

Antimicrobial susceptibility of *Mycoplasma genitalium* isolates from Pretoria, South Africa in 2012 and 2016

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Background: *Mycoplasma genitalium* is a sexually transmitted pathogen associated with non-gonococcal urethritis and cervicitis. Azithromycin regimens have been considered first-line treatment for *M. genitalium* infections, with fluoroquinolone regimens effective as second-line treatments. However, the proportion of *M. genitalium* harbouring macrolide or fluoroquinolone resistance-associated mutations has been increasing worldwide. This study was done to compare the genotypic macrolide and fluoroquinolone resistance of *M. genitalium* strains obtained from women attending a termination of pregnancy clinic five years apart.

Methods: *M. genitalium* was detected by PCR in vaginal swab samples from 100 and 104 termination of pregnancy attendees at a tertiary hospital in Pretoria, South Africa during 2012 and 2016 respectively. Genes associated with macrolide and fluoroquinolone resistance in the *M. genitalium* isolates were sequenced and analysed.

Results: The prevalence of *M. genitalium* was 6.0% (6/100) in 2012 and 7.7% (8/104) in 2016. No resistance-associated mutations were seen in the 2012 isolates. Among the 2016 *M. genitalium* isolates, two (25%) harboured a macrolide-associated resistance mutation and one (12.5%) a fluoroquinolone resistance-associated mutation in the *parC* gene.

Conclusions: There is an increase in macrolide and fluoroquinolone resistance among local *M. genitalium* strains. This highlights the need for improved surveillance.

Keywords: fluoroquinolone resistance, macrolide resistance, *Mycoplasma genitalium*, South Africa, termination of pregnancy clinic

Background

Mycoplasma genitalium is a sexually transmitted pathogen associated with non-gonococcal urethritis, cervicitis, and related upper genital tract conditions such as pelvic inflammatory disease and infertility.^{1,2} *M. genitalium* is the smallest self-replicating prokaryote, but despite its small size, it is capable of causing disease, evades host immune responses through antigenic variability, and develops resistance to antimicrobial agents.³

Routine screening for *M. genitalium* is performed in only a few countries. As the organism cannot readily be cultured on standard laboratory media, detection relies on nucleic acid amplification tests. Although there are a few commercial tests available, there are still no assays approved by the FDA for routine diagnostic testing.⁴ In most cases of sexually acquired urethritis and cervicitis, tests are only performed for *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.

The treatment of *M. genitalium* infection, either individually or as part of syndromic management, differs globally. Although no genetic determinant such as *tetM* has been found in this mycoplasma, tetracyclines are not useful for in vivo eradication of *M. genitalium*, with reported cure rates of around 30%.^{5,6} The efficacy of doxycycline, used extensively to treat non-gonococcal urethritis and cervicitis in the past, is relatively poor for *M. genitalium* infection. Azithromycin has been the preferred treatment for several years in many countries.

As with other bacterial sexually transmitted infections (STIs), such as gonorrhoea, antimicrobial resistance poses a threat to the effective treatment of *M. genitalium*. Treatment failure due to

the rapid emergence of macrolide resistance is now very common and widespread.⁷ Where studies have shown that older fluoroquinolones such as ciprofloxacin and levofloxacin are less active against *M. genitalium*, moxifloxacin has been demonstrated to be effective in patients with macrolide-resistant strains of *M. genitalium*.⁸ However, fluoroquinolone resistance, including resistance to moxifloxacin, has also been described.⁸

In South Africa, the recommended management of urogenital infections is syndromic. The recommended seven-day course of doxycycline for genital discharge syndromes (i.e. male urethritis and vaginal discharge) was changed in 2015 to include single-dose oral azithromycin only, with doxycycline no longer in the guidelines for use other than for penicillin allergy in syphilis and balanitis.

Mutations in specific genes may be associated with resistance to antimicrobial drugs. Resistance to macrolides was shown to be due to mutations occurring in region V of the 23S rRNA gene,⁹ and fluoroquinolone resistance due to mutations in the *gyrA* and *parC* genes¹⁰ of *M. genitalium*.

In our study, we compared the macrolide and fluoroquinolone resistance profiles of *M. genitalium* strains obtained from woman attending a termination of pregnancy clinic in 2012 with those attending in 2016.

Materials and methods

Patient samples and processing

Patients visiting the termination of pregnancy clinic (TOP) at the Dr George Mukhari Academic Hospital (DGMH) in Pretoria,

South Africa were recruited for the study. Consecutive consenting women were enrolled both in 2012 (August) and in 2016 (September). Both groups of patients had not received any antibiotic therapy in the preceding month and gave verbal consent for participation. After a general examination, vaginal swab specimens were collected by a clinician and delivered to the laboratory within 24 hours. DNA was extracted from the swabs using the ZR Genomic DNA™-Tissue MiniPrep kit (Zymo Research Corp, Irvine, CA, USA) according to the manufacturer's instructions for biological liquids and cell suspensions.

Molecular assays

DNA from the ATCC *M. genitalium* G37 strain (33530D) was included in all PCR assays as positive control, and PCR grade water (Bioline) was used as negative control. The presence of *M. genitalium* was detected using conventional PCR as described previously.¹¹ The primers targeted a 281 bp region of the 140 kDa adhesion gene (*MgPa*) of *M. genitalium*.

Antimicrobial resistance genes were analysed in all *M. genitalium* positive isolates. Potential macrolide resistance was detected by amplifying and sequencing a unique 147 bp region of the V region of the 23S rRNA of *M. genitalium*.⁹ BLAST technology was used to compare sequences with the *M. genitalium* G37 complete genome [L43967.2]. Sequences of strains with known mutations were also used in the analysis: LA141 [HF572938.1] (A2058G); LA088 [HF572933.1] (A2059G) and LA202 [HF572946.1] (A2059C). *GyrA* and *parC* genes were amplified and sequenced to detect fluoroquinolone-associated resistance mutations (M95I and D99 N in *gyrA*; S83I, S83R, and D87 N in *parC*). Primers targeting a 230 bp region of the *gyrA* gene and a 220 bp region of the *parC* gene were used.¹⁰ BLAST technology was used to compare sequences with DNA gyrase subunit A [*M. genitalium* G37; ID: L43967.2]; the whole genome [*M. genitalium* MG6320; ID: CP003772.1]; and topoisomerase IV, A subunit [*M. genitalium* strain LA107; ID: HF947096.1].

Descriptive statistical analysis was performed using SPSS® version 16 (SPSS Inc, Chicago, IL, USA) to compare results obtained in 2012 and in 2016. Sequences obtained were edited using Chromas Lite and BioEdit. Sequences were aligned with MAFFT.

Results

One hundred samples were collected in September 2012 and 104 in October 2016. The median age of the women was 23 years (range: 18–42 years); with the mean ages of the women 24 and 23 years in 2012 and 2016 respectively. *M. genitalium* was detected in six of the 100 specimens (6.0%) in 2012 and in eight of 104 (7.7%) specimens in 2016.

The V region of the 23S rRNA of *M. genitalium* was amplified and sequenced for 13 isolates (five in 2012 and eight in 2016). None of the 2012 isolates harboured macrolide resistance-associated mutations. Among the 2016 isolates, two isolates had the A2059G mutation. This is one of the three different mutations at positions 2058 and 2059 (*E. coli* numbering) in region V of the 23S rRNA gene that have been associated with the macrolide-resistance phenotype.

Twelve *gyrA* and *parC* positive amplicons were sequenced (five in 2012 and seven in 2016). Neither the 2012 nor 2016 strains harboured mutations in the *gyrA* gene associated with fluoroquinolone resistance as reported previously.¹²

Table 1. Genotypic resistance of *M. genitalium* isolates

Year	Patient ID	23S rRNA mutation	Mutant fluoroquinolone QRDR amino acid change	
			<i>GyrA</i>	<i>ParC</i>
2012	M13	WT	WT	WT
	M35	WT	ND	ND
	M63	WT	WT	C234T (no change)
	M72	WT	WT	WT
	M78	ND	WT	WT
	M98	WT	WT	WT
2016	T25	A2059G	WT	WT
	T30	A2059G	WT	C234T (no change)
				G248T (Ser→Ile [83])
	T37	WT	ND	ND
	T63	WT	WT	C234T (no change)
	T71	WT	WT	WT
	T75	WT	WT	WT
	T76	WT	WT	C234T (no change)
T101	WT	WT	WT	

Note: WT: wild type; ND: not done.

None of the 2012 isolates harboured the *parC* alterations associated with fluoroquinolone resistance, while one strain from 2016 had the G248T resistance-associated mutation (Ser→Ile 83) which has been previously described for *M. genitalium*.¹² One strain in 2012 and three strains in 2016 shared the same silent mutation (C234T) in the *parC* gene. Results are summarised in Table 1.

Discussion

The prevalence of *M. genitalium* among women attending for TOP at DGMHAH did not change much over the five years between the studies (6.0% vs. 7.7%). This was similar to the prevalence of vaginal *M. genitalium* (8.7% [52/601]) in women visiting primary health care clinics across the Mopani District of Limpopo province in 2015.¹⁴ The *M. genitalium* prevalence patterns tend to differ between studies, with rates ranging from 0% to as high as 47.5% in different population samples.^{15,16} Data collected from various studies concerning the prevalence of *M. genitalium* infections are somewhat inconsistent and contradictory and this is mainly due to the heterogeneity in populations studied, sampling methods used, underlying risk factors for infection and clinical presentation (symptomatic or asymptomatic).¹⁵ In a study at a TOP clinic in Denmark, *M. genitalium* was detected in less than 1% of the 102 participants¹⁷ while the organism was detected in 22.4% of women recruited from an urban medical centre in Cincinnati, USA.¹⁸ As our study was not done among patients with vaginal discharge syndrome but rather among women seeking termination of pregnancy, the prevalence and probably bacterial loads of *M. genitalium* were relatively low. A South African study conducted on women with vaginal discharge syndrome from Cape Town and Johannesburg reported a significantly higher *M. genitalium* prevalence among participants from Johannesburg than those from Cape Town (11.2% vs. 2.1%; $p = 0.0066$).¹⁹

