Molecular epidemiological analysis of Mycoplasma genitalium shows low prevalence of azithromycin resistance and a well-established epidemic in South Africa

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Abstract

Objectives: Macrolide resistance in *Mycoplasma genitalium* is emerging globally. There is paucity of data from sub-Saharan Africa where syndromic management is used to treat sexually transmitted infections (STIs). We conducted a molecular epidemiological study to determine the prevalence of azithromycin resistance and epidemic diversity of *M. genitalium* infections in South Africa.

Methods: We analysed 90 *M. genitalium*-positive specimens that had been collected consecutively from men and women (50% symptomatic) from geographically diverse communities across the northern part of South Africa between 2015 and 2019. Melting curve analysis followed by targeted sequencing of the 23S rRNA gene was performed to detect azithromycin resistance. Molecular typing was done through single nucleotide polymorphism (SNP) analysis of the MG191 gene and short tandem repeats (STR) assessment of the MG309 gene. An overview of all published *M. genitalium* sequence types was generated and novel sequence types identified in this study were allocated numbers accordingly.

Results: Azithromycin resistance was detected in 1/90 *M. genitalium*-positive specimens (1.1%; 95% CI 0% to 3.3%) as conferred by A2071G mutation; this strain also harboured a

C234T mutation in the *parC* gene with wild type *gyrA* gene. SNP typing and STR assessment was successful in 38/90 specimens (42%) and showed a genetically diverse epidemic, without geographic clustering, with eight novel sequence types identified.

Conclusion: This is the first study that determines resistance in *M. genitalium* infection since introduction of azithromycin in the syndromic management regimen for STIs in South Africa in 2015. Despite a well-established epidemic, azithromycin-resistant *M. genitalium* infection is still uncommon in the public healthcare sector. However, it has the potential to undermine the effectiveness of syndromic management. Introduction of molecular diagnostics and continuous surveillance are warranted for early detection emergence of resistance.

Keywords: Africa; azithromycin; molecular epidemiology; molecular typing; mycoplasma.

Introduction

Mycoplasma genitalium is a sexually transmitted, fastidious bacterium associated with genital tract infection in men and women.[1] Infection may present as discharge or dysuria but can also occur without symptoms. Untreated *M. genitalium* infection is associated with various reproductive tract complications. In South Africa, the prevalence of *M. genitalium* infection in the sentinel aetiological surveillance was reported at 8.6% among women with vaginal discharge and 8.1% in men with urethral discharge;[2, 3] research studies reported asymptomatic *M. genitalium* infection among 7.4% of women and 5.3% of men.[4, 5]

In South Africa, sexually transmitted infections (STIs) including *M. genitalium* are treated syndromically, that is, with an empirical combination of broad-spectrum antibiotics based on the presenting symptoms. Azithromycin and ceftriaxone, often combined with metronidazole, are the current syndromic regimen of choice for urethral and vaginal discharge.

Treatment of *M. genitalium* infection has become increasingly challenging worldwide due to emergence of resistance to azithromycin, the first-line therapeutic choice.[1] Resistance to azithromycin and other macrolides is mainly mediated by mutations in the V-domain of the 23S ribosomal RNA (rRNA) gene; these mutations can be detected reliably using various molecular methods. Two brief reports from South Africa describing a total of six asymptomatic women with azithromycin-resistant *M. genitalium* infection confirms that such infections might occur.[6, 7] However, in the absence of routine diagnostics, the prevalence of azithromycin resistance in *M. genitalium* infections in sub-Saharan Africa is unknown. In order to optimally support clinical management of patients, guide the design of syndromic management algorithms, and to inform public health programmes, measuring the population-level prevalence of azithromycin resistance is essential.

This study measured the prevalence of azithromycin resistance in *M. genitalium* among infected patients from a wide geographic area of northern South Africa. Molecular typing of *M. genitalium* was performed and a review of globally reported sequence types (STs) was

compiled with the aim of gaining insight into the *M. genitalium* epidemic and associated azithromycin resistance.

Methods

Description of study cohorts that provided specimens for this analysis

We analysed 90 stored specimens from individuals with *M. genitalium* infection recruited into four studies conducted in Tshwane and Johannesburg Health Districts, Gauteng Province, and in the Mopani District, Limpopo Province, South Africa; these studies are summarised in table 1.[8–12] These cohorts had different inclusion criteria. The frequency of *M. genitalium* detection ranged from 7.0% to 15% in the different studies; two studies only included symptomatic individuals presenting with vaginal discharge syndrome or male urethritis syndrome. The Gauteng Province facilities (Tshwane and Johannesburg Health Districts) were ~90–120 km apart. Mopani District is ~350–450 km north of Tshwane and Johannesburg, and facilities there were up to 100 km apart from each other. In all studies, *M. genitalium* detection was performed at the Department of Medical Microbiology, University of Pretoria, using a validated real-time PCR targeting the MgPa gene on the LightCycler assay (Roche Molecular Diagnostics, Germany) after DNA extraction using the High Pure PCR Template Preparation Kit (Roche Diagnostics, Switzerland).[13] Stored positive DNA specimens from these studies were used in this evaluation; only one specimen from each individual was included. In the few cases where multiple *M. genitalium*-positive specimens were available from one patient, we only used the one that was first obtained in time.

