Macrolide and fluoroquinolone associated mutations in Mycoplasma genitalium in a retrospective study of male and female patients seeking care at an STI Clinic in Guangzhou, China, 2016-2018.

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Abstract

Background

Antimicrobial resistance in *M. genitalium* is a growing clinical problem. We investigated the presence of mutations for macrolide and fluoroquinolone, two commonly used medical regimens for treatments in China. Our aim is to analyze the prevalence and diversity of mutations among *M. genitalium*-positive clinical specimens in Guangzhou, Guangdong, south China.

Methods

A total of 154 stored *M. genitalium* positive specimens from men and women attending an STI clinic were tested for macrolide and fluoroquinolone mutations. *M. genitalium* was detected via TaqMan MGB real-time PCR with a sensitivity of five genome equivalents (geq)/reaction. Mutations associated with macrolide resistance were detected using primers targeting region V of the 23S rRNA gene. Fluoroquinolone resistant mutations were screened via primers targeting topoisomerase IV (parC) and DNA gyrase (gyrA).

Results

98.7% (152/154), 95.5% (147/154) and 90.3% (139/154) of *M. genitalium* positive samples produced sufficient amplicon for detecting resistance mutations in 23S rRNA, gyrA and parC genes, respectively. 66.4% (101/152), 0.7% (1/147) and 77.7% (108/139) samples manifested mutations in 23S rRNA, gyrA and parC genes, respectively. A2072G (59/101, 58.4%) and S83I (79/108, 73.1%) were highly predominating in 23S rRNA and parC genes, respectively. Two sample had amino acid alteration in gyrA (M95I and A96T, respectively). Two sample had two amino acid alterations in parC (S83I + D87Y). 48.6% (67/138) samples harbored both macrolide and fluoroquinolone resistance-associated mutations. The most common combination of mutations was A2072G (23S rRNA) and S83I (parC) (40/67, 59.7%). One sample had three amino acid changes in 23S rRNA, gyrA and parC genes (A2072G + A96T + S83I).

Conclusions

The high antimicrobial resistance rate of *M. genitalium* shows a worrisome trend in Guangzhou and suggests antimicrobial resistance testing and the development of new antibiotic regimens are crucial.
Background
Antimicrobial resistance (AMR) of Mycoplasma genitalium (M. genitalium) to antibacterial regiments is a growing problem with global implications for clinical guidelines and treatment [1-6]. As Jensen and Bradshaw (2015) argue, clinical monitoring and effective reporting on antimicrobial resistance-mediating mutations in M. genitalium across geographic regions and populations are crucial for developing effective treatments in managing M. genitalium infections and AMR-mediation across global settings [7]. Yet, despite being one of the most populous countries in the world, there is sparse data on the circumstances of AMR-related mutations in China. Here, we contribute to global efforts to address this gap in AMR surveillance by investigating the presence of mutations to macrolide and fluoroquinolone, two commonly used medical regiments for bacterial intervention treatments in Guangzhou, China.

What diseases are caused by or associated with M. genitalium?
M. genitalium is commonly associated with sexually transmitted infections (STIs) and often presents as a co-infection other STIs [5, 8-10]. It was first isolated from urethral specimens in male patients with Nongonococcal urethritis (NGU) in 1981 by Tully et al [11]. M. genitalium belongs to the order Mycoplasma in the class Mollicute, which is the smallest known prokaryotic microorganism capable of self-replication [12]. Substantial evidence supports research of M. genitalium being a major cause of STI, including urethritis [13], Mucopurulent cervicitis (MPC) [14], endometritis [15], and pelvic inflammatory disease (PID) [16, 17]. M. genitalium is also a suspected cause of reactive arthritis and proctitis [18]. The 2009 European guidelines on NGU management identified M. genitalium and Chlamydia trachomatis (C. trachomatis) as leading causes of NGU [19]. In reviewing recent prevalence rates collected from studies across diverse medical facilities in England and reported in journals from 2006-2016, Horner and Martin [20], argue that NGU is the most common clinical manifestation of M. genitalium [20]. Based on available data, they project that if 125,000 male patients were to become infected with M. genitalium in England, 5.2% would likely develop NGU during a one-year infection period [20]. In Australia, a report published in 2006 provides support for this pattern from earlier periods – male patients with NGU tested significantly more frequently for M.
genitalium compared to male patients without NGU at a rate of 9–1% [21]. In the US, a study from 2009 also supports this trend– males with urethritis tested positive for M. genitalium at 22.4% vs males without urethritis having positive results at a rate of 7.3% [22].

What Are Known Or Suspected Factors For M. Genitalium?

According to a recent meta-analysis of 63 epidemiological studies published from 1991 to 2016 [23], the incidence of M. genitalium infection appears related to the income-level of reporting localities. In high-income countries (HIC) in US and Western Europe such as Denmark, Great Britain, Norway, and Russian Federation, the calculated summary incidence rate was extremely low, at 1.3% [23], ranging between 1% to 3% for both men and women in general populations [24–26]. However, in comparison, general populations living in low-and-middle-income countries (LMIC), such as Honduras, Vietnam, Kenya, Madagascar, and Tanzania experienced much higher disease burden. The calculated summary incident rate was almost three times higher, at 3.9%, for the same period [23].

Many studies from earlier waves of research, like the meta-analysis described above [23], report not finding significant differences in M. genitalium prevalence and risk factors between men and women. However, mounting evidence suggest differences by sex for acquiring an M. genitalium infection [17, 27]. According to Wang et al., among 2753 outpatients (2161 males, 592 females) seeking treatment at an STI clinic in Nanjing, the M. genitalium infection rate was 7.95% [28]. Focusing solely on M. genitalium infection alone, male patients had a significantly higher infection rate at 62.30% compared to female patients at 36.84% [28]. Similarly, Qin et al [29] from our STI facility found an infection rate of 7.94% (209/2,633) from swabs of patients’ genital tract, with males experiencing significantly higher positive tests at 8.94% (n = 1958) compared to female patients at 5.04% (n = 675) [29].

Moreover, there is a growing consensus from research in China and internationally, of other characteristics contributing to increased risk of M. genitalium infections. Characteristics linked to increased infection include experiencing problems with fertility for both men [9, 30] and women [31], abnormal pregnancy status [32], and from being members of vulnerable populations, including men-who-have-sex-with men (MSM) [33], female sex workers (FSW) [34], and people living with HIV [35].

As reported in this journal, a study from Shenyang, a city in north China, reported that M. genitalium
infection is a significantly correlated to HIV infection among MSM living in China [33].

Overall, the high rates of infection in LMIC, and patterns of co-infecting STIs coupled with high disease burden among vulnerable populations, even for people living in HIC, are causes for concern. These considerations have spurred clinicians and public health agencies around the globe to call for concerted management of M. genitalium guidelines and treatment as a means of mitigating AMR-related problems [1–6].

