JAC Antimicrob Resist
doi:10.1093/jacamr/dlab091

JAC-Antimicrobial Resistance

High prevalence of circulating dual-class resistant *Mycoplasma genitalium* in asymptomatic MSM in Tokyo, Japan

Naokatsu Ando [1,2], Daisuke Mizushima[1], Misao Takano[1], Morika Mitobe[3], Hirofumi Miyake[3], Keiko Yokoyama[3], Kenji Sadamasu[3], Takahiro Aoki[1], Koji Watanabe[1], Haruka Uemura[1], Yasuaki Yanagawa[1], Hiroyuki Gatanaga[1,2]* and Shinichi Oka[1,2]

[1]AIDS Clinical Center, National Center for Global Health and Medicine, Tokyo, Japan; [2]Joint Research Center for Human Retrovirus Infection, Kumamoto University, Kumamoto, Japan; [3]Department of Microbiology, Tokyo Metropolitan Institute of Public Health, Tokyo, Japan

*Corresponding author. E-mail: higatana@acc.ncgm.go.jp

Received 4 February 2021; accepted 6 June 2021

Objectives: To assess the prevalence and antibiotic resistance profile of *Mycoplasma genitalium* detected from urogenital/rectal swab samples obtained from MSM in Tokyo, Japan.

Methods: We performed PCR-based screening for *M. genitalium* urogenital/rectal infection in 982 asymptomatic MSM between 1 January 2019 and 5 November 2020. Mutations in the antibiotic resistance-associated genes *gyrA* and *parC* and the 23S rRNA of *M. genitalium* were analysed.

Results: The prevalence of *M. genitalium* infection was 6.1%: the prevalence of rectal and urogenital infection was 4.7% and 1.4%, respectively. Among the cases, 48 were successfully analysed for 23S rRNA, 41 for *parC* mutations and 37 for *gyrA* mutations. Macrolide- and quinolone-resistance associated mutations (23S rRNA and *parC* mutations) were observed in 43 (89.6%) and 28 (68.3%) cases, respectively. The quinolone-resistance associated mutation-harbouring variants also harboured macrolide-resistance associated mutations. The S83I mutation in the *parC* gene was most commonly identified (24 cases, 58.5%), and its combination with M95I or D99N mutation in the *gyrA* gene was observed in 9 of 36 successfully analysed cases (25.0%). No significant association was observed between the presence of antibiotic resistance and antibiotic exposure for either macrolides or fluoroquinolones ($P = 0.785$ and 0.402, respectively).

Conclusions: In Tokyo, there is an alarmingly high prevalence of *M. genitalium* harbouring macrolide and/or quinolone resistance-associated mutations in MSM, irrespective of antibiotic exposure. The high prevalence of *M. genitalium* strains with both *parC* and *gyrA* mutations limits the efficacy of sitafloxacin. Therefore, suitable alternatives are required to treat such *M. genitalium* infections.

Introduction

Mycoplasma genitalium causes a sexually transmitted infection (STI). This pathogen was initially identified in a case of non-gonococcal urethritis and it is associated with other urogenital conditions in both men and women.[1,2] Frequent reports of asymptomatic *M. genitalium* infections[1,3] have led to speculations that the rectum may be an important reservoir of *M. genitalium*.[3] However, routine screening for *M. genitalium* is not recommended in asymptomatic individuals, because screening has not been shown to prevent the infection or related complications.[4] Moreover, empirical antibiotic treatment for *M. genitalium* infection or concurrent STIs, like those caused by *Chlamydia trachomatis*, raises concerns about the spread of *M. genitalium* strains harbouring macrolide and quinolone resistance-associated mutations.[3,5] Azithromycin failure is associated with mutations at positions 2071 or 2072 of the 23S rRNA gene;[6] fluoroquinolone failure, especially that of moxifloxacin, is associated with the S83I, S83R, D87N or D87Y mutations in *parC* and might be associated with the M95I or D99N mutations in *gyrA*.[7,8] Combined mutations in the *parC* and *gyrA* genes, such as S83I plus D99N, G93C or M95I, are associated with increased MIC values for quinolones[9] and S83I combined with M95I or D99 are more strongly associated with quinolone failure, compared with the S83I mono-mutations.[10] In fact, multidrug resistance in *M. genitalium* has become a global threat. A recent