Table 1 Overview of study cohorts from which *Mycoplasma genitalium* specimens (n=90) are included in this analysis

Year	Geographic location	Setting	Sample size	Study population	M. genitalium prevalence	Symptomatic infection*	Ref
2015–2019	Johannesburg Health District	Male sexual health services at three PHC facilities	129	Men (≥18 years) with urethral or anal discharge	7.0% (n=9)	9/9 (100%)	9 10
2016	Mopani District	Mobile clinic service in deep-rural areas	251	Women (≥18 years) attending for any type of health services	8.4% (n=21)	11/21 (52%)	11
2016–2017	Tshwane Health District	Three PHC facilities in urban township	294	HIV-infected pregnant women (≥18 years) attending ANC	15% (n=44)	9/44 (20%)	12
2017–2018	Mopani District	Six PHC facilities in rural areas across the district	177	Men and women (≥18 years) with STI-associated symptoms* mobilised for care	9.0% (n=16)†	16/16 (100%)	13

*Individuals presenting with vaginal discharge syndrome or male urethritis syndrome were defined as symptomatic.
†Eleven women and five men.
ANC, antenatal care; PHC, primary healthcare; STI, sexually transmitted infection.

Detection of macrolide resistance-associated mutations

We used the modified real-time PCR assay as described by Xiao *et al* for detection of macrolide resistance-associated mutations.[14] This assay uses real-time PCR based on fluorescence resonance energy transfer technology targeting the relevant section of the 23S rRNA gene of *M. genitalium* coupled with melting curve analysis for detection of point mutations. Amplification curves were analysed using the Abs quant/2nd Derivative Max and Melting temperatures (T_m) values were determined by using the manual T_m function. Specimens with a temperature peak other than the wild-type were sent for sequencing of the 23S rRNA gene as well as the quinolone resistance-determining regions of the *parC* and *gyrA* genes.[15, 16]

To confirm reproducibility of the melting curve analysis results, we performed the multiplex ResistancePlus qPCR assay (SpeeDx, Australia) on a random subset of 30 specimens at the Department of Medical Microbiology at the Maastricht University Medical Centre.

Molecular epidemiology of *Mycoplasma genitalium*

We used single nucleotide polymorphism (SNP) analysis of the MG191 (*mgpB*) gene in the MgPa operon combined with short tandem repeat (STR) assessment of the putative lipoprotein MG309 gene for molecular typing of *M. genitalium* at the Department of Medical Microbiology at the Maastricht University Medical Centre. These typing methods have been used by various researchers to report STs.[17]

Following SNP analysis, we generated a dendrogram to visualise the relationship between *M. genitalium* specimens using the unweighted pair-group method with arithmetic mean. A ST was assigned to each *M. genitalium* strain using the numbering system developed by Cazanave *et al*.[17] This system includes ST numbers 1–56 from a study conducted by Hjorth *et al* and STs 57–60 by Ma *et al*.[18, 19] We allocated number 64–80 to STs described by Musatovova, 81–88 for STs published by Cazanave and 89–98 for those reported by Pond *et al*.[17, 20, 21] As such, the numbering of new STs identified in our study starts from 99 onwards. As for STR analysis, the number of tandem repeats in the MG309 gene was counted and reported.

M. genitalium strains with macrolide resistance-associated mutations were visualised in the dendrogram to identify potential clustering and assess genetic relatedness.

Statistical analysis

Descriptive statistics are provided as number with proportion, median with range and mean with SD. We did not perform any comparative statistics.