AMR Problems Related To M. Genitalium Treatment Interventions

Due to the lack of a cell wall, M. genitalium is hardy, readily surviving the onslaught of antibiotics, such as penicillin, typically used in first-wave and multi-wave medical treatment interventions prescribed in clinical setting [36]. Taking advantage of in vitro tests that demonstrate M. genitalium sensitive to tetracyclines, macrolides, and new fluoroquinolones [37], current treatment interventions and guidelines often recommend these medications. According to the 2016 European NGU guidelines, patients who test positive for urethritis should be tested for C. trachomatis and M. genitalium via nucleic acid amplification testing [38]. Since a single-dose treatment of azithromycin may result in the development of antimicrobial resistance in M. genitalium, the 2015 UK NGU guidelines and the 2016 European M. genitalium guidelines were no longer recommended azithromycin 1 g as first line therapy [39, 40]. Hence, it is troubling that mounting evidence illustrating M. genitalium drug-resistance increases with even just a single-dose treatment of azithromycin [2, 41–43]. The macrolide sensitive M. genitalium eradication rate was 85% (82–88%, in the 12 studies prior to 2009) compared to 67% (57–77%, in the 9 studies since 2009) [44]. In locations with high incidence of NGU, the practice of widespread use of a single-dose antimicrobial treatment can be problematic. This is the case for Greenland, where 55,000 inhabitants have tested positive for NGU, with common treatment intervention consisting of single dose azithromycin therapy, from 1998 to 2005. Unfortunately, macrolide resistance rate of M. genitalium has been reported to be 100% in the region [45].

Fluoroquinolone moxifloxacin, another medication extensively used as a second-line bactericidal, has a cure rate approaching 100% in infections with susceptible strains [46]. In recent years, an increasing moxifloxacin treatment failure rate has also been noted, first in the Asia-Pacific region [7]
and later in Australia [47]. Indications point to an emergence of fluoroquinolone resistant strains [47–49]. The elimination rate of moxifloxacin for M. genitalium infection has decreased from 100–89% since 2010 [47].

Genetically, mutations resulting in macrolide resistance are primarily attributed to single-nucleotide polymorphism (SNP) at positions A2058 or A2059 in region V of the 23S rRNA gene [2, 50]. Fluoroquinolone resistance is attributed to alternations the GyrA subunit in DNA gyrase (which is composed of two gyrA and two gyrB subunits), or the ParC subunit of topoisomerase IV (which is composed of two parC and two parE subunits) [51]. Moxifloxacin resistant M. genitalium isolates, primarily causing amino acid changes at positions S83 and D87 (M. genitalium numbering) of parC, are similar to those found in other fluoroquinolone resistant bacteria [41, 52–54]. AMR studies of fluoroquinolone resistance in M. genitalium DNA tend to amplify the quinolone-resistance determining region (QRDR) of the gyrA gene and the corresponding region of the parC gene from M. genitalium DNA [55].

Multidrug resistance is present in both macrolide and fluoroquinolone resistance-associated mutations in M. genitalium being reported in Japan, Australia and New Zealand since 2008 [41, 52, 56, 57]. This disturbing trend suggesting that the AMR dilemma attributable to M. genitalium is spreading and becoming even more virulent [41, 52, 56, 57].

Clinical Research On M. Genitalium Antimicrobial Resistance In China

Although many published reports focus on M. genitalium prevalence and risk-factors across diverse sub-populations, at the time of this study, there are only three locations actively conducting AMR-related research in clinical settings in China. There are two published reports, one study of macrolide and quinolone resistant among men attending an STI center in the Nanjing vicinity, a city on the coastline central coastline, near the China Centers for Disease Control and Prevention [58, 59], published in Chinese and English. The second study is on macrolide and tetracycline mutations among men seeking care at an infertility clinic in Changsha, in the interior of China [60], published in English. Our location, an STI center based in a hospital in Guangzhou, constitutes the third AMR study site. Our facility is situated in Guangdong, the biggest province in China, with more than 100 million people
living within this region of south China. Our hospital is located in Guangzhou, a city at the geographic center of Guangdong. As a major city in the Pearl River Delta, Guangzhou is well-integrated with nearby metropolises of Hong Kong, Macau, and Shenzhen, each within a one-hour commuting radius. Guangzhou is the earliest foreign trade port, so there are many foreign exchanges and high population movement. This region is an international hub for travel, trade, and commerce and a major destination for migrants and their concomitant illnesses. As a provincial level STI center, our doctors are referred patients from all over the region when care providers from feeder hospitals throughout the region are unable to resolve medical ailments locally.

Servicing the medical needs of such a diverse population, our study focuses on macrolide and fluoroquinolone resistance-associated mutations in M. genitalium. While the two published studies are the first to address AMR mutations in China, we build on current knowledge in two keyways. First, we continue monitoring and reporting efforts on macrolide and fluoroquinolone resistance, expanding on reports from the two prior studies based in central and interior China, by contributing data on a major urban migration destination in south China. Second, we expand on AMR surveillance by being the first to report macrolide and fluoroquinolone-associated mutations in men and women population in China.

Looming AMR dilemma: A near clinical failure for treating M. genitalium

Along with conducting this study, our team recently shared a case-report describing the dire conditions of AMR confronted by patients [Unpublished]. A particularly virulent case was treated at our facility in Guangzhou in 2017 - a patient experiencing a 2-month-long history of persistent urethral irritation and urethral discharge was referred to our clinic. From the referral clinic, we collected the medical history for a 48-year-old male who presented at our clinic for C. trachomatis. Doctors at prior facility had prescribed one combination treatment of a 7-day course of azithromycin and a subsequent 7-day course of minocycline. After these initial treatments, the patient reported mild improvement of urethral irritation, but the symptoms never fully resolved, and urethral discharge persisted despite the C. trachomatis tests coming back with negative results in post-treatment assessments.

Unfortunately, after 8 weeks of treatment, the patient was referred to our provincial-level STI center.
We conducted a test for C. trachomatis from the first void urine sample via Cobas 4800 chlamydia/NG Amplification/Detection Assay (Roche Molecular Systems Inc, New Jersey, USA). Physical examination of the genital area revealed redness of the penile meatus, and his underwear was contaminated with a small amount of discharge, symptomatic indicators of M. genitalium infection. Collection of first void urine sample was then analyzed and was negative for C. trachomatis and N. gonorrhoeae. During the following 10 months, the patient was treated with a series of antibiotics to resolve M. genitalium symptoms. There were a total of six treatment regiments. When the diagnostic lab work indicated M. genitalium positive results, the patient was (1) started on antofloxacin (a fluoroquinolone antibiotic produced in China) 200 mg once a day for 7 days, followed by azithromycin 500 mg once a day for 7 days; (2) when M. genitalium results came back positive, the second regiment consisted of moxifloxacin 400 mg once daily for 7 days, followed by minocycline 100 mg twice daily for 14 days; (3) persistent M. genitalium infection after the second round of treatments resulted in a prescription for antofloxacin at 200 mg once daily combined with rifampin 450 mg once a day for 21 days; (4) when this regimen also failed to clear the M. genitalium infection, the patient was put on azithromycin 500 mg once a day for 21 days. (5) After exhausting orally administered medications, when the results came back positive again, our team started the patient on intramuscular spectinomycin 2 g once a day for 7 days. Although there were promising results initially, with M. genitalium test screening coming back negative on day 11 and 22, the infection results came back positive again on day 52. (6) The last regiment, doxycycline 100 mg twice a day for 14 days, yielded M. genitalium negative results during screenings on day 19, 81 and 116 after doxycycline treatment. His symptoms almost resolved except for occasional urethral irritation. During the more than 10 months of treatment, he occasionally had protected vaginal sex with his wife. Cervical swabs from his wife were negative for C. trachomatis, N. gonorrhoeae, and M. genitalium. During his intensive course of care, our team collected and analyzed several laboratory specimens. A urethral swab sample was taken prior to starting the 21-day treatment of azithromycin. M. genitalium had amino acid changes of S83I in parC, and G93C in gryA. M. genitalium had A2059G mutation in positions 2059 (E. coli numbering) of the 23S rRNA gene. No mutations were found in the gyrB.
Despite interventions using a combination of azithromycin, minocycline, moxifloxacin and spectinomycin, this case was a near clinical failure for effective treatment of persistent M. genitalium infection. From his extended schedule of treatments, it is evident that this patient suffered from AMR complications, and is a harbinger of looming AMR problems in China.

