

# Antimicrobial resistance in *Neisseria gonorrhoeae* and *Mycoplasma genitalium* isolates from the private healthcare sector in South Africa: A pilot study

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**Background.** Reports have emerged globally of antimicrobial resistance (AMR) in *Neisseria gonorrhoeae* and *Mycoplasma genitalium* infections. In South Africa (SA), there are substantial differences between private and public healthcare with regard to antimicrobial drug prescribing practice, which could affect AMR patterns of private and public healthcare patients.

**Objectives.** To perform a pilot study to determine the frequency of AMR of *N. gonorrhoeae* and *M. genitalium* in patients accessing SA's private healthcare sector.

**Methods.** In this cross-sectional study, *N. gonorrhoeae*-positive cultures and *M. genitalium* DNA samples were collected from a private healthcare reference laboratory from August 2018 to August 2019. In *N. gonorrhoeae*-positive cultures, antimicrobial susceptibility testing was performed, followed by *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) to determine genetic relatedness of the isolates. To determine macrolide and fluoroquinolone resistance rates, *M. genitalium*-positive samples were analysed by sequencing the 23S rRNA, *gyrA* and *parC* genes.

**Results.** Twenty-one *N. gonorrhoeae*- and 27 *M. genitalium*-positive specimens were included in this analysis. High rates of resistance were detected among gonococcal isolates, with 90% resistance to tetracycline, 86% to penicillin and 62% to ciprofloxacin, but no resistance to azithromycin, cefixime and ceftriaxone. NG-MAST revealed genetically diverse isolates with 83% novel NG-MAST sequence types. Macrolide and fluoroquinolone resistance-associated mutations were detected in 18.5% ( $n=5/27$ ) and 7.4% ( $n=2/27$ ) of *M. genitalium* strains, respectively.

**Conclusions.** We observed high frequencies of ciprofloxacin, penicillin and tetracycline resistance in *N. gonorrhoeae* and macrolide resistance-associated mutations in *M. genitalium* in private healthcare sector patients in SA. This finding highlights the need to use diagnostics for sexually transmitted infections and to include the private healthcare sector in antimicrobial surveillance and stewardship programmes.

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The increasing prevalence of antimicrobial resistance (AMR) in the sexually transmitted infections (STIs) *Neisseria gonorrhoeae* and *Mycoplasma genitalium* is a major concern worldwide.<sup>[1]</sup> In South Africa (SA), STIs are treated syndromically based on the patient's presenting symptoms and using a combination of broad-spectrum empirical antibiotics. The standard regimen for urethral and vaginal discharge syndrome is ceftriaxone and azithromycin with or without metronidazole.<sup>[2]</sup> An important limitation of the syndromic management approach is that there are limited data on the occurrence of AMR in *N. gonorrhoeae* and *M. genitalium*, owing to the lack of routine laboratory diagnostics.

The healthcare system in SA is two-tiered: the public healthcare sector provides free healthcare and serves the majority of the population (>80%), while private healthcare is accessed by the minority (15 - 17%) and is largely paid for through private health insurance.<sup>[3]</sup> Both sectors use syndromic treatment for STI management.<sup>[2]</sup> However, most of the AMR data have been reported from the public healthcare sector. The national STI sentinel surveillance is conducted

only in public sector facilities, and research studies have focused on that sector as well.<sup>[4-7]</sup> Research on the occurrence of AMR in STIs in the private healthcare sector therefore remains limited.<sup>[8,9]</sup>

There are substantial differences between the private and public healthcare sectors with regard to patients' healthcare-seeking behaviour, antimicrobial drug prescribing practice, and socioeconomic status of the patient population.<sup>[10]</sup> These factors could all impact on the occurrence of antimicrobial-resistant STIs and result in different epidemiological profiles in the two sectors.

## Objectives

To conduct a pilot study to determine the frequency of AMR of *N. gonorrhoeae* and *M. genitalium* in patients accessing SA's private healthcare sector.

## Methods

In this cross-sectional study, *N. gonorrhoeae*-positive cultures and *M. genitalium* DNA samples with linked anonymous clinical

information were collected from a private healthcare sector diagnostic reference laboratory in Pretoria, SA, from August 2018 to August 2019. This reference laboratory receives specimens for pathology services from all provinces in SA. The laboratory uses New York City (NYC) agar to culture and matrix-assisted laser desorption/ionisation time-of-flight mass spectrometry (MALDI-TOF MS; Bruker, USA) to identify *N. gonorrhoeae*, and the Anyplex II STI-7 assay (Seegene, Korea) to detect *M. genitalium*. Cultured *N. gonorrhoeae* and *M. genitalium* DNA were transported to the Department of Medical Microbiology at the University of Pretoria for further resistance testing.

