Simplifying molecular approaches to detect *Mycoplasma genitalium parC* resistance mutations, to facilitate individualized fluoroquinolone treatment.

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Background: *Mycoplasma genitalium* infections have rapidly become resistant to mainstay treatments. While individualised treatment approaches have improved treatment outcomes for macrolides, individualised therapy for fluoroquinolones has not yet been explored, due to scarcity of commercial assays and a lack of confidence in specific mutations associated with resistance. However, our recent work defines the clear role and diagnostic utility of specific markers to inform microbial cure with fluoroquinolone antimicrobials, specifically where the wildtype sequences inferring susceptibility are targeted (rather than resistance markers inferring resistance). Current suitable molecular methods are however lacking.

Methods: To facilitate individualized fluoroquinolone treatment, we developed proof-of-concept molecular tests. As per above, these assays were designed to focus on detection of *M. genitalium* and characterisation of wildtype *parC* sequences, that are strongly linked with fluoroquinolone susceptibility. Targeting ParC wildtype sequences are also simpler to design, such that samples containing sequences with a complete match to the wildtype probe will have identical melting peak temperatures, while any mismatches (including resistance mutations) result in significantly lower melting peak temperatures. These differences are easily differentiated for characterizing both ParC wildtype and mutants.

Results: The method was validated using 227 *M. genitalium*-positive samples, and the results compared to DNA sequencing. These assays were able to detect *M. genitalium* and characterise fluoroquinolone susceptibility in 143/227 (63%) of samples. We saw 100% agreement between “gold standard” DNA sequencing and the results of our novel probe-based assays and there was a clear shift (decrease) in melting peak temperatures for samples harbouring ParC mutations linked with fluoroquinolone resistance.

Conclusion: These proof-of-concept assays have considerable potential to improve individualised treatment approaches and rationalize tests of cure for *M. genitalium* infections. The ability to initiate individualised treatment in up to two-thirds of cases will enhance antimicrobial stewardship for this challenging pathogen.

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