Clinical monitoring and effective reporting of findings and research of antimicrobial resistance-mediating mutations in M. genitalium across geographic regions and populations are crucial for the development efficacious regiments for combating M. genitalium infections and managing AMR across global settings [7]. Unfortunately, there is sparse data and low awareness of the trends of antimicrobial resistance of M. genitalium in China. The aim of this study is to support and contribute to AMR research in south China by analyzing the prevalence and diversity of mutations associated with macrolide and fluoroquinolone resistance among M. genitalium in positive clinical specimens in Guangzhou, China.

**Methods**

**Study population and specimens**

A total of 154 M. genitalium positive clinical specimens were collected from patients initially attending an STI clinic at Dermatology Hospital, Southern Medical University, Guangzhou, China. The collecting period was from December 2016 to December 2018. The samples included urethral swabs and/or rectal swabs from male patients and cervical swabs from female patients. M. genitalium was detected via TaqMan MGB real-time polymerase chain reaction (PCR) with a sensitivity of five genome equivalents (geq)/reaction as described by Jensen et al [61]. DNA extracted from M. genitalium positive samples were tested the same day or stored at -20°C prior to use. Samples used in our study were collected with the permission of STI patients as part of conventional protocol for diagnostics and treatment. The specimens were then processed and stored with no identifiable data from our patients. As part of hospital protocol, de-identified patient samples are stored in the STI clinic biobank for surveillance, diagnosis, and research purposes. This study utilities specimens from this biobank. In our hospital protocol, the department who collected the samples are subjected to use the stored samples, under these conditions, no ethical review was required in implementing clinical analysis of
Detection of macrolide and fluoroquinolone resistance-associated mutations in 23S rRNA, gyrA and parC

From extracted DNA, mutations associated with macrolide resistance were detected using primers targeting region V of the 23S rRNA gene (nucleotides 1992–2138) [50]. Fluoroquinolone resistance mutations in the gyrA (nucleotides 172–402) and parC (nucleotides 164–483) genes were screened using primers as reported previously [62, 63]. Details of the primer sequencing and thermo-cycling parameters for amplification are provided in Table 1.

| Primer sequences and thermo-cycling parameters for amplification of resistance-determining regions |
|----------------------------------------------------------------------------------|------------------|-----------------|-------------------|
| Forward primer (5’-3’) | Reverse primer (5’-3’) | Fragment Length | Thermo-cycling parameters |
| 23 s-1992F | 23S-2138R | 147 bp | 95°C-3 min–denaturing |
| CCATCTCCTTGACTGTCTCGGCTAT | CCTACCTATTTCTTACATGGTGGTGT | 147 bp | 95°C-3 min–denaturing |
| gyrA-F | gyrA-R | 300 bp | 94°C-30sec |
| CCTATGCTAGAGTGACTTTAA | ATTATCTAAACTTGCAGCAACTT | 300 bp | 94°C-30sec |
| parC-F | parC-R | 214 bp | 58°C-30sec 35 cycle |
| TGGGCTTAAAACCCGCCACT | CGGGTTTGCTGTAAACGCAT | 214 bp | 72°C-10 min–extension |

bp: base pair

Each reaction volume of 25 μl contained 10 × PCR buffer (without Mg²⁺: 100 mM Tris-HCl pH 8.8 at 25°C; 500 mM KCl, 0.8%(v/v) Nonidet), 0.5 μl each forward and reverse primer, 0.5 μl dNTP 10 mM, 1 U of Pfu DNA polymerase (Invitrogen) and sterile water. Fragment Lengths were between 118–300 base pairs (bp). Forward primers included 23 s-1992F, gyrA-F, and parC-F. Reverse primers included 23 s-2138R, gyrA-R, and parC-R. Confirmation of PCR product was achieved by using an agarose gel (1.5%) electrophoresis. The amplified fragments were purified by QIAquick PCR Purification Kit (QIAGEN). Sequencing services were purchased and outsourced to Sangon Biotech, China. All sequencing results were compared with the sequence of reference strain G37 (GenBank accession number: NC_000908.2).

Data Analysis

23S rRNA, GyrA and parC genes were assembled using contiguous and FASTA sequences. Contiguous sequences were assembled using the PRABI-Doua: CAP3 Sequence Assembly Program (http://doua.prabi.fr/software/cap3) and FASTA sequences were obtained via BLASTN, an open-source software available online (https://blast.ncbi.nlm.nih.gov/).

Ethics Approval

The study was approved by the Ethics Committee of Dermatology Hospital of Southern Medical University (approval no. GDDHLS – 20171203, 13/12/2017). Informed consent was waived as for the committee believe that the research presents no potential risk to identify the harm resulting from a
breach of confidentiality

Results

Summary of AMR Clinical Studies in China

As M. genitalium testing is not part of routine screening or even opportunistic screening protocols in China, relevant studies are usually retrospectively designed or conducted on stored samples. This is the case for clinical studies on AMR in China. Table 2 summarizes the characteristics and macrolide resistant mutations across these three studies.

| Table 2 | Clinical studies of macrolide resistance-associated mutations in 23S rRNA in China, 2012–2018 |
|---------|-------------------------------------------------------------------------------------------|
| Author  | Liu et al [58] | Li et al [59] | Li et al [60] | Ke et al [present study] |
| Reporting language | Chinese | English | English | English |
| Study location | Nanjing, Jiangsu | Nanjing, Jiangsu | Changsha, Hunan | Guangzhou, Guangdong |
| Study period | 2012 (Apr-May) | 2011(Apr) – 2015 (Aug) | 2016 (Oct) – 2017 (Dec) | 2016–2018 |
| Study type | Retrospective | Cohort | Retrospective | Retrospective |
| Patient Population | NGU | NGU | Infertile | STI |
| Sample size | 18 | 341 | 60 | 152 |
| Sex | Men | Men | Men | Men + Women |
| 23S rRNA mutation rate | 94.4% | 88.9% | 96.7% | 66.4% |
| A2058G | 27.8% | 17.6% | 20.0% | 13.2% |
| A2058T | 11.1% | 0% | 1.7% | 13.2% |
| A2058C | 0% | 0.3% | 0% | 0% |
| A2059G | 55.6% | 61.9% | 60.0% | 39.5% |
| A2059C | 0% | 0.3% | 0% | 0.7% |
| A2059T | 0% | 8.8% | 0% | 0% |
| A2058T + A2059G | 0% | 0% | 11.7% | 0% |
| A2058G + A2059G | 0% | 0% | 1.7% | 0% |
| A2059G + T2086C | 0% | 0% | 1.7% | 0% |

