Detection of *Mycoplasma genitalium parC* gene mutations associated with quinolone resistance – evaluation of a multiplex real-time PCR assay

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Background:
Treatment for *Mycoplasma genitalium* infection is increasingly being complicated by rising levels of antibiotic resistance. Resistance to fluoroquinolones is associated with mutations in the *parC* gene. Although the precise mutations conferring resistance are not fully understood, the single nucleotide polymorphism (SNP) G248T/S83I is most implicated.

Methods:
This study evaluated the MG+*parC*(beta2) assay (SpeeDx, Australia), which detects single nucleotide polymorphisms (SNPs) in the *parC* gene at amino acid position S83 (A247C/S83R, G248T/S83I, G248A/S83N) and D87 (G259A/D87N, G259T/D87Y, G259C/D87H). Samples were collected from patients receiving sequential doxycycline-moxifloxacin, with known treatment outcomes. Assay performance was compared to Sanger sequencing. Sensitivity, specificity, and predictive value for treatment failure were calculated.

Results:
From analysis of 205 samples, the MG+*parC*(beta2) assay performed with a high sensitivity 98.2% (95%CI:90.3-100) and specificity 99.3% (95%CI:96.3-100) for *parC* SNP detection with a kappa of 0.97 (95%CI:0.94-1.00). The predictive value of G248T/S83I detection (the most common SNP, prevalence of 13% in the study population) was analysed with respect to treatment failure. The positive-predictive-value for moxifloxacin failure after detection of S83I was only 44% (95%CI:24.4-65.1), while negative-predictive-value was high at 96.9% (95%CI:92.7-99.0), suggesting that other SNPs are contributing to resistance.

Conclusion:
MG+*parC*(beta2) performed with high concordance compared to Sanger sequencing. Such qPCR assays can assist in understanding causes of treatment failure, inform the development of diagnostic assays, and can be applied to surveillance of mutations in populations. Due to an incomplete understanding of the basis for

fluoroquinolone resistance, such tests do not appear to be ready for clinical application.

Disclosure of Interest Statement: