Antibiotic susceptibilities and resistance genes of *Ureaplasma parvum* isolated in South Africa

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Objectives: There is only limited information on the antimicrobial susceptibilities and resistance genes of *Ureaplasma parvum* in South Africa. This study was designed to detect and characterize resistance genes in *U. parvum*.

Methods: Fifteen *U. parvum* isolates were investigated employing the broth microdilution method (tetracycline, doxycycline, ofloxacin, erythromycin, azithromycin and josamycin). Gene analyses were performed on target regions of: tet(M); gyrA, gyrB, parC and parE; erm(A), erm(B), erm(C) and erm(E); msr(A), msr(B), msr(C) and msr(D); 23S rRNA operons; and L4 and L22 ribosomal proteins.

Results: Seven of the *U. parvum* isolates were fully susceptible to the antibiotics tested. Five strains exhibited resistance to tetracycline (MICs 16–256 mg/L), one strain was resistant to ofloxacin (MIC 128 mg/L) and four strains were resistant to macrolides (MICs 128 mg/L); two strains showed dual resistance to tetracycline and erythromycin. The five tetracycline-resistant strains were found to have mosaic tet(M) genes, with one strain containing different specific regions to those previously described. Mutations in the L22 ribosomal protein were seen in three strains that were resistant to erythromycin (two strains) and erythromycin+azithromycin (one strain). For a further strain that was resistant to erythromycin and azithromycin, possible mechanisms of resistance remained elusive.

Conclusions: This is the first report of quinolone, erythromycin and azithromycin resistance development in *U. parvum* from South Africa. A point mutation in parC (Pro-57→Leu) and two novel mutations in parE (Ile-73→Thr and a methionine insertion at codon 86) were found in an ofloxacin-resistant strain. The study reinforces the adaptability of *U. parvum* to develop resistance and acquire, modify and maintain transposon-located resistance genes.

Keywords: ureaplasmases, erythromycin, azithromycin, ofloxacin, tetracycline

Introduction

There is only limited information on antimicrobial susceptibilities and resistance development in *Ureaplasma parvum*. Only nine *Ureaplasma urealyticum* and three *U. parvum* tetracycline-resistant strains that are concurrently resistant to doxycycline have been described.1–3 The characterization of tet(M) genes present in ureaplasmases from South Africa has revealed that they can be highly mosaic in structure.3 Fluoroquinolone resistance has been reported to be associated with gene mutations in DNA gyrase and topoisomerase IV in six strains.2,4,5 Mutations in 23S rRNA6 or in ribosomal protein L4 and L22 genes2,7 have been associated with macrolide resistance development in 19 strains. Lu et al.8 reported that 21/72 *U. urealyticum* strains harboured erythromycin ribosome methylase (erm) and 31/72 had macrolide/streptogramin resistance (msr) genes.

"This report describes antibiotic susceptibility profiles of *U. parvum* strains isolated in South Africa and antibiotic resistance genes associated with resistance development or gene acquisition.

Materials and methods

Clinical isolates

One hundred and fifty-seven vaginal swabs (Dacron) were collected from consenting women presenting for termination of pregnancy at the Dr George Mukhari Tertiary Hospital, Pretoria, during the period 2009–10. The ethics approval reference number for the study is MREC/P/60/2009:IR. DNA was extracted using the High Pure PCR Template Preparation Kit (Roche) as for the isolation of nucleic acids from bacteria and *U. parvum* PCR detection was performed.5 PCR detected *U. parvum* and
U. urealyticum in 31 and 5 specimens, respectively, which were then processed for isolation and preliminary identification in U9 broth (Bio-Rad) and ATCC specific broths, with preliminary screening using the SIR Mycoplasma Kit (Bio-Rad). Fifteen presumptive U. parvum isolates were obtained and resubjected to PCR detection/identification confirmatory testing. No Mycoplasma hominis contamination was detected.

**Determination of MICs**

MICs of tetracycline, doxycycline, ofloxacin, erythromycin, azithromycin and josamycin (Sigma) were determined by microbroth dilution. CLSI breakpoints as for U. urealyticum were used: quinolones, susceptible ≤2 mg/L and resistant ≥4 mg/L; tetracyclines, susceptible ≤1 mg/L and resistant ≥2 mg/L; and macrolides, susceptible ≤4 mg/L and resistant ≥16 mg/L.

**DNA extraction, resistance gene amplification and sequencing**

U. parvum DNA was extracted from culture medium using the High Pure PCR Template Preparation Kit (Roche), and antibiotic resistance gene regions were amplified employing the following primers and conditions. PCR Template Preparation Kit (Roche), and antibiotic resistance gene regions were amplified employing the following primers and conditions.

**Results**

Seven U. parvum strains were susceptible to all the antibiotics tested and all strains were susceptible to josamycin. Five strains were resistant to tetracycline (MICs 16–256 mg/L), with two strains exhibiting intermediate doxycycline resistance (MICs 4 mg/L). One strain was resistant to ofloxacin (MIC 128 mg/L). Four strains were resistant to erythromycin (MICs 128 mg/L), with two of the strains being resistant to azithromycin (MICs 128 mg/L). Dual resistance to different antibiotic groups was seen in two strains, with resistance to erythromycin (MICs 128 mg/L) and tetracycline (MICs 256 mg/L). They also exhibited intermediate resistance to doxycycline (MICs 4 mg/L).