At the University of Pretoria, *N. gonorrhoeae* was confirmed by Gram stain, rapid oxidase test and API NH (bioMérieux, France). Minimum inhibitory concentration (MICs) were determined using E-tests (bioMérieux, France) on the GC-agar base (Oxoid, UK) for tetracycline, ciprofloxacin, penicillin G, azithromycin, ceftriaxone, cefixime and spectinomycin. MICs were interpreted according to the European Committee of Antimicrobial Susceptibility Testing breakpoints, except for azithromycin, where epidemiological cut-off (ECOFF) values were used because no resistance breakpoints exist (<http://www.eucast.org>). *N. gonorrhoeae* ATCC 49266 strain was used as quality control. Molecular typing of gonococcal isolates was done using the *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) as previously described.<sup>[11]</sup> Sequences were submitted to the NG-MAST database (<http://www.ng-mast.net>) for assignment of sequence types (STs).

The presence of *M. genitalium* DNA was confirmed using a validated real-time polymerase chain reaction (PCR) assay targeting the *MgPa* adhesin gene.<sup>[11]</sup> Macrolide drug resistance-associated mutations in the 23S rRNA gene were identified using real-time PCR assay coupled with melting curve analysis for detection of point mutations.<sup>[12]</sup> Samples with a temperature peak different from the wild-type strains were sequenced to confirm mutations in the 23S rRNA gene.<sup>[13]</sup> Quinolone resistance-associated mutations were detected by sequencing of quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes in macrolide-resistant strains, as previously described.<sup>[13]</sup> Ethical approval was obtained from the Research Ethics Committee at the Faculty of Health Sciences, University of Pretoria (ref. nos 253/2017, 207/2018 and 171/2019).

## Results

Twenty-one *N. gonorrhoeae* isolates were collected over the study period, 19 from urethral swabs and 2 from vaginal swabs. Gonococcal isolates showed high rates of resistance to tetracycline ( $n=19/21$ ; 90%), penicillin ( $n=18/21$ ; 86%) and ciprofloxacin ( $n=13/21$ ; 62%). All isolates were susceptible to the cephalosporins and spectinomycin; MICs for azithromycin were all below the ECOFF.

NG-MAST classified 21 isolates into 12 different STs; 10 ( $n=10/12$ ; 83%) of these STs have not been described previously. Seven ( $n=7/12$ )

STs were identified as unrelated singletons. Frequent STs were ST3609 ( $n=3$  isolates), ST6216 ( $n=2$ ), a novel ST containing *porB* allele 1320 and *tbpB* allele 623 ( $n=4$ ), and a novel ST containing *porB* allele 11132 and *tbpB* allele 136 ( $n=3$ ).

*M. genitalium* was confirmed in 27/27 (100%) of DNA samples received; 21 were from male urine samples, 4 from vaginal swabs and 2 from urethral swabs. Melting curve analysis of DNA identified five strains that had a different melting temperature (62 - 64.7°C) from the wild-type strain (69°C). Sequence analysis confirmed macrolide resistance-associated mutations in the 23S rRNA in 18.5% ( $n=5/27$ ) of *M. genitalium* strains; an A-to-G transition was detected at nucleotide position 2071 in 3 strains and at position 2072 in 2 (Table 1). Various amino acid alterations were observed in the ParC and GyrA of the QRDR; two strains had a Gln-132→Arg substitution in the GyrA and the other one had a silent mutation (A318G). In addition, one strain had a Ser83→Ile substitution in the ParC and a concurrent *gyrA* mutation (Gln-132→Arg) that has been associated with moxifloxacin and sitafloxacin treatment failures.<sup>[12]</sup> One isolate had a novel Met-55→Leu substitution in the ParC of unknown clinical significance. Coexistent macrolide and quinolone resistance-associated mutations were detected in 2 patients.

