A multiplex PCR pipeline for antimicrobial resistance typing in *Neisseria gonorrhoeae* in Queensland

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Background: *Neisseria gonorrhoeae* has been identified by the Centre for Disease Control as an urgent antimicrobial threat. Similarly, the World Health Organisation has identified *N. gonorrhoeae* as a priority for enhanced, quality-assured antimicrobial resistance (AMR) surveillance. Current point-of-care diagnostics for Gonorrhea are not widely available and currently cannot inform antimicrobial selection. Therefore, detection of resistance and decreased susceptibility in circulating strains is critical to maintain effective antimicrobial stewardship. To fulfill this aim, a molecular AMR screen for *N. gonorrhoeae* was developed.

Methods: In collaboration with New Zealand and Queensland government health departments, primers targetting 15 genes from N. gonorrhoeae Multiantigen Sequence Typing (NG-MAST), Neisseria Multi-locus Sequence Typing (MLST), and Neisseria gonorrhoeae Sequence Typing for Antimicrobial Resistance (NG-STAR) typing schemes were utilised, alongside primers targetting plasmid-mediated penicillin resistance. Amplicon-based gene targets were selected to improve cost effectiveness compared to whole genome sequencing, and were amplified in a multiplexed manner for efficiency. Validation *in silico* and through wet lab verification against local and global *N. gonorrhoeae* sequences is ongoing.

Results: An automated sequencing pipeline was established to process NextSeq Illumina sequencing and implement downstream bioinformatic analysis, specifically looking for *N. gonorrhoeae* antimicrobial resistance markers. Submission into the globally maintained typing schemes allows prediction of resistance phenotype, and identification of novel resistance types.

Conclusion: This work demonstrates viability and utility of a broad but affordable sexually transmitted infection AMR surveillance pipeline. This will be implemented into routine use as part of Queensland Health's molecular surveillance of *N. gonorrhoeae* isolates. This pipeline will assist by informing on emerging *N. gonorrhoeae* resistance to major antibiotics in Queensland, as well as underpinning enhanced antimicrobial stewardship to preserve antibiotics. This workflow also paves the way for further metagenomic multiplex pipelines for use with the more prevalent clinical (uncultured) samples, expanding AMR surveillance in remote regions.

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