O05 - Detecting antimicrobial resistance and treatment failure

O05.1 REAL-TIME PCR AND MELT CURVE ANALYSIS TARGETING GYRA GENE FOR PREDICTION OF CIPROFLOXACIN RESISTANCE IN CLINICAL *NEISSERIA GONORRHOEAE* ISOLATES

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Introduction Increasing antimicrobial resistance in *Neisseria gonorrhoeae* has been a major problem worldwide, limiting effective empirical therapeutic options in patients infected with multidrug resistant strains. Guidelines from U.S. CDC no longer recommend treatment with fluoroquinolones (FQs) due to emergence of resistance nationally. However, current prevalence of resistance in the US is still low at 12% and treatment with FQs may be a viable option for susceptible isolates. A rapid molecular test predicting FQ susceptibility in *N. gonorrhoeae* isolates would help physicians in determining an effective treatment plan for each patient.
Methods Twenty-three ciprofloxacin (CIP)-susceptible and 77 CIP-resistant clinical *N. gonorrhoeae* isolates were obtained from Neisseria Reference Laboratory at University of Washington and grown on chocolate agar plates. To determine the association between mutations in gyrA gene and CIP resistance in those isolates, we extracted DNA from culture and performed real-time PCR on a Lightcycler 480 based on the HybProbe system targeting gyrA gene followed by melt curve genotyping.
Results Melt curve genotyping analysis demonstrated wild-type melt patterns in all (100%) 23 CIP-susceptible isolates, while all 77 (100%) CIP-resistant isolates demonstrated mutant melt patterns.
Conclusion There was a 100% concordance between PCR melt genotypes and CIP susceptibility among all clinical isolates tested. The assay is currently being validated for testing on DNA extracted directly from clinical specimens ultimately to be offered for use in clinical practice.
Disclosure of interest statement This study was funded in part by NIH R21AI109005.

O05.2 DIAGNOSTIC AND CLINICAL IMPLICATIONS OF GENOTYPIC FLUOROQUINOLONE SUSCEPTIBILITY DETECTION FOR *NEISSERIA GONORRHOEAE*

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Introduction Antibiotic resistance poses major challenges to empirical treatment of *Neisseria gonorrhoeae* (NG), potentially addressable if antimicrobial susceptibility point of care (POC) tests were available. The performance of a POC compatible real-time PCR assay (GCSNP), enabling detection of fluoroquinolone susceptible NG directly on clinical samples from multiple anatomical sites and on diverse circulating strains, was evaluated.
Methods Residual routine nucleic acid amplification test samples, derived from patients who were also culture positive for NG at the same clinical attendance were GCSNP tested. Assay performance was further verified using a phenotypically characterised fluoroquinolone resistant and susceptible strain panel which was sequence-typed using NG-MAST (NG Multi Antigen Sequence Typing).
Results 290 residual samples derived from 222 clinical episodes (56 female; 166 male) were tested by GCSNP, yielding result in 90% (n = 262/290), with assay failure more likely in non-genital compared to genital samples (16.4% vs. 5.2%, p = 0.002). 29.7% (n = 66/222) of NG cases were attributable to fluoroquinolone resistant strains in at least one anatomical site. GCSNP predictive values for fluoroquinolone susceptibility were 100% (95% CI: 95.9–100%) and 100% (82.8–100%), respectively for urogenital (n = 173) and rectal samples (n = 37). In four episodes of multi-anatomical-site infection (3 male, 1 female) different antimicrobial susceptibility profiles were observed across sample sites but all were correctly genotyped using GCSNP. GCSNP panel testing correctly identified all of 92 phenotypically susceptible (n = 52) and resistant (n = 40) strains. A total of 66 diverse NG-MAST sequence types were observed in the panel.
Conclusion GCSNP testing enables accurate genotypic detection of fluoroquinolone susceptible NG from clinical samples, from multiple anatomical sites and across diverse circulating gonococcal strains, enabling use of tailored anti-gonococcal therapy following NAAT positivity. If multi-site infection is suspected, genotypic testing on all anatomical sites is necessary in order to account for the presence of infections with mixed susceptibility.
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O05.3 MULTIPLEX REAL-TIME PCR WITH HIGH RESOLUTION MELTING ANALYSIS FOR DETECTING RESISTANCE MECHANISMS IN *NEISSERIA GONORRHOEAE*

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Background Molecular tests to detect antimicrobial resistance in *Neisseria gonorrhoeae* (NG) are urgently needed. Genetic methods are the mainstay of NG detection in many settings, but resistance testing still requires conventional phenotypic tests. The objective of this study was to develop an assay to detect the

most clinically important genetic markers conferring resistance to antibiotics used for the treatment of gonorrhoea.

Methods We designed a fluorescent dye real-time PCR assay combined with high resolution melting (HRM) analysis. Several triplex and duplex reactions included target sequences for: two NG-specific genes (*porA* and *opa*); targets specific for the *penA* mosaic XXXIV (A501, G545S), associated with reduced cephalosporin susceptibility; single nucleotide polymorphisms conferring resistance to ciprofloxacin (GyrA S91F), azithromycin (23S rRNA A2059G and C2611T) and spectinomycin (16S rRNA C1192T and 5S rRNA T24P). We tested >50 characterised NG isolates, including: clinical isolates resistant to ciprofloxacin, azithromycin, spectinomycin, ceftriaxone (strain F89), strains with reduced cephalosporin susceptibility, the wild-type reference strain ATCC49226, and commensal *Neisseria* spp.

