



***Mycoplasma genitalium* Resistance assays**

Mycoplasma genitalium (commonly referred to as MGen and formally known as *Mycoplasma genitalium*) is a Gram-positive bacterium emerging as a significant causative agent of sexually transmitted infections in both males and females¹. *M. genitalium* was initially isolated from the urethral swabs of males with non-gonococcal urethritis in 1981 and was identified as a new species in 1983². Infection is closely associated with urethritis in men, and cervicitis in women, with links also been made to pelvic inflammatory disease. Through use of polymerase chain reaction (PCR), *M. genitalium* has been established as a key source of non-chlamydial, non-gonococcal urethritis in men and mucopurulent cervicitis in women³. The bacterium is one of the smallest known self-replicating organisms that proliferates on the skin cells of the human genital and urinary tracts. Bacterial infections such as those caused by *M. genitalium* are treated with antibiotics, however recent studies have found alarming rates of resistance to macrolide treatments. Recent changes to the British Association for Sexual Health and HIV (BASHH) guidelines have stated that all patient samples containing *M. genitalium* DNA should, where feasible, additionally be tested for macrolide resistance mediating mutations. Where macrolide resistance associated mutations exist treatment is typically followed up with a fluoroquinolone antimicrobial.

The recommended second line fluoroquinolone is moxifloxacin for 7-10 days; 14-days for complex infections⁴. Treatment failure of this second-line option has also been reported and is likely to increase⁵. Recent studies suggesting that moxifloxacin success rates range from 69 to 100%⁶. Therefore, detection of resistance mutations may become increasingly important in the treatment of these infections.

Signs and Symptoms

M. genitalium infection yields a vast range of clinical symptoms, though asymptomatic colonisation may occur. Infection can cause urethral inflammation, referred to as urethritis, in both men and women, which can result in pain when urinating (dysuria), the frequent need to urinate and pain during sex. Resistance should be suspected when the symptoms or the organism persists despite appropriate therapy.

Diagnosis and Treatment

Micropathology Ltd. accept both genital swabs and urine (males only) as validated sample types. Initial treatment against *M. genitalium* infection is typically a singular 1 g dose of azithromycin, a macrolide antibiotic which has been shown to be considerably more effective at treating infection than the standard doxycycline. However, individuals whose infections are not cleared following this primary stage of treatment are often found to host macrolide resistant strains of *M. genitalium*⁴. These infections can be followed up with additional, more intensive, azithromycin treatments or by an alternative group of antimicrobials the fluoroquinolones.

Where macrolide resistance testing is requested, all samples will be routinely tested for *M. genitalium* DNA in the first instance.

Macrolide resistance assay (Mg23)

Macrolides are bacteriostatic antibiotics that by binding to the 50S subunit of the ribosome, inhibit bacterial protein synthesis of a range of bacteria including Gram-positives. Mutations associated with macrolide resistance in *M. genitalium* have been identified at positions 2058 and 2059 (Escherichia coli numbering) on region V of the 23S rRNA gene. At Micropathology Ltd., our in-house single round assay amplifies a region of the 23S rRNA gene using sequencing to interrogate the bases found at 2058 and 2059. Where changes associated with macrolide resistance are observed, results are reported as “Resistance associated mutation(s) observed”, followed by the mutation.

Fluoroquinolone resistance assay (ParC)

Fluoroquinolones target topoisomerase enzymes responsible for regulation of DNA supercoiling. Mutations in the quinolone resistance determining region (QRDR) of the *parC* gene (encoding the ParC subunit A of topoisomerase IV) have been associated with resistance to fourth generation fluoroquinolones such as moxifloxacin. The best characterised resistance associated mutations cause changes in serine 83 (S83), and aspartic acid 87 (D87) (*M. genitalium* numbering) in the QRDR of the *parC* gene. Other single nucleotide polymorphisms (SNPs) in the *parC* and *gyrA* (encodes subunit A in DNA gyrase) may also contribute to fluoroquinolone treatment failure, however their role in mediating resistance is currently not well defined⁷.

At Micropathology Ltd. our single round ParC PCR assay amplifies a region of *parC* in *M. genitalium*. Analysis is based upon the analysis of sequence data generated to investigating positions 247, 248, 249 and 259 of the *M. genitalium parC* gene only, which confer amino acid changes in S83 and D87 strongly associated with resistance to fluoroquinolones. Not all mutations capable of causing fluoroquinolones resistance are known and/or are fully characterised. Consequently, inferred resistance based upon genotypic data may not correlate directly with empirically determined resistance based upon phenotypic analyses.

References

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