fluoroquinolones, had a strong activity to ciprofloxacin-resistant N. gonorrhoeae strains. The MIC90 of ciprofloxacin or sitafloxacin were 16 μg/ml or 0.25 μg/ml, respectively.

Purpose In this study, the relationship between genetic mutations of QRDR and antimicrobial susceptibilities of sitafloxacin against ciprofloxacin-resistant N. gonorrhoeae strains was examined.

Methods The subjects were 12 N. gonorrhoeae strains which were gotten by the Japanese national surveillance by three Japanese societies including the Japanese Association of Infectious Diseases, the Japanese Society of Chemotherapy and the Japanese Society of Clinical Microbiology. MICs of sitafloxacin to these 12 strains were more than 2 μg/ml, but MICs of sitafloxacin to these strains were less than 0.125 μg/ml. The base sequence of QRDR on gyrA or parC genes of these strains were examined

Results On QRDRof gyrA of 12 strains, mutations of 2 amino-acids were found, such as Ser91to Phe, Asp95 to Ala or Asp95 to Gly. Regarding parC gene, mutations of 4 amino-acids were found, such as Asp86 to Asn in 1 strain, Ser87 to Asn in 6 strains, Ser87 to Arg in 5 strains, Glu91 to Lys, Gln or Gly in 3 strains and Ala123 to Ser in 3 strains.

Conclusion Sitafloxacin had a strong activity to ciprofloxacin-resistant N. gonorrhoeae which had at least more than 3 mutations of amino-acids on QRDR on gyrA and parC genes.

P1.018 ANALYSIS OF MOSAIC PENICILLIN-BINDING PROTEIN 2 VARIANTS WITH ALA501 MUTATIONS THAT CONFER HIGH-LEVEL RESISTANCE TO EXPANDED-SPECTRUM CEPHALOSPORINS IN *NEISSERIA GONORRHOEAE*

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Decreased susceptibility to the expanded-spectrum cephalosporins cefixime and ceftriaxone in *Neisseria gonorrhoeae* has increased dramatically over the past decade globally and recently resistance was reported to the last remaining recommended treatment option, ceftriaxone, raising fears that gonorrhoea may become untreatable. The major reason for decreased susceptibility has been mosaic *penA* alleles encoding penicillin-binding protein 2 (PBP2), the major target for these antibiotics, with up to 70 mutations relative to wild-type. Less prevalent in most settings has been non-mosaic *penA* alleles containing A501V or A501T mutations just downstream of the KTG active site motif that also confer decreased susceptibility. We have shown that Ala501 mutations, when introduced into mosaic *penA* alleles, confer resistance to expanded-spectrum cephalosporins, and recently, a novel mosaic *penA* allele containing an A501P mutation and resulting in ceftriaxone resistance was described. To understand the role of Ala501 mutations in mosaic *penA* alleles, we transformed FA19 with the mosaic *penA* allele from 35/02 harbouring a randomised codon at position 501 and selected for increased cefixime resistance. From this screen, we identified five Ala501 mutations (Val, Thr, Ser, Pro and Arg) that resulted in increased cefixime resistance, indicating that only a small subset of mutations are capable of conferring resistance. Surprisingly, only one clone with an A501P mutation was selected, perhaps suggesting a fitness defect with this mutation. MIC analyses showed that mutation of Ala501 to Val, Ser or Thr conferred ~2.5-fold increases in resistance, whereas mutation to Arg and Pro increased resistance nearly 5-fold. PBP2–6140CT (PBP2 containing four C-terminal mutations) harbouring A501V or A501T mutations was crystallised and revealed major ordering and some reorganisation of the β3-β4 hairpin that is immediately adjacent to the active site. Modeling of β-lactams into the crystal structure indicates that the mutations likely introduce a steric clash with the R1 substituent of expanded-spectrum cephalosporins.

P1.019 IDENTIFICATION OF THE AMINO ACIDS CONFERRING HIGH-LEVEL RESISTANCE TO EXPANDED-SPECTRUM CEPHALOSPORINS IN THE *PENA* GENE FROM THE *NEISSERIA GONORRHOEAE* STRAIN H041

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The recent identification of a high-level ceftriaxone-resistant (MIC = 2–4 μg/ml) isolate of *Neisseria gonorrhoeae* from Japan (H041) portends the loss of ceftriaxone as an effective treatment for gonococcal infections. This is of grave concern because ceftriaxone is the last remaining option for first-line empiric antimicrobial monotherapy. The *penA* gene from H041 (*penA41*) is a mosaic *penA* allele similar to mosaic *penA* alleles conferring intermediate-level cephalosporin resistance (Ceph[I]) worldwide, but has 13 additional mutations compared to the mosaic *penA* gene from the previously studied Ceph[I] strain, 35/02 (*penA35*). When transformed into the wild-type strain FA19, the *penA41* allele confers 300- and 570-fold increases in the MIC of ceftriaxone and cefixime, respectively. In order to understand the mechanisms involved in high-level ceftriaxone resistance and to improve the surveillance and epidemiology during the potential emergence of ceftriaxone resistance, we sought to identify the minimum number of amino acid alterations above those in *penA35* that confer high-level resistance to ceftriaxone. Using restriction-fragment exchange and site-directed mutagenesis, we identified three mutations - A311V, T316P, and T483S - that, when incorporated into the mosaic *penA35* allele, confer essentially all of the increased resistance of *penA41*. Mapping these onto the crystal structure of PBP 2 shows that A311V and T316P are close to the active-site nucleophile, Ser310, that forms the acyl-enzyme complex, while Thr483 lies on a loop close to the active site and is predicted to interact with the carboxylate of the beta-lactam antibiotic. These three mutations have thus far only been described in *penA41*, but dissemination of these in other mosaic alleles would spell the end of ceftriaxone as an effective treatment for gonococcal infections.

P1.020 PHENOTYPIC AND GENETIC CHARACTERIZATION OF THE FIRST THREE CASES OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANT *NEISSERIA GONORRHOEAE* INFECTION IN SOUTH AFRICA AND ASSOCIATION WITH CEFIXIME TREATMENT FAILURE

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Objectives To describe the phenotypic and genetic characteristics of the first three cases of extended-spectrum cephalosporin (ESC) resistant *Neisseria gonorrhoeae* in South Africa which were associated, in one case, with a verified cefixime treatment failure.

Methods Three ESC resistant *N. gonorrhoeae* isolates were cultured from the urethral discharge of three men-who-have-sex-with-men (MSM), two residing in Johannesburg and one in Cape

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 GONORRHOEAE

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Introduction Strain-typing and characterisation of associated resistance mechanisms is pivotal to understanding the development and spread of Neisseria gonorhoeae (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. gonorhoeae 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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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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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