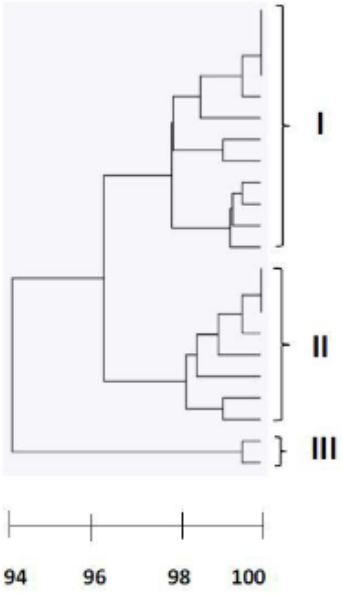
Results

Prevalence of macrolide resistance

We included 90 *M. genitalium*-positive specimens in our analysis. In melting curve analysis, 89/90 (99%) of *M. genitalium* specimens showed a peak melting temperature in similar range to the wild-type strain suggesting the absence of macrolide resistance-associated mutations. Only one specimen (1.1%; 95% CI 0% to 3.3%) showed a melting peak (63.7°C) different from that of the wild type strains (68.2°C±0.5). Sequence analysis of the 23S rRNA gene confirmed an A2071G mutation associated with macrolide resistance. Sequencing of the quinolone resistance-determining regions revealed a wild type *gyrA* gene and a C234T mutation in the *parC* gene resulting in a proline to serine substitution. Macrolide resistance results were concordant between melting cure analysis and the multiplex ResistancePlus qPCR assay.

Molecular epidemiology of *Mycoplasma genitalium*

Genotyping was successful in 38/90 (42%) specimens; successfully genotyped specimens had cycle threshold values<38 in the initial diagnostic *mgpA*-targeted real-time PCR. SNP analysis of MG191 was successful in 22/90 specimens (24%) and revealed 17 different STs comprising three clusters (figure 1). Strain clustering by geographic location was not observed; ST-2 and ST-7 were observed in specimens from all three geographic locations. We identified eight novel STs, numbered 99–106, and deposited sequences into GenBank under the accession numbers MN543061, MN543062, MN543063, MN543064, MN543065, MN543066, MN543067 and MN543068. All *M. genitalium* STs previously assigned in the literature, and newly identified in this study, can be found in online supplementary table S1 (online supplementary data A).



Study site	MG191 ST	MG309 STR	Gender	HIV status
Pretoria	7	11.1	Female	Positive
Mopani District PHC	7	11.1	Male	Positive
Johannesburg	7	15.1	Male*	Positive
Johannesburg	7	11.2	Male	Unknown
Johannesburg	89	12.1	Male	Negative
Mopani District PHC	6	17.1	Male	Positive
Mopani District MHC	3	14.1	Female	Negative
Johannesburg	99	8.1	Male*	Positive
Pretoria	65	19.1	Female	Positive
Johannesburg	4	14.2	Male*	Negative
Pretoria	100	9.1	Female	Positive
Johannesburg	101	11.2	Male	Negative
Pretoria	2	15.1	Female	Positive
Mopani District PHC	2	14.3	Male	Positive
Johannesburg	2	-	Male*	Negative
Pretoria	102	-	Female	Positive
Pretoria	64	13.1	Female	Positive
Pretoria	103	-	Female	Positive
Pretoria	104	8.1	Female	Positive
Mopani District MHC	105	10.1	Female	Negative
Pretoria	51	16	Female	Positive
Mopani District MHC	106	9.2	Female	Negative
Pretoria	-	8.1	Female	Positive
Pretoria	-	9.3	Female	Positive
Pretoria	-	9.3	Female	Positive
Pretoria	-	9.3	Female	Positive
Mopani District MHC	-	10.2	Female	Negative
Pretoria	-	11.2	Female	Positive
Pretoria	-	11.2	Female	Positive
Mopani District MHC	-	12.2	Female	Positive
Pretoria	-	13.2	Female	Positive
Mopani District PHC	-	14.2	Female	Positive
Mopani District PHC	-	14.4	Female	Negative
Mopani District PHC	-	15.2	Female	Positive
Pretoria	-	16.2	Female	Positive
Pretoria	-	16.3	Female	Positive
Mopani District MHC	-	16.4	Female	Negative
Mopani District MHC	-	16.5	Female	Negative

Figure 1. Dendrogram of *Mycoplasma genitalium* specimens obtained from different areas and participant populations in South Africa. Underlined ST numbers (99–106) indicate novel STs. *Men reporting having sex with other men. MHC, mobile healthcare clinic; PHC, primary healthcare clinic; ST, sequence type; STR, short tandem repeat

MG309STR analysis was successful in 35/90 (39%) specimens of which 19 (54%) had MG191 STs previously allocated. The number of STRs observed varied between 8 and 19. As MG309 contains two different repeat units, AGT and AAT, we examined the distribution patterns of these units between different specimens. The AGT/AAT distribution was identical in specimens with 8 STRs. Two different patterns were observed for those with 10, 11, 12, 13

and 15 STRs. Specimens with 9, 14 and 16 STRs showed three, four and five different AGT/AAT distribution patterns respectively. Combining the number of SNPs and STR resulted in identification of unique STs. The azithromycin-resistant *M. genitalium* strain was a novel ST (# 101) and had 11 STRs.