meta-analysis by Machalek et al.[11] reported that the rate of macrolide resistance-associated mutations (MRM; in the 23S rRNA gene) in *M. genitalium* was approximately 10.0%, while that of quinolone resistance-associated mutations (QRM; in *parC*) was 4.8% before 2010; these rates increased to 51.4% and 9.3% for macrolide and quinolone resistance, respectively, in 2016–17.[11] The prevalence of MRM and QRM is higher in the Western Pacific region, including Australia and Japan, compared with that in European countries.[11] A more recent study from Belgium on samples collected in 2015–18 showed higher frequencies of 74.3% and 39.7% for MRM and QRM, respectively; 17.8% of the samples harboured S83I mutations.[12] However, in individuals with asymptomatic rectal infections, the prevalence of antibiotic resistance-associated mutations remains unclear,[3] and rectal infections, rather than urethral infections, may help to better detect circulating strains due to higher prevalence.[3] Here, we report the current epidemiology of *M. genitalium* rectal and urethral infections among MSM in Tokyo, Japan, an endemic area of MDR *M. genitalium*.

Materials and methods

Study population

We conducted two prospective cohort studies at two outpatient clinics at the AIDS Clinical Center (ACC) and the Sexual Health Clinic (SHC), National Centre for Global Health and Medicine (NCGM), Tokyo, Japan, between 1 January 2019 and 5 November 2020. MSM who regularly visited the ACC (age >20 years) or the SHC (age >16 years) were eligible to participate. The ACC cohort comprised HIV-positive MSM, while the SHC cohort comprised HIV-negative but high-risk MSM who regularly tested for HIV and other STIs. All participants provided written informed consent and completed a questionnaire regarding their recent sexual behaviour. Data on participant demographics were obtained from the medical records. This study was approved by the Human Research Ethics Committee of NCGM (NCGM-G-003350-00; NCGM-G-002091-00) and conducted according to the principles expressed in the Declaration of Helsinki of 1964 and the later amendments.

Detection of M. genitalium and macrolide and quinolone resistance

Ten millilitres of first void urine and/or a rectal swab were collected from each participant and screened for *M. genitalium* using a commercial PCR assay kit (LSI Medience Co., Tokyo, Japan).[13] *M. genitalium*-positive samples were used for resistance detection. Resistance-associated mutations were detected by targeted amplification of the 23S rRNA V domain and of the relevant regions of the *gyrA* and *parC* genes, as previously described,[10] followed by Sanger sequencing, performed using the ABI PRISM BigDye Terminator v3.1 Cycle Sequencing Kit (Thermo Fisher Scientific, Japan) on the Applied Biosystems 3500 Genetic Analyzer (Thermo Fisher Scientific). The sequences were compared with the corresponding target regions of the *M. genitalium* G37 complete genome (GenBank: L43967.2) to detect and identify mutations.

Statistical analysis

The prevalence of *M. genitalium* infection was estimated from the number of infected individuals among the study population. Statistical analyses were conducted using STATA V.16 (StataCorp, College Station, TX, USA) and GraphPad Prism V9 (GraphPad Software, CA, USA). The χ^2 or Mann–Whitney *U* tests and the Kruskal–Wallis test were used for the categorical and continuous variables, respectively; $P < 0.05$ was considered statistically significant.