## Discussion

This pilot study reports on antimicrobial-resistant *N. gonorrhoeae* and *M. genitalium* infections in patients accessing the private healthcare sector in SA. We demonstrated high rates of AMR in *N. gonorrhoeae* isolates for some of the previously used antibiotics and concurrent macrolide and quinolone resistance-associated mutations in some of the *M. genitalium* strains. Although specimen collection was done over a 1-year period in a large reference laboratory, the number of strains obtained was limited, highlighting that diagnostic testing for STIs is performed only on a small scale in the private healthcare sector.

High frequencies of tetracycline and ciprofloxacin resistance were detected for gonococcal isolates; these drugs have been extensively used in the syndromic management of STIs in SA in the past two decades.<sup>[2]</sup> These findings are consistent with national surveillance data and a recent study in KwaZulu-Natal Province showing high rates of resistance to these antimicrobials in the public sector.<sup>[7,4]</sup> In addition, a higher frequency of resistance to penicillin (89%) was observed in gonococcal isolates in our study compared with the national sentinel surveillance, which reports a penicillin resistance prevalence of 51%.<sup>[4]</sup> This finding may be explained by a higher consumption of amoxicillin and amoxicillin/clavulanic acid in the private healthcare sector for other unrelated infections such as respiratory tract infections.<sup>[14]</sup> We did not observe azithromycin resistance in this selected group of gonococcal isolates from the

**Table 1. Macrolide resistance-associated mutations and fluoroquinolone resistance-associated amino acid changes in GyrA and ParC in *Mycoplasma genitalium* from private healthcare sector patients in South Africa**

Patient no.	Province	Gender	Specimen type	23SrRNA* mutation	Amino acid change*	
					GyrA	ParC
1	Western Cape	M	Urine	A2071G	Wild type	Wild type
2	Western Cape	M	Urine	A2071G	Gln-132→Arg	Met-55→Leu
3	Gauteng	M	Urine	A2072G	Wild type	Wild type
4	Gauteng	M	Urethral swab	A2072G	Silent mutation†	Wild type
5	Gauteng	M	Urethral swab	A2071G	Gln-132→Arg	Ser83→Ile

M = male.

\*Nucleotide positions or amino acid changes numbering according to *M. genitalium* G37 genome (NC\_000908.2).

†Silent mutation (A318G).

private healthcare sector, which is somewhat unexpected as there are concerns about emerging azithromycin resistance in the public healthcare sector.<sup>[7]</sup> Molecular epidemiological analysis of the gonococcal isolates showed genetic diversity, with a high number of novel NG-MAST STs that have not been described elsewhere.

A high frequency of macrolide resistance-associated mutations was detected in *M. genitalium* strains; this is in stark contrast to data from the public sector, where a low prevalence of macrolide resistance-associated mutations has been reported in several studies.<sup>[5,6,15]</sup> The observed high rate of macrolide resistance in the private healthcare sector could be due to selection bias, i.e. patients with urethritis not responding to empirical treatment may be more likely to have a diagnostic test. It could also result from different prescribing behaviour; azithromycin is commonly used for treatment of respiratory infections in the private healthcare sector, but not in the public sector.<sup>[14]</sup> Of concern is that we detected *M. genitalium* strains with coexistent macrolide and quinolone resistance-associated mutations in two patients.

### Study limitations

This pilot study has several limitations. First, the relatively small sample size and geographical spread may not be fully representative of the population in the private sector; however, it does give an important insight into the situation and highlights the need for further research. Second, there was probably selection bias of patients who had specimens sent for laboratory diagnostics, as these are expensive, patients can generally not afford such tests, and syndromic treatment remains the cornerstone of STI management. Lastly, the therapeutic outcomes of the patients with dual-class resistance in *M. genitalium* associated with treatment failures were not available owing to the anonymised study design.

### Conclusions

High frequencies of AMR in *N. gonorrhoeae* and macrolide resistance in *M. genitalium* were detected from patients in the private healthcare sector. This study highlights the importance of introducing routine STI diagnostics and inclusion of the private sector in AMR surveillance programmes.

**Declaration.** None.

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**Author contributions.** LDM, RPHP and MMK designed, initiated and co-ordinated the study. LDM, CK and K-AS performed all the laboratory analyses. CK and K-AS supplied patient data. LDM, RPHP and MMK analysed and interpreted all the data, and wrote a first draft of the manuscript. LDM, RPHP, CK, K-AS and MMK read, commented on and approved the final manuscript.

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**Conflicts of interest.** None.

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