A custom amplicon sequencing approach to detect resistance associated mutations and sequence types in *Mycoplasma genitalium*

Authors:
Plummer EL[1, 2], Murray GL[1, 2, 3], Bodiyabadu K[1, 2], Su J[1, 2], Garland SM[1, 2, 3], Bradshaw CS[4, 5], Read TRH[4], Tabrizi SN[1], Danielewski JA[1, 2]

[1] Women's Centre for Infectious Diseases, The Royal Women's Hospital, Parkville Victoria
[2] Murdoch Children's Research Institute, Melbourne, Victoria
[3] Department of Obstetrics and Gynaecology, The University of Melbourne, Parkville, Victoria
[4] Melbourne Sexual Health Centre, Alfred Hospital, Carlton, Victoria, Australia
[5] Central Clinical School, Monash University, Melbourne, Victoria, Australia

Background:
Mycoplasma genitalium resistance to antibiotics is increasing, for both macrolides (first line) and fluoroquinolones (second line), with very limited treatment alternatives on the horizon. Surveillance via sequencing of multiple *M. genitalium* loci would allow: monitoring of known antibiotic resistance mutations, associations between resistance/treatment failure and specific mutations, and strain typing for epidemiological purposes. In this study we assessed the performance of a custom amplicon sequencing approach, which negates the cost of library preparation for next generation sequencing (NGS).

Methods:
Fifty-two *M. genitalium* positive samples (cervical, vaginal, anal and rectal swabs, and urine) were used. Three regions associated with *M. genitalium* antibiotic resistance (23S rRNA, *parC* and *gyrA* genes) were targeted, in conjunction with a locus used for differentiation of sequence types in the *mgpB* gene, and findings compared to Sanger sequencing.

Results:
Amplicon sequencing provided adequate sequence read coverage (>30x) for the majority of samples for 23S rRNA gene (96%) and *mgpB* (97%), *parC* (78%) and *gyrA* (75%). Single nucleotide polymorphisms (SNPs) were characterised in samples for 23S rRNA gene (94%), parC (56%) and gyrA (4%). Unlike Sanger sequencing, mixed mutations could be identified by the amplicon sequencing method, and ratios of mutation types determined. All results, with one exception, were concordant to Sanger sequence results. Sequence diversity in the *mgpB* region was represented by 15 sequence types, 4 being observed in multiple samples. No clear association between antibiotic resistance SNPs and *mgpB* sequence types was determined.

Conclusion:
The utility of this custom amplicon sequencing approach for generating highly informative datasets with the capacity to identify and determine ratios of mixed sequences is demonstrated. The use of this customisable amplicon sequencing method enables cost effective, scalable amplicon sequencing of multiple target regions of interest in *M. genitalium*.

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