Results

Participants' demographic and clinical characteristics

A total of 982 MSM were analysed. The median age was 39 (31–47) years. Among them, 49.2% (483/982) and 50.8% (499/982) were HIV-positive and -negative, respectively. All study participants were asymptomatic, as per the questionnaires or direct examination. The median CD4+ T cell count in patients with HIV (PLWH) was 585.5 (452.5–758) cells/mm^3; 94.4% of PLWH showed well-controlled viral replication (HIV-1 RNA < 200 copies/mL).

Prevalence of M. genitalium infection

The prevalence of *M. genitalium* infection among the study participants was 6.1% (60/982). *M. genitalium* was significantly more prevalent in rectal samples compared with that in urogenital samples (4.7% [46/977] versus 1.4% [14/980], $P < 0.0001$; Figure 1). No cases of urogenital and anorectal coinfection were observed. The difference in the prevalence of *M. genitalium* infection between HIV-positive (7.5% [36/483]) and -negative MSM (4.8% [24/499]) was not statistically significant ($P = 0.083$). The prevalence of rectal and urogenital *M. genitalium* infections in PLWH was 6.0% (29/482) and 1.4% (7/483), respectively, while that in HIV-negative participants was 3.4% (17/495) and 1.4% (7/497), respectively; the differences were not significant ($P = 0.057$ and 0.957, respectively).

Resistance

Among the 60 isolates, 48 were successfully analysed for MRM, 41 for *parC* mutations, and 37 for *gyrA* mutations (Figure 1). MRM, including A2071G, A2071T, or A2072G, were detected in 89.6% (43/48) of samples. MRM were detected in 89.5% (34/38) and 90% (9/10) of rectal and urogenital samples, respectively. Regarding QRM, 68.3% (28/41) isolates harboured *parC* mutations: 63.6% (21/33) and 87.5% (7/8) in rectal and urogenital samples, respectively (Table S1, available as Supplementary data at *JAC-AMR* Online). Most of them (58.5% [24/41]) harboured the S83I mutation. In contrast, *gyrA* mutations were detected in 27.0% (10/37) of the isolates: 25% (7/28) and 33.3% (3/9) in rectal and urogenital samples, respectively. The S83I in the *parC* combined with the M95I or D99N mutations in the *gyrA* were detected in 25.0% (9/36) of the successfully analysed cases. Among the study participants, 28.3% (17/60) with *M. genitalium* infections had a history of azithromycin therapy, while 6.7% (4/60) had undergone fluoroquinolone therapy in the 90 days before enrolment. However, no significant associations were observed between antibiotic exposure and resistance to either of these antibiotics ($P = 0.785$ and 0.402, respectively).

Discussion

We found a high prevalence of *M. genitalium* carrying MRM (89.6%), QRM (68.3%) and dual-class resistance-associated mutations (68.3%) in asymptomatic MSM in Tokyo, irrespective of antibiotic exposure.

Almost all *M. genitalium* carried MRM, and 68.3% of the detected *parC* mutations, including S83I, S83R and D87N, limit moxifloxacin-based therapy.[7–10] The use of sitafloxacin, an