*Escherichia coli numbering; rRNA: ribosomal ribonucleic acid; NGU: Non-gonococcal urethritis; STI: Sexually Transmitted Infection

The earliest published clinical research conducted on macrolide-associated mutations conducted on samples collected on a group of men (n = 18) seeking medical care for NGU symptoms in Nanjing [58]. The 23S rRNA mutation rate was 94.4%, with A2059G being the most common (55.6%), A2058G being second most (27.8%), and A2058T as the third most common mutation (11.1%), with no double-mutations detectable [58]. Then, 358 M. genitalium positive samples were collected, the 23S rRNA, parC and gyrA genes were successfully amplified and sequenced in 341 samples (95.3%), 344 samples (96.1%) and 339 samples (94.7%), respectively [59]. The 23S rRNA mutation rate was 88.9%,
with A2059G being the most common (61.9%), A2058G being second most (17.6%) no double-mutations were detectable [59]. The parC mutation rate was 90.4%, with Ser83→Ile being the most common (83.7%), a double mutation in G248A + G259T were detectable [59]. The gyrA mutation rate was 13.0%, with Met95→Ile being the most common (5.3%), three double-mutations in G244A + G285A, G285A + A309G, and G285A + A317G were detectable [59]. In comparison to the Nanjing study, the Changsha study conducted macrolide and tetracycline associated mutations on samples collected from men attending an infertility clinic (n = 60) [60]. Macrolide and tetracycline resistances were found in 58 samples (96.7%) and 27 samples (45.0%), respectively [60]. The macrolide mutations rate was similarly extremely high at 96.7% [60]. However, it is noteworthy that the percentage distribution and type of mutations reported differ between the two studies. The two most common mutations in the Nanjing study are also the most frequent mutations in Changsha, that is, A2059G (60.0%) and A2058G (20.0%) [60]. Unlike the earlier study, the analysis conducted on specimens from Changsha detected double-mutations, and these mutations are frequent enough to be third most common set of mutations (A2058T + A2059G at 11.7%) [60].

In our study, we examine both men and women (n = 152), with our results yielding much lower aggregated combined rates of mutations (66.4%). Mutations at A2059G are still the most common, but at a much lower rate (39.5%), A2058G and A2058T are present in samples in the same percentages (13.2%), with A2059C (0.7%) being introduced in this study as a new mutation. Double mutation was not detected for among our samples.

Laboratory Results Of AMR Mutations In M. Genitalium

A total of 154 M. genitalium DNA-positive samples were stored from the collection period (December 2016-December 2018). Of these, 98.7% (152/154) of positive samples produced sufficient amplicon for detecting macrolide resistance mutations in the 23S rRNA gene. Results are summarized in Table 3.
Table 3
Prevalence of SNPs related to macrolide-resistance in domain V of the 23S rRNA gene and fluoroquinolone resistance-associated mutations in QRDR of GyrA and ParC from 154 M. genitalium positive samples in Guangzhou, China, 2016–2018

| Gene     | SNPa (E. coli numbering) | Amino acid change (E. coli numbering) | Frequency, % (No of samples containing mutation(s) or wild type / No of successfully sequenced samples) |
|----------|--------------------------|--------------------------------------|-----------------------------------------------------------------------------------------------------|
| 23S rRNA | A-2071 (2058)→G          | b                                    | 13.8 (21/152)                                                                                        |
|          | A-2071 (2058)→T          |                                      | 13.2 (20/152)                                                                                        |
|          | A-2072 (2059)→G          |                                      | 38.8 (59/152)                                                                                        |
|          | A-2072 (2059)→C          |                                      | 0.7 (1/152)                                                                                         |
|          | Wild type                |                                      | 33.6 (51/152)                                                                                        |
| GyrA     | G-285→C                  | Met-95(83)→Ile                       | 0.7 (1/147)                                                                                         |
|          | G-286→A                  | Ala-96(84)→Thr                       | 0.7 (1/147)                                                                                         |
|          | Wild type                |                                      | 98.6 (145/147)                                                                                      |
| ParC     | C-234→T                  | Silent mutation                      | 0.7 (1/139)                                                                                         |
|          | G-241→T                  | Gly-81(78)→Thr                       | 0.7 (1/139)                                                                                         |
|          | A-247→C                  | Ser-83(80)→Arg                       | 2.2 (3/139)                                                                                         |
|          | G-248→A                  | Ser-83(80)→Asn                       | 5.8 (8/139)                                                                                         |
|          | G-248→T                  | Ser-83(80)→Ile                       | 56.8 (79/139)                                                                                       |
|          | T-249→A                  | Ser-83(80)→Arg                       | 1.4 (2/139)                                                                                         |
|          | G-259→T                  | Asp-87(84)→Tyr                       | 2.9 (4/139)                                                                                         |
|          | G-259→A                  | Silent mutation                      | 2.2 (3/139)                                                                                         |
|          | A-260→G                  | Asp-87(84)→Gly                       | 2.9 (4/139)                                                                                         |
|          | T-267→C                  | Silent mutation                      | 0.7 (1/139)                                                                                         |
|          | G-248→T                  | Ser-83(80)→Ile + Asp-87(84)→Tyr     | 1.4 (2/139)                                                                                         |
|          | + G-259→T                | Wild type                            | 22.3 (31/139)                                                                                        |

a Nucleotide positions in 23S rRNA and in GyrA and ParC gene are given according to the M. genitalium G37 genome (GenBank accession no. NC_000908.2). E. coli numbering is shown in parentheses. SNP: single-nucleotide polymorphism; rRNA: ribosomal ribonucleic acid; b -., no amino acid change.

Among 152 samples, 66.4% (101/152) manifested mutations in the 23S rRNA gene, and 33.6% were wild type. The mutation A2072G (n = 59) was highly predominating in Guangzhou, accounting for 58.4% (59/101) of the cases positive for nucleotide substitutions in the 23S rRNA gene. Other detectable mutations include A2071G (n = 21), A2071T (n = 20), and A2072C (n = 1). Two nucleotide substitutions in the 23S rRNA gene were not detected.

The quinolone resistance determining regions (QRDR) of gyrA and parC genes could be successfully sequenced in 95.5% (147/154) and 90.3% (139/154) of samples, respectively (Table 3). Amino acid alterations in gyrA (M95I, A96T) were only detected in two samples. To our knowledge, this specific amino acid change (A96T) has not been reported elsewhere in the AMR literature. However, mutation at the next position (99 in gyrA) has been described in the previous reports, and the positions are within the QRDRs, indicating its association with fluoroquinolone resistance [53]. parC mutations typically associated with fluoroquinolone resistance was detected in 77.7% (108/139) of samples. Of
these, the most frequent mutation was S83I (n = 79), accounting for 73.1% of 108 samples with SNPs in parC. In two samples, two amino acid alterations in parC (S83I + D87Y) were present. As shown in Table 3, alterations in parC are more common than in gyrA.

Of the 138 samples undergoing complete analysis for both the 23 s RNA and parC genes, 48.6% (67/138) samples harbored both macrolide and fluoroquinolone resistance-associated mutations. Combining the 23S rRNA and parC mutations, 15 genotypes were identified (Table 4).

