countries.

Who and when should *M. genitalium* be checked are still in debate. To identify *M. genitalium* in general population is unpractical for its low prevalence. Previous studies have shown that presence of STIs was the most important associated factor with *M. genitalium* infection, especially in male patients.[2,3] In the United States, only persistent or recurrent urogenital infections warrant testing for *M. genitalium*; in contrast, testing for *M. genitalium* is suggested in any symptomatic urogenital infection in European countries.[10,11] However, testing for asymptomatic persons are discouraged in all currently available guidelines.[10,11] In our study, 88.0 % of participants in PWoH group were asymptomatic (PrEP user and VCT for HIV), which accounted for one third cases of *M. genitalium* infection diagnosed in this study, but with a significant higher rate of RAMs to

Table 2 Profiles of resistance-associated mutations of *Mycoplasma genitalium* detected in their first episode of infection and during follow-up.

	Infection detected in the first visit (n = 83)	New infection detected during follow-up (n = 34)	All (n = 117)
People with HIV, n (%)	54 (65.1)	17 (50.0)	71 (60.7)
Sites of detection, n (%)			
Rectal only	51 (61.5)	27 (79.4)	78 (66.7)
Urethra only	23 (27.7)	6 (17.7)	29 (24.8)
Oral only	2 (2.4)	0	2 (1.7)
Vagina only	3 (3.6)	0	3 (2.6)
Rectal + urethra	1 (1.2)	1 (2.9)	2 (1.7)
Urethra + oral	2 (2.4)	0	2 (1.7)
Urethra + vagina	1 (1.2)	0	1 (0.9)
RAMs to macrolides, n/N (%)	17/78 (21.8)	9/29 (31.0)	26/107 (24.3)
A2058, n (%)	10 (58.8)	6 (66.7)	16 (61.5)
A2059	7 (41.2)	3 (33.3)	10 (38.5)
RAMs to fluoroquinolones, n/N (%)	20/83 (24.1)	6/33 (18.2)	26/116 (22.4)
parC, n/N (%)	20/83 (24.1)	6/33 (18.2)	26/116 (22.4)
Gly81, n (%)	2 (10.0)	0	2 (7.7)
Asp82	0	0	0
Ser83	16 (80.0)	5 (83.3)	21 (80.8)
Asp87	2 (10.0)	1 (16.7)	3 (11.5)
Val103	0	0	0
gyrA, n/N (%)	0/82 (0)	0/33 (0)	0/115 (0)
RAMs to tetracycline, n/N (%)	6/82 (7.3)	3/32 (9.4)	9/114 (7.9)
G966, n (%)	2 (33.3)	1 (33.3)	3 (33.3)
C967	4 (66.7)	3 (100)	7 (77.8)
C1192	1 (16.7)	0	1 (11.1)
Combined RAMs to antimicrobials, n/N (%)	11/83 (13.3)	6/34 (17.7)	17/117 (14.5)
Fluoroquinolones + macrolides, n (%)	10 (90.9)	4 (66.7)	14 (82.4)
Fluoroquinolones + tetracycline	1 (9.1)	0	1 (5.9)
Macrolides + tetracycline	0	2 (33.3)	2 (11.8)
All combined	0	0	0
Treatment, n (%)			
Doxycycline only	69 (83.1)	29 (85.3)	98 (83.8)
Doxycycline + azithromycin	1 (1.2)	0	1 (0.9)
Doxycycline + moxifloxacin	1 (1.2)	0	1 (0.9)
No treatment	12 (14.5)	5 (14.7)	17 (14.5)
Microbiologic outcome			
Cure/clearance, n (%)	19 (22.9)	8 (23.5)	27 (23.1)
Failure, n (%)	25 (30.1)	11 (32.4)	36 (30.8)
No follow-up testing, n (%)	39 (47.0)	15 (44.1)	54 (46.2)

N = total number of participants tested; n = number of participants with data. Abbreviation: RAMs, resistance-associated mutations.