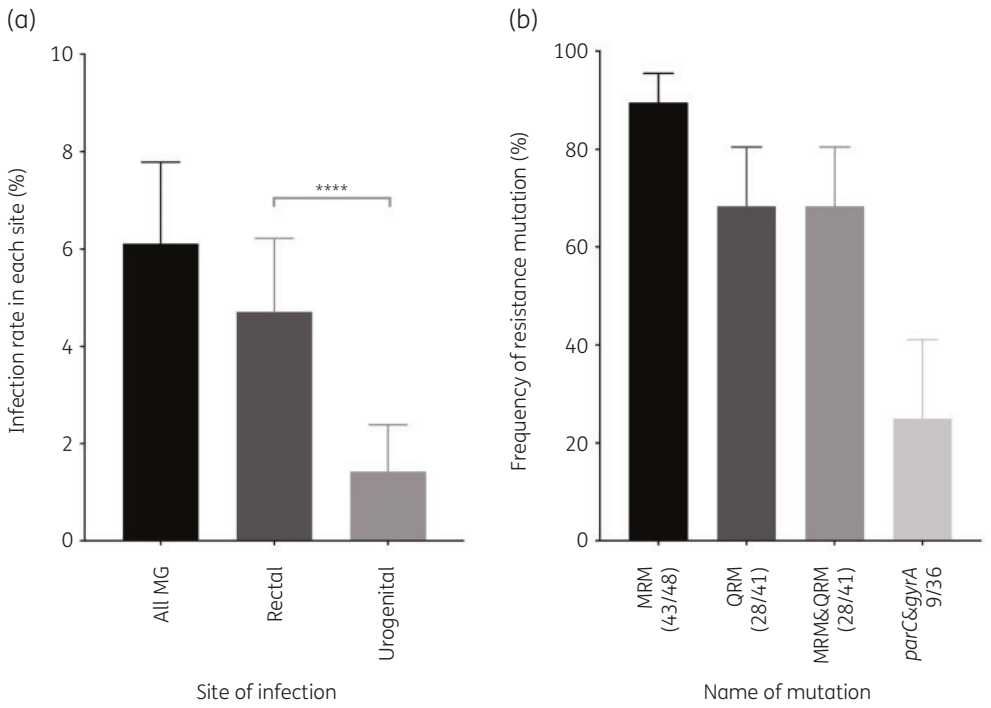

Figure 1. (a) Asymptomatic *M. genitalium* infection rate; ****$P < 0.0001$. (b) The rate of mutations associated with macrolide and/or fluoroquinolone resistance (MRM or QRM, respectively), defined as *parC* (excluding D82N) or *gyrA* mutations. MG, *Mycoplasma genitalium*.

alternative antibiotic for the treatment of resistant *M. genitalium* strains carrying *parC* mono-mutations, is approved in some countries, including Japan.[9,10,14] However, sitafloxacin-based treatment may not be as effective against the strains co-harbouring the *parC* and *gyrA* mutations reported here (25.0%). To the best of our knowledge, this is the highest ever reported prevalence of strains with mutations associated with dual-class resistance. One study has reported a prevalence of 6.1% of dual-class resistance,[15] whereas other studies have reported a prevalence of 7.4%–10.4% in the context of triple mutations in 23S rRNA, *parC* and *gyrA*.[5,16] Another alternative is to focus on quinolone resistance-based treatment strategies; however, the availability of commercial diagnostic tools to investigate quinolone resistance-associated mutations is a major challenge.[17] Importantly, both *parC* and *gyrA* mutations should be monitored as potential markers of fluoroquinolone treatment failure.

Doxycycline and sitafloxacin combination therapy and pristinamycin exhibit high efficacy in the treatment of resistant *M. genitalium*.[14,18] Therefore, further research on combination therapy using doxycycline, which is a low-cost antibiotic, and new potential drugs such as zoliflodacin, lefamulin and gepotidacin, which are effective against resistant *M. genitalium in vitro*,[19–21] is essential for establishing effective alternate treatments for *M. genitalium*.

Here, we report *M. genitalium* infection rates of 4.7% and 1.7% in the rectum and urethra of MSM in Japan. These results are in line with reports from Australia[3,22] and Japan,[23] with rectal and urethral infection rates of 7.0%–8.5% and 2.7%–4.0%, respectively. The high prevalence of *M. genitalium* in asymptomatic MSM necessitates considering routine screening in high-risk MSM. However, there are no consistently effective treatments against resistant strains of *M. genitalium*. Unlike in females, in whom pelvic inflammatory diseases and infertility are linked to infections, there is no sufficient evidence of sequelae to justify routine screening in MSM. Moreover, evidence that macrolides induce selective pressure for resistance-associated strains is limited. Therefore, at present, routine screening of *M. genitalium* in asymptomatic MSM is not fully justified.[4]

Urogenital and anorectal *M. genitalium* infections are more prevalent in HIV patients, compared with those in non-HIV patients;[24,25] this was not observed in this study, probably because of the small sample size in this study. Additionally, in contrast to the results of a previous study on symptomatic patients from China,[5] no association between resistant strains and antibiotic exposure in asymptomatic patients was observed in this study. Our results imply that MDR *M. genitalium* strains are already in circulation in Japan. Therefore, there is an urgent need to optimize antibiotic use based on antimicrobial stewardship programmes to prevent the spread of MDR *M. genitalium* strains.