**Table 4**

| Mutation(s)         | Count (%) |
|---------------------|-----------|
| A2071G + S83I       | 8 (5.8)   |
| A2071G + S83R       | 2 (1.4)   |
| A2071G + S83N       | 1 (0.7)   |
| A2071G + D87G       | 1 (0.7)   |
| A2071G + D87N       | 1 (0.7)   |
| A2071T + S83I       | 1 (0.7)   |
| A2071T + S83R       | 2 (1.4)   |
| A2071T + D87N       | 1 (0.7)   |
| A2071T + D87Y       | 1 (0.7)   |
| A2072G + G81C       | 1 (0.7)   |
| A2072G + S83I       | 40 (29.0) |
| A2072G + S83R       | 3 (2.2)   |
| A2072G + S83N       | 1 (0.7)   |
| A2072G + D87Y       | 3 (2.2)   |
| A2072G + S83I + D87Y| 1 (0.7)   |

*a Nucleotide positions in 23S rRNA and in parC gene are listed in accordance to the *M. genitalium* G37 genome (GenBank accession no. NC_000908.2). b Only 138 successfully sequenced samples included.

The most common combination of mutations was A2072G (23S rRNA) + S83I (parC) (59.7%, 40/67). The second most common combination being A2071G + S83I (5.8%, 8/67). Combinations based on A2072G (73.1%, 49/67), A2071G (19.4%, 13/67), and A2071T (7.5%, 5/67). One sample was observed with amino acid changes both in gyrA and parC and mutations in the 23S rRNA gene (A2072G + S83I + A96T).

**Discussion**

*M. genitalium* is an emerging but undiagnosed pathogen of NGU, as prevalent as *C. trachomatis* in high-risk populations. Other countries have found 1 to 2.8% *M. genitalium* prevalence rates among sexually active adolescents and 14.1% in FSW [26, 64, 65]. Our previous study reported that the number of *M. genitalium*-infected patients in STI clinics of Guangzhou was 7.9(209/2,633) from 2010 to 2013 [29]. Within the last 10 years, *M. genitalium* eradication rate has become declined gradually [66, 67]. The resistance rate of *M. genitalium* has been described as a rising phenomenon.
substantially in many countries [68, 69]. This study aimed to determine a data of the prevalence of macrolide and fluoroquinolone resistance-associated mutations in M. genitalium in the Guangzhou region, 2016–2018.

In the present study, we found that SNPs in region V of the 23S rRNA gene was observed in 101 (66.4%) samples from male and female patients with M. genitalium-positive infection in 2016–2018. The majority of mutations occurred at positions A2058 and A2059 mainly to G (C or T is relatively less), and with the exception of a study from Greenland, the mutation frequency (66.4%) [70] observed was relatively higher than frequencies reported by Russia and Estonia (0.7–10%) [71], South Africa (10%) [72], southern Sweden (13%) [73], France (17%) [74], Japan (42.2%) [68], southern USA (48%) [75], Norway (56%) [76], and Denmark (57%) [76]. However, the rate of 66.4% in our study is lower than rates reported across studies from England (82.4%) [77], the US (Alabama: 74.1% HIV positive MSM) [35], and Australia (79.4%) [78]. These single nucleotide mutations confer high-level macrolide resistance and are predicted to result in treatment failure for M. genitalium infections [79].

The single-dose regimen of azithromycin 1 g has been recommended as first-line treatment for NGU (include M. genitalium and C. trachomatis) infection in many regions and in country-specific guidelines [70, 80]. However, with the trend of increasing macrolide resistance rate for M. genitalium, there is a steady concomitant rise in treatment failures being reported worldwide [48, 65, 72, 73, 81]. In China, antibiotics are easily and readily available to the public as an over-the-counter medication purchased in drug stores. As a self-administered treatment for people feeling unwell, it may well be overused, exacerbating and worsening the public health and medical problems of antibiotics resistance confronting health facilities today [82].

It is widely reported that M. genitalium expressed a diversity of mutations linked to fluoroquinolone resistance-associated in gyrA and parC gene [65, 76, 83]. Similar to findings from other studies, mutations in the QRDR of the gyrA gene of all samples were rarely detected in our study [65, 84]. The amino acid changes (Met95→Ile and Ala96→Thr) in gyrA were found in our specimens. The Met to Ile transition at position 95 of gyrA (G to C at nucleotide position 285) was first reported in 2013 by Tagg
et al [83], most commonly observed from 2013 to 2017 in Japan, have been reported in moxifloxacin-resistant strains of M. pneumoniae, M. hominis, and Ureaplasma spp [69, 83, 85, 86]. To our knowledge, a gyrA Ala96→Thr mutation in the core of the QRDR has not previously been described in M. genitalium and its association with resistance to fluoroquinolone remains unknown. M. genitalium resistance to fluoroquinolones is increasing and shows the same trend as macrolide resistance. In our region among the 139 samples successfully amplified DNA sequences of parC gene, we observed an exorbitantly high mutation rate of 77.7%. The amino acid changes at G81, S83 and D87, have been previously reported as being associated with fluoroquinolone resistance in M. genitalium and other closely related organisms [83, 85, 87, 88].

Although the majority of published reports have shown the ParC S83N and S83I alteration as the two most prevalent base change at position 248, our study revealed that the S83I alteration were predominating accounting for 71.8% (79/110), significantly higher than those from Japan (13.0-23.2%) [68], New Zealand (16.7%) [56], and southwestern France (9.1%) [74]. Although present, mutation S83N (n = 8) might not significantly increase the moxifloxacin minimum inhibitory concentrations (MIC; 0.125 mg/L; Jensen et al., unpublished data). To our knowledge, the amino acid changes G81T and R91I in the parC identified in this study are new findings and have not been reported previously in M. genitalium or in any closely related organisms. Tagg et al. reported G81C for the first time from 2008 to 2011, then having liked this mutation to fluoroquinolone resistance in M. pneumoniae [83]. Double amino acid changes of S83I and D87Y in parC were observed in two specimens. However, in our study, the prevalence of fluoroquinolone resistance-associated mutations in ParC is higher in comparison to macrolide resistance-associated mutations. Aside from previous studies reported from Japan [88], this trend is rarely found in extant prior studies.

49.3% (68/138) samples were multidrug resistant contained both macrolide and fluoroquinolone resistance related SNPs. If SNP on parC is strictly limited to S83I, the multidrug resistance rate was 36.1% (50/138). In Japan, the prevalence of multidrug resistance with A2058G or A2059G in the 23S rRNA and amino-acid change in Ser83 or Asp87 of parC has been reported up to 21.8% in 2010-2017 [68]. Our data shown very high prevalence (47.8%) of the same mutation. This trend of multidrug
resistance presents challenges the clinicians because of lacking a suitable alternative therapy after azithromycin and moxifloxacin failure. Pristinamycin as the only third-line treatment has been reported to be only about 75% effective and is not readily available in China [89].