macrolides (41.0% vs 14.7%, $p < 0.01$) before receiving treatment. A higher prevalence of *M. genitalium* infection than that of *N. gonorrhoeae* or *C. trachomatis* infection in asymptomatic persons and high rates of RAMs to macrolides in these persons are not surprising, especially in the general population who had ever reported diagnosis of STIs in the remote past.[20,25–27] In a previous transmission-dynamic model study, testing for *M. genitalium*, regardless of symptoms or not, might reduce the cumulative incidence of *M. genitalium*-associated pelvic inflammatory disease.[28] While our study did not reveal a higher rate of *M. genitalium* infection in PWoH, almost all of whom were asymptomatic, investigations for *M. genitalium* infection in this risk group may be warranted to avoid further spread of resistant strain, given the increasing trends of STIs among PrEP users in recent years.[29]

Resistance of *M. genitalium* to macrolides and fluoroquinolones is increasing worldwide, which have raised concerns about appropriate management of *M. genitalium* infection. The prevalence of *M. genitalium* resistance to macrolides and fluoroquinolones is also high in Asia-Pacific region; for example, the respective rate was 25% and 37.5% in Singapore, 42.1–47.4% and 52.8–65% in Hong Kong, 58.4% and 73.1% in Guangdong, China, and 89.6% and 68.3% in Japan.[1,20,30–33] However, current guidelines only focus on the RAMs to macrolides and only suggested the routine test of RAMs to macrolides, but not fluoroquinolones.[10,11,34] Though our study revealed the

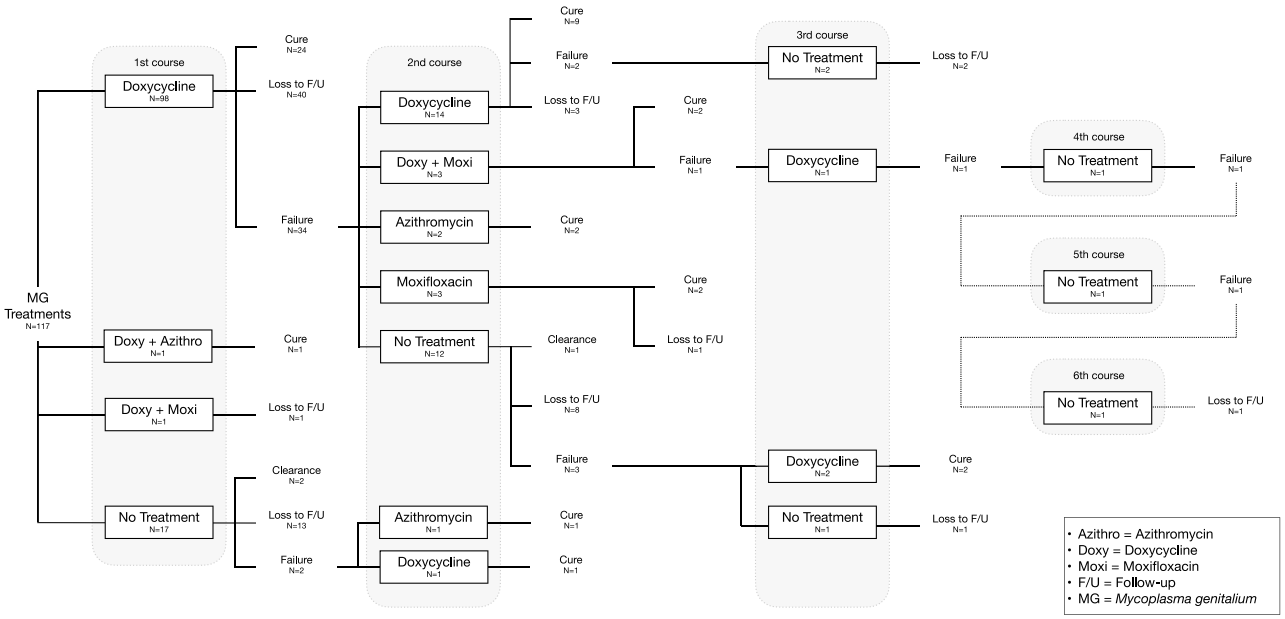

Figure 2. Outcomes of all 117 participants with *Mycoplasma genitalium* infection.