This study is not without limitations. Not all samples were successfully sequenced; therefore, the reported rates may be slightly different from the actual ones.

In conclusion, we report an alarmingly high prevalence of dual-class resistant *M. genitalium* in asymptomatic MSM from Tokyo. Moxifloxacin-based therapy may not be sufficient in this context. Therefore, alternative approaches are needed to treat infections caused by MDR *M. genitalium*.

Acknowledgements

We thank Teruya Katsuji, Kunihisa Tsukada, Junko Tanuma and Daisuke Shiojiri for data collection and Yoshimi Deguchi and Mikiko Ogata, the study coordinators, for their assistance.

Funding

This work was supported by the National Center for Global Health and Medicine (grant numbers 19A1002 and 20A1020) and by ViiV Healthcare Supported International Clinical Research (ViiV Ref. 209459).

Transparency declarations

None to declare.

Supplementary data

Table S1 is available as Supplementary data at *JAC-AMR* Online.

References

1 Taylor-Robinson D, Jensen JS. *Mycoplasma genitalium*: from Chrysalis to multicolored butterfly. *Clin Microbiol Rev* 2011; **24**: 498–514.

2 Lis R, Rowhani-Rahbar A, Manhart LE. *Mycoplasma genitalium* infection and female reproductive tract disease: a meta-analysis. *Clin Infect Dis* 2015; **61**: 418–26.

3 Read TRH, Murray GL, Danielewski JA *et al.* Symptoms, sites, and significance of *Mycoplasma genitalium* in men who have sex with men. *Emerg Infect Dis* 2019; **25**: 719–27.

4 Golden MR, Workowski KA, Bolan G. Developing a public health response to *Mycoplasma genitalium*. *J Infect Dis* 2017; **216** Suppl 2: S420–S426.

5 Li Y, Su X, Le W *et al. Mycoplasma genitalium* in symptomatic male urethritis: macrolide use is associated with increased resistance. *Clin Infect Dis* 2020; **70**: 805–10.

6 Jensen JS, Bradshaw CS, Tabrizi SN *et al.* Azithromycin treatment failure in *Mycoplasma genitalium*-positive patients with nongonococcal urethritis is associated with induced macrolide resistance. *Clin Infect Dis* 2008; **47**: 1546–53.

7 Murray GL, Bradshaw CS, Bissessor M *et al.* Increasing macrolide and fluoroquinolone resistance in *Mycoplasma genitalium*. *Emerg Infect Dis* 2017; **23**: 809–12.

8 Couldwell DL, Tagg KA, Jeoffreys NJ *et al.* Failure of moxifloxacin treatment in *Mycoplasma genitalium* infections due to macrolide and fluoroquinolone resistance. *Int J STD AIDS* 2013; **24**: 822–8.

9 Hamasuna R, Le PT, Kutsuna S *et al.* Mutations in ParC and GyrA of moxifloxacin-resistant and susceptible *Mycoplasma genitalium* strains. *PLoS One* 2018; **13**: e0198355.

10 Murray GL, Bodiyabadu K, Danielewski J *et al.* Moxifloxacin and sitafloxacin treatment failure in *Mycoplasma genitalium* infection: association with parC mutation G248T (S83I) and concurrent gyrA mutations. *J Infect Dis* 2020; **221**: 1017–24.