On alignment and comparison of the tet(M) gene sequences of the five tetracycline-resistant U. parvum strains with GenBank strains N. gonorrhoeae 6418, S. pneumoniae and U. urealyticum SV9-Seattle, the leader 600 nucleotides including

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**Figure 1.** Schematic representation of aligned tet(M) gene sequences of the five tetracycline-resistant U. parvum strains. Shading format: white, regions identical to N. gonorrhoeae; dark grey, regions shared with S. pneumoniae; light grey, regions shared with U. urealyticum SV9; black, regions specific to U. parvum (Up-15). The genes aligned are: N. gonorrhoeae (plasmid pOZ100; base numbers correspond to nucleotides 674–2174), S. pneumoniae (transposon Tn5251; base numbers correspond to nucleotides 2553–4040) and U. urealyticum SV9-Seattle (transposon Tn916; base numbers correspond to nucleotides 2436–3877).
Table 1. Mutations in the L22 protein of three *U. parvum* strains exhibiting resistance to erythromycin and one strain co-resistant to azithromycin

<table>
<thead>
<tr>
<th>Strains</th>
<th>MIC (mg/L)</th>
<th>Nucleotide base changes (amino acid changes)</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>erythromycin</td>
<td>azithromycin</td>
</tr>
<tr>
<td>Up-8</td>
<td>128</td>
<td>≤0.125</td>
</tr>
<tr>
<td>Up-38</td>
<td>128</td>
<td>≤0.125</td>
</tr>
<tr>
<td>Up-71</td>
<td>128</td>
<td>128</td>
</tr>
</tbody>
</table>

the promoter region of the tet(M) gene exhibited diversity with truncated *S. pneumoniae* and *U. urealyticum* regions (Figure 1). The tet(M) sequences for Up-20, Up-38 and Up-56 were identical, with Up-8 showing 97% similarity. Three different regions were evident for strain Up-15, with only 82% similarity. On characterizing int-Tn genes, strains Up-15, Up-20, Up-38 and Up-56 had identical sequences, which differed from *S. pneumoniae* Tn1545 int-Tn by 58 nucleotides. The int-Tn of strain Up-8 had the same 58 nucleotide changes as those of the other four strains, but with an additional nucleotide alteration.

Sequence comparisons of the ofloxacin-resistant strain (MIC 128 mg/L) were conducted with four *U. parvum* serovars (serovar 1 (ATCC 27813), serovar 3 (ATCC 27815), serovar 6 (ATCC 27818) and serovar 14 (ATCC 33697)). There were no mutations in the *gyrA* and *gyrB* genes; however, mutations were observed in the *parC* and *parE* genes. A point mutation in *parC* (Pro-57 → Leu) and two novel mutations in *parE* (Ile-73 → Thr and a methionine insertion at codon 86) were found.

L22 protein alterations of two U. parvum strains exhibiting resistance to erythromycin and one strain additionally resistant to azithromycin are shown in Table 1. Strain Up-8 exhibited six amino acid alterations; strain Up-38, three alterations; and strain Up-71, six alterations. In a further erythromycin-azithromycin-resistant strain, no L22 protein changes were detected. No mutations were found (four strains) following sequence analyses of: (i) the two 235 rRNA operons; (ii) macrolide modification genes *erm(A), erm(B), erm(C)* and *erm(E);* (iii) efflux pump genes *msr(A), msr(B), msr(C)* and *msr(D).*

**Discussion**

The integrase gene regions investigated were identical or very similar for five tetracycline-resistant strains, as were the tet(M) sequences for four strains. One strain harboured a diverse recombinant tet(M) gene. However, *int-Tn* and tet(M) genes from seven tetracycline-resistant ureaplasmas isolated at the Tygerberg Hospital, South Africa, in 2006, were seen to be highly diverse and mosaic in structure. This may be attributed to the different geographical regions from which specimens were collected and according to prior tetracycline exposure. Pretoria is located in the highveld, a ‘closed community’ setting with little tourist activity. The predominance of a single *int-Tn/tet(M)* gene type would be expected, the findings being similar to those of a recent Tunisian study. In contrast, the earlier South African study was conducted in Tygerberg, which is in the vicinity of cosmopolitan Cape Town. Cape Town is a major city with extensive national and international business, trade and tourism, which would result in the presence of multiple and diverse transposon and resistance gene types. To comprehensively assess the prevalence of tetracycline resistance and fully characterize the transposon/tet(M) gene types of *U. parvum* in South Africa, more extensive studies are required from other provinces/regions.

The most commonly reported resistance-mediating mutation, which is Ser83Leu in *parC,* as well as the triple mutation which was recently found to be associated with species-specific polymorphism in ureaplasmas, were not identified in the ofloxacin-resistant isolate. Therefore, the point mutation in *parC* and the two novel mutations in *parE* may be linked to the elevated ofloxacin MIC of 128 mg/L.

Although requiring further studies with additional isolates, some of the mutations observed in L22 proteins may have contributed to macrolide resistance. Xiao et al. found three L22 protein point mutations that resulted in amino acid changes in three different *U. parvum* isolates, but these were stated to be atypical polymorphisms and not considered to contribute to macrolide resistance. Beeton et al. reported a 6 bp deletion in the L4 protein to be associated with macrolide resistance, while Xiao et al. described a five amino acid insertion in the extended loop of the L4 protein near the macrolide-binding site as contributing to macrolide resistance. No mutations in any of the gene targets employed for macrolide resistance assessment were detected in one strain that was co-resistant to erythromycin and azithromycin; the mechanisms of resistance remain unknown.

The findings of *U. parvum* resistance to major antibiotics employed in sexually transmitted infection syndromic management approaches in South Africa, lends to continued prevalence surveillance studies, with analyses of resistance development and resistance gene acquisitions.

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**Transparency declarations**

None to declare.
References


13 Bebear CM, Renaudin H, Charron A et al. In vitro activity of trovafloxacin compared to those of five antimicrobials against mycoplasmas including Mycoplasma hominis and Ureaplasma urealyticum fluoroquinolone resistant isolates that have been genetically characterised. Antimicrob Agents Chemother 2000; 44: 2557–60.


