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 QRDR of gyrA of 12 strains, mutations of 2 amino-acids were found, such as Ser91 to Phe, Asp95 to Ala or Asp95 to Gly. Regarding parC gene, mutations of 4 amino-acids were found, such as Asp26 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.

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

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 (Ceph1) worldwide, but has 13 additional mutations compared to the mosaic pena gene from the previously studied Ceph1 strain, 35/02 (penA35). When transformed into the wild-type strain FA19, the penaA41 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 penaA41. Mapping these onto the crystal structure of PBP2 shows that A311V and T316P are close to the active-site nucleophile, Ser510, 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 spoil the end of ceftriaxone as an effective treatment for gonococcal infections.

**PT.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**

The objective of this study was 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 failure due to cefixime treatment failure. In 2012, a resistant isolate of N. gonorrhoeae from a patient attending the Infectious Diseases Clinic at Tygerberg Hospital was identified, which was subsequently shown to be treatment failure.
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, penA, and pilQ1 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 (penB1b) (G101K, A102N) and penA1 (L421F). 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.

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**A NATIONAL STUDY UTILISING THE SEQUENOM MASSARRAY iPLEX PLATFORM FOR HIGH THROUGHPUT MLST-BASED TYPING AND CHARACTERISATION OF RESISTANCE MECHANISMS IN NEISSERIA GONORRHOEAE**


**P1.021**

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**Introduction** Strain-typing and characterisation of associated resistance mechanisms is pivotal to understanding the 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 (PB2P): AS01 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.