11 Machalek DA, Tao Y, Shilling H *et al.* Prevalence of mutations associated with resistance to macrolides and fluoroquinolones in *Mycoplasma genitalium*: a systematic review and meta-analysis. *Lancet Infect Dis* 2020; **20**: 1302–14.

12 De Baetselier I, Kenyon C, Vanden Berghe W *et al.* An alarming high prevalence of resistance-associated mutations to macrolides and fluoroquinolones in *Mycoplasma genitalium* in Belgium: results from samples collected between 2015 and 2018. *Sex Transm Infect* 2021; **97**: 297–303.

13 Takanashi M, Ito S, Kaneto H *et al.* Development and clinical application of an InvaderPlus assay for the detection of genital mycoplasmas. *J Infect Chemother* 2015; **21**: 516–9.

14 Durukan D, Doyle M, Murray G *et al.* Doxycycline and sitafloxacin combination therapy for treating highly resistant *Mycoplasma genitalium*. *Emerg Infect Dis* 2020; **26**: 1870–4.

15 Deguchi T, Ito S, Yasuda M *et al.* Surveillance of the prevalence of macrolide and/or fluoroquinolone resistance-associated mutations in *Mycoplasma genitalium* in Japan. *J Infect Chemother* 2018; **24**: 861–7.

16 Vesty A, McAuliffe G, Roberts S *et al. Mycoplasma genitalium* antimicrobial resistance in community and sexual health clinic patients, Auckland, New Zealand. *Emerg Infect Dis* 2020; **26**: 332–5.

17 Fernández-Huerta M, Bodiyabadu K, Esperalba J *et al.* Multicenter clinical evaluation of a novel multiplex real-time PCR (qPCR) assay for detection of fluoroquinolone resistance in *Mycoplasma genitalium*. *J Clin Microbiol* 2019; **57**: e00886-19.

18 Read TRH, Jensen JS, Fairley CK *et al.* Use of pristinamycin for macrolide-resistant *Mycoplasma genitalium* infection. *Emerg Infect Dis* 2018; **24**: 328–35.

19 Damião Gouveia AC, Unemo M, Jensen JS. *In vitro* activity of zoliflodacin (ETX0914) against macrolide-resistant, fluoroquinolone-resistant and antimicrobial-susceptible *Mycoplasma genitalium* strains. *J Antimicrob Chemother* 2018; **73**: 1291–4.

20 Veve MP, Wagner JL. Lefamulin: review of a promising novel pleuromutilin antibiotic. *Pharmacotherapy* 2018; **38**: 935–46.

21 Jensen JS, Nørgaard C, Scangarella-Oman N *et al. In vitro* activity of the first-in-class triazaacenaphthylene gepotidacin alone and in combination with doxycycline against drug-resistant and –susceptible *Mycoplasma genitalium*. *Emerg Microbes Infect* 2020; **9**: 1388–92.

22 Couldwell DL, Jalocon D, Power M *et al. Mycoplasma genitalium*: high prevalence of resistance to macrolides and frequent anorectal infection in men who have sex with men in western Sydney. *Sex Transm Infect* 2018; **94**: 406–10.

23 Kato Y, Kawaguchi S, Shigehara K *et al.* Prevalence of *N. gonorrhoeae*, *C. trachomatis*, *M. genitalium*, *M. hominis* and *Ureaplasma* spp. in the anus and urine among Japanese HIV-infected men who have sex with men. *J Infect Chemother* 2020; **26**: 403–6.

24 Mavedzenge SN, Weiss HA. Association of *Mycoplasma genitalium* and HIV infection: a systematic review and meta-analysis. *AIDS* 2009; **13**: 611–20.

25 Crowell TA, Lawlor J, Lombardi K *et al.* Anorectal and urogenital *Mycoplasma genitalium* in Nigerian men who have sex with men and transgender women: prevalence, incidence, and association with HIV. *Sex Transm Dis* 2020; **47**: 202–6.