# PHENOTYPIC ANTIBIOTIC RESISTANCE OF *MYCOPLASMA GENITALIUM* AND ITS ASSOCIATION TO MACROLIDE RESISTANCE-ASSOCIATED MUTATIONS

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**Background:**
*Mycoplasma genitalium* (MG) is a sexually transmitted bacterium that is associated with urethritis in men. In the Netherlands, over 60% of MG strains harbor different single nucleotide polymorphisms (SNPs) of macrolide resistance-associated mutations (MRAMs). We aimed to establish *in vitro* cultivation and phenotyping of genetically diverse MG-strains to better understand the association between macrolide resistance and genotype and to assess minimal inhibitory concentrations (MICs) for alternative antibiotics.

**Methods:**
Urine samples positive for MG by transcription mediated amplification and RT-PCR were cultured in the presence of Vero cells. RT-PCR on cultured samples was used to determine MICs for 8 different antibiotics. MICs were defined as the minimum concentration of antibiotic that inhibited growth by 99%. Laboratory-developed PCR was used to determine known MRAMs. Growth inhibition of MG by azithromycin was compared for the different MRAM SNPs.

**Results:**
MG strains were successfully grown from 18/41 (44%) MG-positive urine samples, with phenotypic resistance analysis performed in 15 of these strains. The MIC results ranged as follows: azithromycin (<0.016->16), levofloxacin (1-4), moxifloxacin (<0.25-1), sitafloxacin (<0.032-0.25), minocycline (<0.25-1), doxycycline (<0.125-2), spectinomycin (<2.5->25), lefamulin (<0.004-0.064). Growth of strains harboring the A2058T MRAM was significantly more inhibited at azithromycin concentrations ranging from 4-32 mg/L compared to strains harboring the A2058G MRAM ($p$<0.01).

**Conclusion:**
MG strains showed significant differences in inhibition of MG growth induced by azithromycin between MRAM SNPs, suggesting different levels of macrolide resistance aligned with specific mutations. This may support the notion that macrolide treatment in patients infected with MG harboring particular MRAM SNPs is associated with differences in treatment efficacy. Based on MICs only, fourth generation fluoroquinolones, tetracyclins and lefamulin may be considered as an effective treatment. With ongoing increase of antibiotic resistance in MG, this study emphasizes the need to culture genetically diverse MG strains to associate genotypic to phenotypic antibiotic resistance.

**Disclosure of Interest Statement:**