ANALYSIS OF MYCOPLASMA GENITALIUM STRAINS ISOLATED FROM PREGNANT WOMEN AT AN ACADEMIC HOSPITAL IN PRETORIA, SOUTH AFRICA

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Background

- *M. genitalium* = smallest self-replicating prokaryote (580kb) [*E. coli* = 4600kb]
- 1980: first isolated from 2 men with NGU
- Sexually transmitted pathogen
- Causes cervicitis, abnormal vaginal discharge, urethritis, pelvic inflammatory disease (PID) and infertility in women.
  - Association with adverse pregnancy not confirmed
- In men: urethritis, proctitis, infertility
Background Cont.

- Lacks a cell wall, it cannot be cultured on standard laboratory medium
- Serology insensitive = cross reaction with *M. pneumoniae*
- Nucleic acid amplification tests (NAATs) have become the gold standard of detection [PCR]
Background Cont.

• Absence of the cell wall: resistant to penicillins, cephalosporins, vancomycin
• Cannot readily be cultured → difficult to test antimicrobial susceptibility
• CDC recommends tetracyclines, macrolides or fluoroquinolones for treatment
• Treatment mainly syndromic for genital discharge (MUS and VDS)
• In South Africa: Guidelines revised in 2015
  Doxycycline → Azythromycin
Background Cont.

- Antimicrobial resistance and typing of strains rely on DNA sequence data
- High levels of sequence variability between clinical isolates are seen which may be associated with antimicrobial resistance
- Data is scanty regarding prevalence, antimicrobial resistance and circulating types of *M. genitalium* in South Africa due to the syndromic treatment approach
Aim

• To determine the prevalence and the molecular characteristics of *M. genitalium* strains from pregnant women attending the termination of pregnancy and antenatal clinics at Dr George Mukhari Academic Hospital
Methods

• Protocol approved by the Sefako Makgatho Health Sciences University Research and Ethics Committee (SMUREC) (SMUREC/P/138/2015: PG)

• Vaginal swabs collected from women attending the termination of pregnancy (TOP) and antenatal (ANC) clinics at the DGMAH between June and December 2015

• Screened for *M. genitalium* using a commercial real time PCR assay (Sacace, Italy)
Methods Cont.

• Antimicrobial resistance analysis:
  • Genotypic resistance markers determined by sequence analysis:
    • Macrolides: V-region of the 23S rRNA (Jensen et al, 2008)
    • Fluoroquinolones: gyrA, and parC genes (Deguchi et al, 2002)
  • BLAST/BioEdit/MAFFT technology used to compare/align sequences with the *M. genitalium* G37 complete genome [L43967.2] and strains with macrolide and fluoroquinolone-associated mutations
• **Genotyping:**

  Strains were typed using:

  • *mgpB* single-nucleotide polymorphism typing (SNP)
    
    281-bp fragment of *M. genitalium* adhesion gene amplified and sequences aligned and compared with strain G37 [L43967.2], as well as sequence types 1 to 55 as described previously (Hjorth *et al*, 2006; Pond *et al*, 2014)

  • MG309 short tandem repeat (STR) analysis

    Sequences were aligned with *M. genitalium* strain G37 [KC445182.1] which contains 12 copies of the STR (AGT or AAT) (Cazanave *et al*, 2012).
Results

• Specimens were collected from 100 participating women (TOP:50; ANC:50) with a mean age of 23.0 years (TOP) and 28.5 years (ANC)

• *M. genitalium* detected in 7 (7.0%) of specimens of which one positive sample could not be detected with further methods
Results Cont.

• **Fluoroquinolone resistance:**
  
  • No resistance-associated mutations were seen in the \textit{gyrA} genes
  
  • A \textit{parC} fluoroquinolone resistance-associated mutation was seen in 1 isolate [G248T mutation (Ser→Ile 80)]
# Results Cont.

Amino acid sequence alignment of the *M. genitalium* parC gene of clinical isolates with reference strains

<table>
<thead>
<tr>
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<tr>
<td>CP003772.1 MG 6320</td>
<td>AVGEIMGYHPHCDSIYDARIIRMSQKNN*TTVSIX</td>
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<tr>
<td>HF947096.1 MG parC</td>
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<tr>
<td>M25 ParC Consensus</td>
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<td>*</td>
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<tr>
<td>T30 ParC Consensus</td>
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<tr>
<td>M84 ParC Consensus</td>
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☐ : Mutation (Ser→Ile 80) associated with resistance
Results Cont.

• Macrolide resistance:
  • Macrolide associated mutation A2059G was seen in 2 isolates [2/6: 33.3%]

Mutations associated with macrolide resistance: A2058G
Results Cont.

- **Genotyping:**
  - SNP typing revealed Sequence Types 1, 2, 4 and 39. Two strains were ST 1 (as wild-type), 2 were ST 4 and 1 each was ST 2 and ST 39.
  - Four different types were seen using MG 309 STR analysis; 3 strains had 10 repeats; 2 strains had 12 repeats (as wild-type) and 1 strain each had 11 and 14 repeats.
  - Typing assigned *M. genitalium* to 2 major clusters. Genotypic macrolide and fluoroquinolone resistance was found within cluster B.
Results Cont.
### Results Cont.

**Demographic and genotypic characteristics of patients with *Mycoplasma genitalium* infection**

<table>
<thead>
<tr>
<th>Spec</th>
<th>Clinic</th>
<th>Age</th>
<th>Mutant Fluoroquinolone QRDR: Amino Acid Change</th>
<th>23S rRNA Mutation</th>
<th>mgpB SNP Type</th>
<th>MG309 STR Copy Nr</th>
<th>Cluster</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>GyrA</td>
<td>ParC</td>
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<tr>
<td>T30</td>
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<td>28</td>
<td>WT</td>
<td>S80I WT*</td>
<td>A2059G</td>
<td>ST4</td>
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<td>WT*</td>
<td>WT</td>
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<td>WT</td>
<td>WT*</td>
<td>WT</td>
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<td>WT*</td>
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<td>A2059G</td>
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<tr>
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<td>WT</td>
<td>WT*</td>
<td>WT</td>
<td>ST1</td>
<td>12</td>
</tr>
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</table>

**Legend:**
- TOP: Termination of pregnancy clinic; ANC: Ante-natal clinic;
- QRDR: Quinolone resistant determinant region;
- WT: Wild type; WT*: Silent mutation
Discussion

- *M. genitalium* was present in 7.0% of participants
- Frequent undiagnosed STD in this population
- First report of fluoroquinolone resistance-associated mutation in *M. genitalium* in South Africa
- Also first report of MDR *M. genitalium*
- Macrolide resistance-associated mutations in 2 of the 6 isolates
  - Azithromycin only included in the national guidelines in 2015, alarming to already find resistance-associated genes
- *M. genitalium* strains grouped into two major clusters. The resistant isolates clustered together
Conclusion

• As the sample size was small, this study still does not justify changing the South African treatment guidelines for symptomatic patients

• However, the prevalence of macrolide resistance reported in this study, emphasises the importance of surveillance to adopt the optimal guidelines for syndromic management of MUS and VDS

• In addition, resistance against both macrolides and fluoroquinolones as found in this study and other studies all over the world continues to stress the need for alternative treatment regimes
Acknowledgements

- VLIR funding
- Dept of Obstetrics & Gynaecology
- Sister Makathini (TOP clinic)