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

[1]P Hemarajata*, [1]S Yang, [2]OO Soge, [1]RM Humphries, [3]JD Klausner. [1]*Department of Pathology and Laboratory Medicine, David Geffen School of Medicine, UCLA, USA*; [2]*Neisseria Reference Laboratory, GISP Regional Laboratory, University of Washington Harborview Medical Center, USA*; [3]*Division of Infectious Diseases, Center for World Health and Department of Epidemiology, David Geffen School of Medicine and Fielding School of Public Health, UCLA, USA*

10.1136/sextrans-2015-052270.103

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*

[1]MJ Pond*, [1]C Hall, [2]M Cole, [1]KG Laing, [1]V Miari, [1]H Jagatia, [3]E Harding-Esch, [1,2]I Monahan, [1,4]T Planche, [1]J Hinds, [2]C Ison, [2]S Chisholm, [1]PD Butcher, [1,5]ST Sadiq. [1]*Institute for Infection and Immunity, St George's University of London, London, UK*; [2]*Sexually Transmitted Bacteria Reference Laboratory, Public Health England, London, UK*; [3]*HIV/STI Department, Public Health England, London, UK*; [4]*Department of Medical Microbiology, St George's University Hospitals NHS Foundation Trust, London, UK*; [5]*Department of Genitourinary & HIV Medicine, St George's University Hospitals NHS Foundation Trust, London, UK*

10.1136/sextrans-2015-052270.104

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.
Disclosure of interest statement This study was funded under the UKCRC Translational Infection Research (TIR) Initiative supported by the Medical Research Council (Grant Number G0901608) with contributions to the Grant from the Biotechnology and Biological Sciences Research Council, the National Institute for Health Research on behalf of the Department of Health, the Chief Scientist Office of the Scottish Government Health Directorates and the Wellcome Trust.

O05.3 MULTIPLEX REAL-TIME PCR WITH HIGH RESOLUTION MELTING ANALYSIS FOR DETECTING RESISTANCE MECHANISMS IN *NEISSERIA GONORRHOEAE*

[1]V Dona*, [2]N Low, [1]YN Guilarte, [1]A Lupo, [3]H Furrer, [4]M Unemo, [1]A Endimiani. [1]*Institute for Infectious Diseases, University of Bern, Bern, Switzerland*; [2]*Institute of Social and Preventive Medicine, University of Bern, Bern, Switzerland*; [4]*Örebro University Hospital, Örebro, Sweden*; [3]*Department of Infectious Diseases, Bern University Hospital and University of Bern, Bern, Switzerland*

10.1136/sextrans-2015-052270.105

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