Like all mycoplasmas, *M. genitalium* has natural resistance against all beta-lactam agents and other antibiotics targeting peptidoglycan assembly since they lack a cell wall. Treatment options are tetracyclines, macrolides, and fluoroquinolones. Although doxycycline has been used to treat nongonococcal urethritis and cervicitis for many years, it has been shown to have poor efficacy against *M. genitalium* and isolates with reduced susceptibility have been reported.⁵ The macrolide azithromycin is now preferred for the treatment of nongonococcal urethritis and related clinical syndromes. However, after the first report in 2006 of azithromycin resistance observed in *M. genitalium* isolates among Australian patients,²⁰ the efficacy of the drug in treating *M. genitalium* infections has decreased worldwide.²¹

Resistance to macrolides is believed to be associated with point mutations occurring in region 5 (referred to as 'V region') of the 23S rRNA. Three mutations at positions 2058 and 2059 (*E. coli* numbering) have been reported.⁹ In our study, none of the 2012 isolates harboured macrolide resistance-associated mutations, while two of the 2016 isolates (25.0%) had the A2059G mutation, which is associated with macrolide resistance. This was also the most commonly seen resistance-associated mutation reported from Australia.¹² Ours is the second report of macrolide resistance in South Africa. Macrolide resistance-associated mutations were previously reported in 4/41 (9.8%) in *M. genitalium*-positive specimens collected from women attending primary health care clinics in the Limpopo province of rural South Africa.¹⁴ Contrary to our study, all their mutated specimens harboured the A2058G mutation.

The presence of macrolide resistance is of concern, as the treatment guidelines issued by the South African Department of Health were changed in 2015, when doxycycline was replaced by a single dose of azithromycin for the treatment of male urethritis and vaginal discharge syndromes. Although the prevalence of *M. genitalium* among the patients in our study was similar over the five-year period, macrolide resistance increased. This trend is observed in many countries, with the proportion of cases of *M. genitalium* with these mutations increasing in recent years. Data from Sweden showed the proportion of infections with a mutation increasing from 0% in 2006 to 14% in 2010, and 21% in 2011.²² In Japan an increase from 0% in 2011 and 2012 to 29% in 2013 was reported²³ and in Australia an increase from about 20% between 2007 and 2009 to 36% between 2012 and 2013 was shown.²⁴ It is believed that the continued use of azithromycin 1 g therapy is driving the increase in macrolide antimicrobial resistance as a result of frequent selection of resistant genotypes.²¹ This has led some investigators to use an extended dose of azithromycin, in the belief that it may be less likely to induce resistance than a single 1 g dose.^{8,25}

Following treatment failure by macrolides, patients with macrolide-resistant strains of *M. genitalium* are usually treated with fluoroquinolones, particularly moxifloxacin.⁷ This fourth-generation quinolone drug is bactericidal and generally well tolerated. In our study, none of the strains harboured mutations in the *gyrA* gene. One *M. genitalium* isolate from 2016 harboured a fluoroquinolone resistance-associated mutation in the *parC* gene, which is the first reported in South Africa. This G248T mutation (Ser→Ile 80) is among those that are known to be associated with fluoroquinolone resistance in *M. genitalium* and other closely related organisms.^{13,26} This mutation was common in *M. genitalium* isolates from patients attending sexual health clinics in Sydney, Australia,¹² as well as among Japanese men with male urethritis syndrome²³ and patients from France.²⁷ Although we did not perform susceptibility testing to confirm

phenotypic resistance, based on previous studies it is likely that the mutation observed in our isolate will contribute to moxifloxacin resistance.¹³

One strain in 2012 and three strains in 2016 shared the same silent mutation (C234T) in the *parC* gene of *M. genitalium*. This mutation was also commonly found among isolates in Japan, but the significance of this mutation is unknown.^{13,26}

Resistance of *M. genitalium* to macrolides and fluoroquinolones in our study showed that in South Africa a similar trend is observed as reported for the Asia-Pacific region where escalating resistance to azithromycin and moxifloxacin for *M. genitalium* has been seen. Analysing 140 infections, combined macrolide/fluoroquinolone-resistant mutations were found in 8.6% of specimens, for which recommended therapies would be ineffective.²⁸

Limitations of this study include the small numbers of *M. genitalium* isolates obtained during this period that could be tested, and the absence of a clinical history from participants, especially on the treatment for genital discharge syndrome in the preceding period. More surveillance data over time are needed from both symptomatic and asymptomatic patients to describe trends in macrolide and fluoroquinolone resistance accurately.

In conclusion, macrolide-resistant *M. genitalium* was present in this population and fluoroquinolone resistance was seen for the first time. As the prevalence of *M. genitalium* and the associated number of antimicrobial resistance markers was low, this study still does not justify changing the South African treatment guidelines for symptomatic patients with *M. genitalium*. However, as data for *M. genitalium* are not widely available in South Africa this and future studies should pave the way towards improved antimicrobial surveillance in *M. genitalium*.

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