Table 3 Resistance-associated mutations of *Mycoplasma genitalium* detected in the participants and treatment responses to antimicrobials administered.

RAMs before the 1st course of treatment and results after treatment[a]					RAMs before the 2nd course of treatment and results after treatment[b]				
Outcomes	All (n = 100)				Outcomes	All (n = 24)			
RAMs	Cure (n = 25)	Failure (n = 34)	Loss to f/u (n = 41)	p-value	RAMs	Cure (n = 17)	Failure (n = 3)	Loss to f/u (n = 4)	p-value
Macrolides, n/N (%)	5/25 (20.0)	13/32 (40.6)	3/38 (7.9)	<0.01	Macrolides, n/N (%)	3/13 (23.1)	3/3 (100)	2/4 (50.0)	0.05
Fluoroquinolones	5/25 (20.0)	12/34 (35.3)	7/40 (17.5)	0.17	Fluoroquinolones	5/17 (29.4)	2/3 (66.7)	2/4 (50.0)	0.45
Tetracycline	0/25 (0)	4/33 (12.1)	1/40 (2.5)	0.11	Tetracycline	1/17 (5.9)	0/3 (0)	1/4 (25.0)	0.51
Combined	4/25 (16.0)	10/34 (29.4)	1/41 (2.4)	<0.01	Combined	1/17 (5.9)	2/3 (66.7)	2/4 (50.0)	0.02

Resistance profiles and HIV serostatus				
RAMs before the 1st course of treatment	PWH (n = 71)	PWoH (n = 46)	All (n = 117)	p-value
Macrolides, n/N (%)	10/68 (14.7)	16/39 (41.0)	26/107 (24.3)	<0.01
Fluoroquinolones	14/71 (19.7)	12/45 (26.7)	26/116 (22.4)	0.38
Tetracycline	6/71 (8.5)	3/43 (7.0)	9/114 (7.9)	>0.99
Combined	8/71 (11.3)	9/46 (19.6)	17/117 (14.5)	0.21
Repsonse to the 1st course of treatment[a]	PWH (n = 68)	PWoH (n = 32)	All (n = 100)	p-value
Cure, n (%)	16 (23.5)	9 (28.1)	25 (25.0)	0.02
Failure	18 (26.5)	16 (50.0)	34 (34.0)	
Loss to follow-up	34 (50.0)	7 (21.9)	41 (41.0)	
RAMs before the 2nd course of treatment	PWH (n = 19)	PWoH (n = 17)	All (n = 36)	p-value
Macrolides, n/N (%)	6/16 (37.5)	9/14 (64.3)	15/30 (50.0)	0.14
Fluoroquinolones	7/19 (36.8)	7/16 (43.8)	14/35 (40.0)	0.68
Tetracycline	2/19 (10.5)	1/15 (6.7)	3/34 (8.8)	>0.99
Combined	4/19 (21.1)	5/17 (29.4)	9/36 (25.0)	0.71
Response to the 2nd course of treatment[b]	PWH (n = 13)	PWoH (n = 11)	All (n = 24)	p-value
Cure, n (%)	9 (69.2)	8 (72.7)	17 (70.8)	0.03
Failure	0	3 (27.3)	3 (12.5)	
Loss to follow-up	4 (30.8)	0	4 (16.7)	

[a] After excluding those participants who did not receive antimicrobial therapy for *M. genitalium* (total 17 participants).
[b] After excluding those participants who did not receive antimicrobial therapy for *M. genitalium* (total 12 participants).
N = total number of participants tested; n = number of participants with data.
Abbreviations: PWH, people with HIV; PWoH, people without HIV; RAMs, resistance-associated mutations.

relatively lower rates of antimicrobial resistance in Taiwan (to macrolide, 24.3% and fluoroquinolones, 22.4%) than those in other Asia-Pacific countries, combined resistance was not rare. Concerning the spread of *M. genitalium* with resistance in people at high risk, surveillance of RAMs to both macrolides and fluoroquinolones is warranted to guide the appropriate therapy.

