

Town. One of the MSM reported a persistent urethral discharge which had failed to respond to previous therapy with oral cefixime. Agar dilution minimum inhibitory concentration assays were performed for eight antibiotics. The Johannesburg patients' isolates were further characterised by identification of key β -lactam-associated resistance mutations in *penA*, *mtrR* and its promoter, *porB1b*, *ponA*, and *pilQ* through PCR-based amplification and DNA sequencing. For molecular epidemiological characterisation, all three isolates were typed by *N. gonorrhoeae* multi-antigen sequencing typing (NG-MAST); additionally, full-length *porB* gene sequencing and multi-locus sequence typing (MLST) were performed for the Johannesburg isolates.

Results All three isolates were resistant to cefixime, ciprofloxacin, penicillin and tetracycline, intermediate/resistant to azithromycin but susceptible to ceftriaxone and gentamicin. The Johannesburg isolates had the type XXXIV *penA* mosaic allele in addition to previously described resistance mutations in the *mtrR* promoter (A deletion), *porB1b* (*penB*) (G101K, A102N) and *ponA1* (L421P). All three isolates had an identical *N. gonorrhoeae* multi-antigen sequence type (ST4822). The two Johannesburg isolates had an identical multi-locus sequence type (ST1901).

Conclusions All three strains were resistant to cefixime and were epidemiologically linked with identical NG-MAST sequence types. The Johannesburg isolates possessed a number of key β -lactam-associated resistance mutations and the type XXXIV *penA* mosaic allele. These two isolates belonged to a successful international MSM-linked multi-drug-resistant gonococcal clone (MLST ST1901), associated with several cefixime treatment failures in Europe and North America.

P1.021 A NATIONAL STUDY UTILISING THE SEQUENOM MASSARRAY IPLEX PLATFORM FOR HIGH THROUGHPUT MLST-BASED TYPING AND CHARACTERISATION OF RESISTANCE MECHANISMS IN NEISSERIA GONORRHOEA

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¹E Trembizki, ²M Chen, ³B Donovan, ^{2,4}C Fairley, ³R J Guy, ³J M Kaldor, ⁵M M Lahra, ³D G Regan, ^{1,6}J Ward, ¹D Whiley on behalf of the GRAND study investigators. ¹QPID Laboratory, QCMRI, the University of Queensland, Brisbane, Australia; ²Melbourne Sexual Health Centre, Carlton Victoria, Australia; ³The Kirby Institute, University of New South Wales, Sydney, Australia; ⁴Melbourne School of Population and Global Health, University of Melbourne, Melbourne, Australia; ⁵WHO Collaborating Centre for STD and HIV, Microbiology Department, SEALS, Prince of Wales Hospital, Sydney, New South Wales, Australia; ⁶Baker IDI, Central Australia, Australia

Introduction Strain-typing and characterisation of associated resistance mechanisms is pivotal to understanding the development and spread of *Neisseria gonorrhoeae* (NG) antimicrobial resistance (AMR). In Australia, we have embarked on a national study to determine the molecular basis of AMR in our local isolates with a view to implementing broad-based molecular surveillance for NG AMR.

Methods In this initial phase of the study, called GRAND (Gonorrhoea Resistance Assessment via Nucleic acid Detection), we are using the Sequenom MassARRAY iPLEX MALDI-TOF MS platform to characterise all available isolates (n = 2373) collected throughout Australia in the first half of 2012. To date, two iPLEX methods have been developed and validated: (1) a typing method targeting 14 informative SNPs previously shown to predict an MLST type; and (2) an AMR method targeting 11 common mutations associated with *N. gonorrhoeae* resistance to penicillin, ciprofloxacin, azithromycin and ceftriaxone, including important mutations on the penicillin binding protein (PBP2): A501 substitutions and the mosaic PBP2 sequence.

Results The results to date show that the technology is well suited for high-throughput typing of *N. gonorrhoeae* isolates. In particular, we found it can be used on heat-denatured isolates (removing the

need for a commercial DNA extraction kit) and can genotype (using both iPLEX reactions) up to 384 isolates within one working day for less than \$AUS20.00 (€15.00) per isolate.

Conclusions The data from this study will provide pivotal information to inform the implementation of molecular-based NG AMR surveillance. Validation and testing is ongoing.

P1.022 HUMAN PAPILLOMAVIRUS 16 VARIANTS ANALYSIS IN MULTIPLE INFECTIONS

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¹N Fontecha, ¹M Basaras, ¹E Arrese, ²S Hernandez, ²D Anda, ²V Esteban, ^{1,2}R Cisterna. ¹University of Basque Country, Leioa, Spain; ²Basurto Hospital, Bilbao, Spain

Background/Objectives Human papillomavirus type 16 (HPV 16) is the primary aetiology of cervical cancer.

Risk factors associated to develop of malignant lesions include: infection persistence, specific HPV 16 variants and multiple infections presence.

We had characterised the genomic variability of E6, E7 and L1 genes in HPV 16 multiple infection patients samples and analysed the relationship between intratypic variants and lesion grade.

Methods HPV 16 multiple infection samples were amplified with three region type-specific primers and amplicons were sequenced using the "Big Dye Terminator Cycle Sequencing kit".

Sequences were aligned using Edit Sequence Alignment Editor and ClustalW, and compared with Genbank reported reference sequences: European (E), African (AF1 and AF2) and Asian-American (AA).

Lesions were divided as negative, low-grade (L-SIL) or high-grade (H-SIL).

Results HPV 16 multiple infections were identified in 125 samples and 78 of them were analysed for intratypic variations: 72 E variants (92.3%), 4 AA variants (5.1%), one AF1 (1.3%) and one AF2 variant (1.3%).

In E6 region, missense mutations (A104del and T350G) were defined in 59% and 41% of samples. In E7 region, a mainly synonymous variation (G849A, 41.33%) was detected. In L1 region, non-synonymous replacements were only identified: 6901insCAT (30%), 6902 insATC (65.7%) and GAT6951del (97.1%).

European variants were mainly detected in samples with no lesion while non-european variants were only found in H-SIL or L-SIL.

Conclusions E6, E7 and L1 genes are useful to determinate among E, AA and AF1/AF2 variants. Non-european variants are also present in our population.

Nucleotide variations different to define variants must be studied owing to their potential impact on pathogenesis. T350G nucleotide substitution is associated with elevated risk of cervical carcinomas. These variations should be taken into consideration.

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P1.023 MOLECULAR TYPING OF TREPONEMA PALLIDUM FROM AN ONGOING SYPHILIS OUTBREAK IN DENMARK

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¹K Salgado-Rasmussen, ²T L Katzenstein, ²J Gerstoft, ¹S Cowan, ¹S Hoffmann, ¹J S Jensen. ¹Statens Serum Institut, Copenhagen, Denmark; ²Department of Infectious Diseases, Rigshospitalet, Copenhagen, Denmark

Background Since 1999, the number of persons diagnosed with syphilis has increased dramatically in Denmark. Molecular typing was used to investigate the epidemiology of *Treponema pallidum* aiming to understand the dynamics of the epidemic. In recent years the tp0548 gene sequence has been used to further differentiate the subtypes obtained using the CDC typing system (number of 60-base pair