Limitations
The limitation of this study is that phenotypic macrolide and fluoroquinolone *in vitro* susceptibility testing was not performed on the sequenced isolates. The significance of several novel mutations in the parC and gyrA gene remains unknown. Nonetheless, related to phenotypic testing has been previously reported in several studies for most mutations. The prevalence of resistance was based on the hypothesis that samples were collected at the patients’ first visit without use of azithromycin or moxifloxacin, the mutation rates are probably overestimated. Additionally, we lack the data of large sample epidemiological survey of *M. genitalium* and the samples were collected mainly from a single clinic, might not be representative of the Guangzhou population.

Conclusions
In conclusion, the reported high resistance rate of *M. genitalium* in this study shows a worrisome trend in Guangzhou. For patients with *M. genitalium* infection, antimicrobial resistance testing is crucial. The occurrence of drug-resistant strains is such a great concern and the development of new antibiotic regimens for *M. genitalium* infections are urgently needed.

Abbreviations
AMR: antimicrobial resistance; DNA: Deoxyribonucleic acid; FSW: female sex worker; geq: genome equivalents; HIC: High income countries; HIV: Human immunodeficiency virus; LMIC: Low and middle income countries; MPC: Mucopurulent cervicitis; M. genitalium: Mycoplasma genitalium; M. hominis: Mycoplasma hominis; MRMM: Macrolide resistance mediating mutations; M. pneumonia: Mycoplasma pneumonia; MSM: Men who have sex with men; NAAT: Nucleic acid amplification testing; NGU: Non-gonococcal urethritis; TaqMan MGB: TaqMan minor groove binder; NGU: Non-gonococcal urethritis; PCR: Polymerase Chain Reaction; PID: pelvic inflammatory disease; QRDRs: quinolone resistance-determining regions; rRNA: ribosomal ribonucleic acid; SNPs: single nucleotide polymorphisms; STI: Sexually Transmitted Infection

Declarations
Ethics approval and consent to participate

The study was approved by the Ethics Committee of Dermatology Hospital of Southern Medical University (approval no. GDDHLS - 20171203, 13/12/2017). Informed consent was waived as for the committee believe that the research presents no potential risk to identify the harm resulting from a breach of confidentiality

Consent to publish

Not applicable.

Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interest.

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Authors' Contributions

Authors are cited in the same order that they are cited in the title page. WJK, DLL, RW analyzed the data and drafted the manuscript. ZYC, XHZ, LYW curated the data reviewed and edited the manuscript. YYL, HRC, YHL performed the laboratory testing, collected and validated the data. CML, YYL collected and curated the data, drafted the manuscript, secured funding. LST, HPZ, LGY provided overall leadership to the study, participated in the analysis and secured funding. All authors have read and approved the final manuscript.

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Authors' Information

Authors are cited in the same order that they are cited in the title page.

References

1. Golden MR, Workowski KA, Bolan G. Developing a Public Health Response to Mycoplasma genitalium. J Infect Dis. 2017;216(suppl_2):S420–6.

2. Unemo M, Jensen JS. Antimicrobial-resistant sexually transmitted infections: gonorrhoea and Mycoplasma genitalium. Nat Rev Urol. 2017;14(3):139–52.

3. Guschin A, Ryzhikh P, Rumyantseva T, et al. Treatment efficacy, treatment failures and selection of macrolide resistance in patients with high load of Mycoplasma genitalium during treatment of male urethritis with josamycin. BMC Infect Dis. 2015;15:40.

4. Mondeja BA, Couri J, Rodríguez NM, et al. Macrolide-resistant Mycoplasma genitalium infections in Cuban patients: an underestimated health problem. BMC Infect Dis. 2018;18(1):601.

5. Campos GB, Lobão TN, Selis NN, et al. Prevalence of Mycoplasma genitalium and Mycoplasma hominis in urogenital tract of Brazilian women. BMC Infect Dis. 2015;15:60.

6. Muller EE, Mahlangu MP, Lewis DA, et al. Macrolide and fluoroquinolone resistance-associated mutations in Mycoplasma genitalium in Johannesburg, South Africa, 2007–2014. BMC Infect Dis. 2019;19(1):148.

7. Jensen JS, Bradshaw C. Management of Mycoplasma genitalium infections - can we hit a moving target?. BMC Infect Dis. 2015;15:343.

8. Nakashima K, Shigehara K, Kawaguchi S, et al. Prevalence of human papillomavirus
infection in the oropharynx and urine among sexually active men: a comparative study of infection by papillomavirus and other organisms, including Neisseria gonorrhoeae, Chlamydia trachomatis, Mycoplasma spp., and Ureaplasma spp. BMC Infect Dis. 2014;14:43.

9. Gdoura R, Kchaou W, Chaari C, et al. Ureaplasma urealyticum, Ureaplasma parvum, Mycoplasma hominis and Mycoplasma genitalium infections and semen quality of infertile men. BMC Infect Dis. 2007;7:129.

10. Moi H, Blee K, Horner PJ. Management of non-gonococcal urethritis. BMC Infect Dis. 2015;15:294.

11. Tully JG, Taylor-Robinson D, Cole RM, et al. A newly discovered mycoplasma in the human urogenital tract. Lancet. 1981;1(8233):1288-91.

12. Dorman CJ. Regulation of transcription by DNA supercoiling in Mycoplasma genitalium: global control in the smallest known self-replicating genome. Mol Microbiol. 2011;81(2):302-4.

13. Jensen JS. Mycoplasma genitalium: the aetiological agent of urethritis and other sexually transmitted diseases. J Eur Acad Dermatol Venereol. 2004;18(1):1-11.

14. Manhart LE, Critchlow CW, Holmes KK, et al. Mucopurulent cervicitis and Mycoplasma genitalium. J Infect Dis. 2003;187(4):650-7.

15. Cohen CR, Manhart LE, Bukusi EA, et al. Association between Mycoplasma genitalium and acute endometritis. Lancet. 2002;359(9308):765-6.

16. Daley GM, Russell DB, Tabrizi SN, et al. Mycoplasma genitalium: a review. Int J STD AIDS. 2014;25(7):475-87.

17. Lis R, Rowhani-Rahbar A, Manhart LE. Mycoplasma genitalium infection and female reproductive tract disease: a meta-analysis. Clin Infect Dis. 2015;61(3):418-26.

18. Taylor-Robinson D, Jensen JS. Mycoplasma genitalium: from Chrysalis to multicolored
butterfly. Clin Microbiol Rev. 2011;24(3):498–514.

19. Shahmanesh M, Moi H, Lassau F, et al. 2009 European guideline on the management of male non-gonococcal urethritis. Int J STD AIDS. 2009;20(7):458–64.

20. Horner PJ, Martin DH. Mycoplasma genitalium Infection in Men. J Infect Dis. 2017;216(suppl_2):S396-405.

21. Bradshaw CS, Tabrizi SN, Read TR, et al. Etiologies of nongonococcal urethritis: bacteria, viruses, and the association with orogenital exposure. J Infect Dis. 2006;193(3):336-45.

22. Gaydos C, Maldeis NE, Hardick A, et al. Mycoplasma genitalium compared to chlamydia, gonorrhea and trichomonas as an aetiological agent of urethritis in men attending STD clinics. Sex Transm Infect. 2009;85(6):438–40.

23. Baumann L, Cina M, Egli-Gany D, et al. Prevalence of Mycoplasma genitalium in different population groups: systematic review and meta-analysis. Sex Transm Infect. 2018;94(4):255–62.