Current treatment guidelines recommend resistance-guided sequential therapy, in which *M. genitalium* infection is first treated with a 7-day course of doxycycline, followed by individualized therapy with either azithromycin or moxifloxacin, pending the results of resistance testing.[10,11,35,36] Initial treatment with doxycycline could decrease bacterial load and was less likely to cause RAMs to emerge.[5,6,37–40] Our study revealed that, in Taiwan, the prevalence of RAMs to tetracycline was still lower than that to macrolides and fluoroquinolones (7.9% vs 24.3% and 22.4%, respectively) and the failure of treatment was not consistently associated with pre-existent RAMs to tetracycline in our study (Table 3, Table S2 and Table S3).

Our study had several limitations. First, a significant proportion (51.8%) of the participants had no follow-up testing in our study, which might preclude us from precisely estimating the rate of cure. Second, because of delayed results of in-house PCR to detect RAMs, treatments for our participants did not follow the guideline of resistance-guided sequential therapy and the TOC assessments were not rigorously conducted at fixed time points, which might contribute to the lower rate of treatment success and the difficulties in differentiating persistence from reinfection. Third, the decision to treat was made at the discretion of primary care physicians and adherence to the recommended treatments might not be ensured. Fourth, the treatment duration of doxycycline was 7 days and the information on adherence to treatment was not collected. Fifth, not every sample tested positive for *M. genitalium* was successfully sequenced by in-house PCR for the detection of RAMs to macrolides, tetracycline and fluoroquinolones, and the prevalence of RAMs could have been underestimated. Finally, some RAMs of the *M. genitalium* strains detected disappeared in subsequent testing. Because of the technical limitations, we were not able to differentiate the strains of *M. genitalium* identified in the first episode of infection and those identified in the test-of-cure assessment or subsequent episodes of infection.

In conclusion, the prevalence of *M. genitalium* in people at high risk in northern Taiwan is similar to those in other countries in Asia-Pacific region. Though the rates of genotypic resistance to macrolides and fluoroquinolones remain lower than those of other countires in Asia-Pacific region, regular surveillance of *M. genitalium* infection and to identify its genotypic resistance to macrolides and fluoroquinolone are warranted to ensure appropriate antimicrobial treatments to be administered.

CRediT authorship contribution statement

Ming-Jui Tsai: Writing — review & editing, Writing — original draft, Validation, Software, Project administration, Methodology, Formal analysis, Data curation. **Hsin-Yun Sun:** Validation, Project administration, Investigation, Data curation. **Li-Hsin Su:** Validation, Investigation, Data curation. **Kuan-Yin Lin:** Validation, Project administration, Investigation, Data curation. **Wang-Da Liu:** Validation, Project administration, Investigation, Data curation. **Yu-Shan Huang:** Validation, Project administration, Investigation, Data curation. **Guan-Jhou Chen:** Validation, Project administration, Investigation, Data curation. **Yi-Ching Su:** Validation, Investigation, Data curation. **Wen-Chun Liu:** Validation, Investigation, Data curation. **Sui-Yuan Chang:** Validation, Methodology, Conceptualization. **Chien-Ching Hung:** Writing — review & editing, Validation, Supervision, Project administration, Methodology, Investigation, Conceptualization.

Declaration of competing interest

The authors declare that there were no conflicts of interest. This research received grant from National Taiwan University Hospital Yunlin Branch, Yunlin, Taiwan (NTUHYL.112.HC001).

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Appendix A. Supplementary data

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