Results HRM analysis correctly identified: all NG strains and all mutations, except for the A501P mutation in strain F89. However, cross-reactions with commensal *Neisseria* spp. occurred for: *penA* mosaic XXXIV (n = 3 species) and 23S rRNA (n = 3 species).

Conclusion Our multiplex PCR assay accurately identified NG and detected the most frequent mutations associated with antimicrobial resistance in cultured isolates. The assay provides results significantly quicker than current culture-based methods. The analytical sensitivity and specificity of the assay for use with urethral, rectal, pharyngeal and vaginal specimens should be evaluated in the near future.

O05.4 MULTIPLEX ASSAY FOR SIMULTANEOUS DETECTION OF *MYCOPLASMA GENITALIUM* AND MACROLIDE RESISTANCE USING PASS MNAZYME QPCR

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Introduction Treatment of *M. genitalium* (Mg) infection with single dose 1 g macrolide antibiotic, azithromycin, is routinely utilised in clinical practice however this has been associated with emergence of macrolide resistance and ineffective cure rates. Mutations at two positions, 2058 and 2059 (E. coli numbering) in the Mg 23S rRNA gene have been associated with macrolide resistance. Simultaneous detection of Mg and mutations associated with azithromycin failure could be used to offer rapid delivery of effective second line regimens. This study evaluates a combined diagnostic-resistance assay for potential use in clinical settings.

Methods Clinical samples diagnosed with Mg were evaluated with a combined-diagnostic resistance assay that employs novel "Primer Assisted Sequence Switching" (PASS) primers coupled with Multi-component Nucleic Acid enzyme" (MNAzyme) detection. PASS primers selectively amplify target sequences resulting in enhanced specificity for mutant over wild-type and MNAzymes allow for efficient detection and discrimination of multiple mutations simultaneously. Multiplexed PASS MNAzyme qPCR was evaluated by comparison to previously screened clinical samples for Mg (MgPa gene) and 23S mutations using Sanger sequencing and HRM analysis.

Results Using artificial templates, this assay was able to detect mutation templates ranging from 10–10,240 copies/reaction, with an associated Mg detection limit comparable to existing assays used in routine diagnostics. Preliminary screening of DNA from 24 clinical samples revealed Mg detection range of 3–300,000 copies/reaction. This assay was able to detect the correct mutation in 21/24 cases (87.5%), however was not readily able to assign a mutation at the lowest concentrations tested, an issue present in all rapid mutation screening tests for Mg.

Conclusion Multiplexed PASS MNAzyme qPCR offers the ability for simultaneous detection of Mg and macrolide resistance mutations. This assay offers considerable advantages in clinical settings with rapid identification of macrolide resistant strains and the ability to implement effective second line agents without delay.

Disclosure of interest statement SpeeDx is the developer and manufacturer of the assay evaluated in this study.

O05.5 *TREPONEMA PALLIDUM* STRAIN-TYPES IN AUSTRALIA AND ASSOCIATION WITH MACROLIDE RESISTANCE

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Introduction Greater understanding of the molecular epidemiology of *Trepomema pallidum* has the potential to enhance control measures. The aim of this study was to determine the strain-types of *T. pallidum* in Sydney, Australia, and investigate clinical and epidemiological associations.

Methods Stored *T. pallidum* DNA from samples between 2004–2011were catagorised into strain-types using analysis of the acidic repeat protein (arp), *T.pallidum* repeat sub- family (tpr) genes, and sequence analysis of the TP0548 gene as described by Marra (2010). Associations between strain-type, the macrolide resistance mutation A2058G, and clinical and demographic characteristics were further investigated.

Results 194 samples from 187 patients were successfully strain-typed into at least 19 separate strains. The predominant strains were 14d/g (91/194; 47%), and 14d/f (18/194; 9%).14d/g remained the commonest strain throughout the study period, and was associated with the A2058G mutation (90/91 vs 70/103 non-14d/g strains: OR 42.4; 95% CI 5.7–317.9 $p < 0.001$). Clinical information was available for 91 samples. 90 were male, of whom 89 reported sex with other men. 21/48 (44%) strains from HIV negative patients were strain 14d/g vs 20/43 (47%) from those HIV positive (OR 1.1; 95% CI 0.5–2.5 $p = 0.79$). When acquisition was reported as being outside Australia 2/13 (15%) cases were strain 14d/g vs 39/78 (50%) of those reporting sex only within Australia (OR 0.18; 95% CI 0.04–0.87 $p = 0.033$.) Both cases of neurosyphilis were attributable to TP0548 gene sequence "f".

Conclusion This is the first time that enhanced strain-typing has been used to define the epidemiology of *Treponema pallidum* infections in the Asia-Pacific region. The most common strain (14d/g) was associated with macrolide resistance and acquisition within Australia. Despite the diversity of strains, the lack of association with HIV status suggests sexual networks between HIV negative and HIV positive men overlap.

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