24. Andersen B, Sokolowski I, Østergaard L, et al. Mycoplasma genitalium: prevalence and behavioural risk factors in the general population. Sex Transm Infect. 2007;83(3):237–41.

25. Oakeshott P, Aghaizu A, Hay P, et al. Is Mycoplasma genitalium in women the "New Chlamydia?" A community-based prospective cohort study. Clin Infect Dis. 2010;51(10):1160–6.

26. Manhart LE, Holmes KK, Hughes JP, et al. Mycoplasma genitalium among young adults in the United States: an emerging sexually transmitted infection. Am J Public Health. 2007;97(6):1118–25.

27. Oakeshott P, Hay P, Taylor-Robinson D, et al. Prevalence of Mycoplasma genitalium in early pregnancy and relationship between its presence and pregnancy outcome.
28. Wang BW, Li S, Su XH, et al. Mycoplasma genitalium infection in patients attending the STD clinic in Nanjing. Zhonghua Nan Ke Xue. 2018;24(12):1073-7. (Chinese)

29. Qin XL, Zheng HP, Huang JM, et al. Study on the infection and pathogenicity of Mycoplasma genitalium in sexually transmitted diseases clinic patients of Guangzhou. Int J Epidemiol Infect Dis. 2015;42(2):99-102. (Chinese)

30. Al-Sweih NA, Al-Fadli AH, Omu AE, et al. Prevalence of Chlamydia trachomatis, Mycoplasma hominis, Mycoplasma genitalium, and Ureaplasma urealyticum infections and seminal quality in infertile and fertile men in Kuwait. J Androl. 2012;33(6):1323-9.

31. Clausen HF, Fedder J, Drasbek M, et al. Serological investigation of Mycoplasma genitalium in infertile women. Hum Reprod. 2001;16(9):1866-74.

32. Esteghamati A, Badamchi A, Naghdalipoor M, et al. Prevalence of Mycoplasma genitalium and Ureaplasma urealyticum in pregnant women. Tehran Univ Med J. 2018;76(8):568-74.

33. Zhao N, Li KT, Gao YY, et al. Mycoplasma Genitalium and Mycoplasma Hominis are prevalent and correlated with HIV risk in MSM: a cross-sectional study in Shenyang, China. BMC Infect Dis. 2019;19(1):494.

34. Coorevits L, Traen A, Bingé L, et al. Macrolide resistance in Mycoplasma genitalium from female sex workers in Belgium. J Glob Antimicrob Resist. 2018;12:149-52.

35. Dionne-Odom J, Geisler WM, Aaron KJ, et al. High Prevalence of Multidrug-Resistant Mycoplasma genitalium in Human Immunodeficiency Virus-Infected Men Who Have Sex With Men in Alabama. Clin Infect Dis. 2018;66(5):796-8.

36. McGowin CL, Totten PA. The Unique Microbiology and Molecular Pathogenesis of Mycoplasma genitalium. J Infect Dis. 2017;216(suppl_2):S382-8.
37. Björnelius E, Anagrius C, Bojs G, et al. Antibiotic treatment of symptomatic Mycoplasma genitalium infection in Scandinavia: a controlled clinical trial. Sex Transm Infect. 2008;84(1):72-6.

38. Horner PJ, Blee K, Falk L, et al. 2016 European guideline on the management of non-gonococcal urethritis. Int J STD AIDS. 2016;27(11):928-37.

39. Horner P, Blee K, O'Mahony C, et al. 2015 UK National Guideline on the management of non-gonococcal urethritis. Int J STD AIDS. 2016;27(2):85-96.

40. Jensen JS, Cusini M, Gomberg M, et al. 2016 European guideline on Mycoplasma genitalium infections. J Eur Acad Dermatol Venereol. 2016;30(10):1650-6.

41. Murray GL, Bradshaw CS, Bissessor M, et al. Increasing Macrolide and Fluoroquinolone Resistance in Mycoplasma genitalium. Emerg Infect Dis. 2017;23(5):809-12.

42. Wold C, Sorthe J, Hartgill U, et al. Identification of macrolide-resistant Mycoplasma genitalium using real-time PCR. J Eur Acad Dermatol Venereol. 2015;29(8):1616-20.

43. Twin J, Jensen JS, Bradshaw CS, et al. Transmission and selection of macrolide resistant Mycoplasma genitalium infections detected by rapid high resolution melt analysis. PLoS One. 2012;7(4):e35593.

44. Lau A, Bradshaw CS, Lewis D, et al. The Efficacy of Azithromycin for the Treatment of Genital Mycoplasma genitalium: A Systematic Review and Meta-analysis. Clin Infect Dis. 2015;61(9):1389-99.

45. Anagrius C, Loré B, Jensen JS. Treatment of Mycoplasma genitalium. Observations from a Swedish STD clinic. PLoS One. 2013;8(4):e61481.

46. Jernberg E, Moghaddam A, Moi H. Azithromycin and moxifloxacin for microbiological cure of Mycoplasma genitalium infection: an open study. Int J STD AIDS. 2008;19(10):676-9.
47. Li Y, Le WJ, Li S, et al. Meta-analysis of the efficacy of moxifloxacin in treating Mycoplasma genitalium infection. Int J STD AIDS. 2017;28(11):1106–14.

48. Couldwell DL, Tagg KA, Jeffreys NJ, et al. Failure of moxifloxacin treatment in Mycoplasma genitalium infections due to macrolide and fluoroquinolone resistance. Int J STD AIDS. 2013;24(10):822–8.

49. Sonnenberg P, Ison CA, Clifton S, et al. Epidemiology of Mycoplasma genitalium in British men and women aged 16–44 years: evidence from the third National Survey of Sexual Attitudes and Lifestyles (Natsal-3). Int J Epidemiol. 2015;44(6):1982–94.

50. 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(12):1546–53.

51. Bebear CM, Renaudin J, Charron A, et al. Mutations in the gyrA, parC, and parE genes associated with fluoroquinolone resistance in clinical isolates of Mycoplasma hominis. Antimicrob Agents Chemother. 1999;43(4):954–6.

52. Kikuchi M, Ito S, Yasuda M, et al. Remarkable increase in fluoroquinolone-resistant Mycoplasma genitalium in Japan. J Antimicrob Chemother. 2014;69(9):2376–82.

53. Deguchi T, Ito S, Yasuda M, et al. Emergence of Mycoplasma genitalium with clinically significant fluoroquinolone resistance conferred by amino acid changes both in GyrA and ParC in Japan. J Infect Chemother. 2017;23(9):648–50.

54. Shimada Y, Deguchi T, Nakane K, et al. Emergence of clinical strains of Mycoplasma genitalium harbouring alterations in ParC associated with fluoroquinolone resistance. Int J Antimicrob Agents. 2010;36(3):255–8.

55. Yoshida H, Bogaki M, Nakamura M, et al. Quinolone resistance-determining region in the DNA gyrase gyrA gene of Escherichia coli. Antimicrob Agents Chemother. 1990;34(6):1271–2.
56. Anderson T, Coughlan E, Werno A. Mycoplasma genitalium Macrolide and Fluoroquinolone Resistance Detection and Clinical Implications in a Selected Cohort in New Zealand. J Clin Microbiol. 2017;55(11):3242–8.