Discussion

This study is among the first to address the molecular epidemiology of *M. genitalium* infection in sub-Saharan Africa. We demonstrate that, although azithromycin resistance is still uncommon, there is a well-established epidemic of *M. genitalium* infections in the northern part of South Africa. This is based on the wide genetic diversity of *M. genitalium* isolates identified from different geographic areas and populations and the detection of eight novel strains.

Although we observed azithromycin resistance in only one *M. genitalium* infection, its presence among specimens from populations in the northern part of South Africa is concerning. This contrasts with two prior studies, including one from the national STI surveillance in Gauteng province that includes Johannesburg and Tswhane Health Districts where no azithromycin resistance was identified.[4, 22] However, unlike those two studies, the *M. genitalium* strains analysed in our study were collected after the introduction of azithromycin in the STI syndromic management regimen in South Africa in 2015; exposure to this drug is considered an important driver of *M. genitalium* azithromycin resistance emergence worldwide.[23] The low rate of azithromycin resistance among our specimens may be a result of the relatively short period between the introduction of azithromycin as part of syndromic management guidelines and when specimens were collected. Consequently, continuous surveillance is warranted to detect potential emergence of resistance at this early stage, as the emergence of resistance could undermine syndromic management for STIs in our region.[24] However, at this stage, azithromycin can still be considered an appropriate drug to cover *M. genitalium* infection in the syndromic regimen prescribed to individuals presenting with dysuria or discharge in South Africa.[9]

Our work is among the first molecular epidemiological studies of *M. genitalium* strains using an established dual method of SNP and STR analysis. To achieve this, we expanded the ST numbering database initiated by Cazanave and colleagues to create a comprehensive, global ST database.[17] A substantial proportion of the *M. genitalium* specimens tested in our study were allocated novel ST numbers and had different STR numbers than those reported from previous studies in Europe, the USA and Tunisia.[17–21] We identified three major clusters with strains from multiple geographic regions allocated to each cluster. This confirms the widespread nature of individual STs, and the presence of a diverse epidemic rather than clonal spread of specific strains. The azithromycin-resistant strain, although assigned a novel ST, belonged to a cluster that occurs throughout the region suggesting that resistance could emerge easily following prolonged exposure to azithromycin.

This study has several limitations. First, we used a convenience sample of specimens from several study cohorts for this analysis instead of performing well-designed surveillance in a cross-sectional or cohort study. This has resulted in a higher proportion of *M. genitalium*-positive specimens from patients with symptomatic infection. We do think, however, that

the diverse cohorts included in this study provide a good representation of the molecular epidemiological situation of *M. genitalium* infections in the northern part of South Africa. Second, we only managed to successfully perform genotyping for a proportion of the specimens due to low microbial load and degradation of DNA that had occurred in some specimens (especially the ones that had been stored for the longest period of time); insufficient remnant material did not play a role. We think that degradation was a random effect and did not introduce any bias other than a potential under-representation of low-load specimens in the dendrogram. As there no indication that low-load specimens are caused by a different genotype than high-load specimens, we do not think that our interpretation of typing results (ie, a well-established genetically diverse epidemic with multiple clusters) would have been any different should typing of all specimens have been successful.

In conclusion, this study confirms the presence of a well-established epidemic of *M. genitalium* infection in South Africa. Though azithromycin resistance is still uncommon, its detection after the introduction of azithromycin for syndromic management in 2015 may portend its increased emergence over time. Surveillance must be performed for the early detection of emergence of resistance following prolonged use of azithromycin as part of syndromic management of STIs.

Key messages

- Molecular typing of *Mycoplasma genitalium* strains demonstrates a genetically diverse epidemic without geographic clustering in South Africa.
- Resistance to azithromycin is still uncommon in *M. genitalium* infections in individuals attending the public healthcare sector.
- Azithromycin resistance, although uncommon, in *M. genitalium* can undermine the effectiveness of syndromic management in these settings.
- Introduction of diagnostics and enhanced surveillance is warranted for early detection of emerging drug resistance.

Contributors

RP conceptualised the study. JL, LvA, LM, CMH, MMK and RP were responsible for collection and microbiological analysis of the sample set. All authors contributed to the writing of the manuscript and approved the final version.

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Ethics approval

The collection of cohorts included in this study was approved through ethical approval by the Research Ethics Committee at the Faculty of Health Sciences of the University of Pretoria (Reference numbers 253/2017, 498/2016, 401/2015) and the Human Research Ethics Committee of the University of the Witwatersrand, Johannesburg, South Africa (Ref: M150352).

Data availability statement

All data relevant to the study are included in the article or uploaded as supplementary information. All data relevant to the study are included in the article; sequences of novel sequence types have been deposited into GenBank. Please contact the corresponding author for access to the raw data.

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