57. Tagg KA, Jeoffreys NJ, Couldwell DL, et al. Fluoroquinolone and macrolide resistance-associated mutations in Mycoplasma genitalium. J Clin Microbiol. 2013;51(7):2245–9.

58. Liu P, Jiang J, Zhang JP, et al. Detection of point mutations associated with macrolide resistance in Mycoplasma genitalium. Chinese Journal of Dermatology. 2014,47(8):551-4. (Chinese)

59. Li Y, Su X, Le W, et al. Mycoplasma genitalium in symptomatic male urethritis: macrolide use is associated with increased resistance. Clin Infect Dis. 2019;ci294.

60. Li WN, Shi L, Long XY, et al. Mycoplasma genitalium incidence, treatment failure, and resistance: a retrospective survey of men of infertile couples from a hospital in China. Andrology. 2019;10.1111/andr.12646.

61. Jensen JS, Björnelius E, Dohn B, et al. Use of TaqMan 5' nuclease real-time PCR for quantitative detection of Mycoplasma genitalium DNA in males with and without urethritis who were attendees at a sexually transmitted disease clinic. J Clin Microbiol. 2004;42(2):683–92.

62. Deguchi T, Maeda S, Tamaki M, et al. Analysis of the gyrA and parC genes of Mycoplasma genitalium detected in first-pass urine of men with non-gonococcal urethritis before and after fluoroquinolone treatment. J Antimicrob Chemother. 2001;48(5):742–4.

63. Pond MJ, Nori AV, Witney AA, et al. High prevalence of antibiotic-resistant Mycoplasma genitalium in nongonococcal urethritis: the need for routine testing and the inadequacy of current treatment options. Clin Infect Dis. 2014;58(5):631–7.

64. Francis SC, Kent CK, Klausner JD, et al. Prevalence of rectal Trichomonas vaginalis
and Mycoplasma genitalium in male patients at the San Francisco STD clinic, 2005-2006. Sex Transm Dis. 2008;35(9):797-800.

65. Deguchi T, Yasuda M, Horie K, et al. Drug resistance-associated mutations in Mycoplasma genitalium in female sex workers, Japan. Emerg Infect Dis. 2015;21(6):1062-4.

66. Bissessor M, Tabrizi SN, Twin J, et al. Macrolide resistance and azithromycin failure in a Mycoplasma genitalium-infected cohort and response of azithromycin failures to alternative antibiotic regimens. Clin Infect Dis. 2015;60(8):1228-36.

67. Tabrizi SN, Su J, Bradshaw CS, et al. Prospective Evaluation of ResistancePlus MG, a New Multiplex Quantitative PCR Assay for Detection of Mycoplasma genitalium and Macrolide Resistance. J Clin Microbiol. 2017;55(6):1915-9.

68. 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(6):e0198355.

69. 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(11):861-7.

70. Gesink DC, Mulvad G, Montgomery-Andersen R, et al. Mycoplasma genitalium presence, resistance and epidemiology in Greenland. Int J Circumpolar Health. 2012;71:1-8.

71. Shipitsyna E, Rumyantseva T, Golparian D, et al. Prevalence of macrolide and fluoroquinolone resistance-mediating mutations in Mycoplasma genitalium in five cities in Russia and Estonia. PLoS One. 2017;12(4):e0175763.

72. Hay B, Dubbink JH, Ouburg S, et al. Prevalence and macrolide resistance of Mycoplasma genitalium in South African women. Sex Transm Dis. 2015;42(3):140-2.
73. Forslund O, Hjelm M, El-Ali R, et al. Mycoplasma genitalium and Macrolide Resistance-associated Mutations in the Skåne Region of Southern Sweden 2015. Acta Derm Venereol. 2017;97(10):1235-8.

74. Le Roy C, Hénin N, Pereyre S, et al. Fluoroquinolone-Resistant Mycoplasma genitalium, Southwestern France. Emerg Infect Dis. 2016;22(9):1677-9.

75. Getman D, Jiang A, O'Donnell M, et al. Mycoplasma genitalium Prevalence, Coinfection, and Macrolide Antibiotic Resistance Frequency in a Multicenter Clinical Study Cohort in the United States. J Clin Microbiol. 2016;54(9):2278-83.

76. Unemo M, Salado-Rasmussen K, Hansen M, et al. Clinical and analytical evaluation of the new Aptima Mycoplasma genitalium assay, with data on M. genitalium prevalence and antimicrobial resistance in M. genitalium in Denmark, Norway and Sweden in 2016. Clin Microbiol Infect. 2018;24:533-9.

77. Pitt R, Fifer H, Woodford N, et al. Detection of markers predictive of macrolide and fluoroquinolone resistance in Mycoplasma genitalium from patients attending sexual health services in England. Sex Transm Infect. 2018;94(1):9-13.

78. 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(6):406-10.

79. Read TRH, Fairley CK, Tabrizi SN, et al. Azithromycin 1.5g over 5 days compared to 1g single dose in urethral Mycoplasma genitalium: impact on treatment outcome and resistance. Clin Infect Dis. 2017;64:250-6.

80. Salado-Rasmussen K, Jensen JS. Mycoplasma genitalium testing pattern and macrolide resistance: a Danish nationwide retrospective survey. Clin Infect Dis. 2014;59(1):24-30.

81. Couldwell DL, Lewis DA. Mycoplasma genitalium infection: current treatment options,
therapeutic failure, and resistance-associated mutations. Infect Drug Resist. 2015;8:147-61.

82. Wang L, Zhang X, Liang X, et al. Addressing antimicrobial resistance in China: policy implementation in a complex context. Global Health. 2016;12(1):30.

83. Tagg KA, Jeoffreys NJ, Couldwell DL, et al. Fluoroquinolone and macrolide resistance-associated mutations in Mycoplasma genitalium. J Clin Microbiol. 2013;51:2245-9.

84. Dumke R, Thürmer A, Jacobs E. Emergence of Mycoplasma genitalium strains showing mutations associated with macrolide and fluoroquinolone resistance in the region Dresden, Germany. Diagn Microbiol Infect Dis. 2016;86(2):221-3.

85. Gruson D, Pereyre S, Renaudin H, et al. In vitro development of resistance to six and four fluoroquinolones in Mycoplasma pneumoniae and Mycoplasma hominis, respectively. Antimicrob Agents Chemother. 2005;49(3):1190–3.

86. Bébéar CM, Renaudin H, Charron A, et al. DNA gyrase and topoisomerase IV mutations in clinical isolates of Ureaplasma spp. and Mycoplasma hominis resistant to fluoroquinolones. Antimicrob Agents Chemother. 2003;47(10):3323–5.

87. Shimada Y, Deguchi T, Nakane K, et al. Emergence of clinical strains of mycoplasma genitalium harbouring alterations in ParC associated with fluoroquinolone resistance. Int J Antimicrob Agents. 2010;36(3):255-8.

88. Kikuchi M, Ito S, Yasuda M, et al. Remarkable increase in fluoroquinolone-resistant Mycoplasma genitalium in Japan. J Antimicrob Chemother. 2014;69:2376-82.

89. Bradshaw CS, Jensen JS, Waites KB. New horizons in Mycoplasma genitalium treatment. J Infect Dis. 2017;216(Suppl 2):S412-9.