fluoroquinolones, had a strong activity to ciprofloxacin-resistant N. gonorrhoeae strains. The MIC90 of ciprofloxacin or sitafloxa
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 sitafloxa 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 sitafloxa to these 12 strains were more than 2 μg/ml, but MICs of sitafloxa 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 QDRDROf 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 Asp96 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** Sitafloxa has a strong activity to ciprofloxacin-resistant N. gonorrhoeae which had at least more than 3 mutations of amino-acids on QRDR on gyrA or parC genes.

**PT019** IDENTIFICATION OF THE AMINO ACIDS CONFERRING HIGH-LEVEL RESISTANCE TO EXPANDED-SPECTRUM CEPHALOSPORINS IN THE PENA GENE FROM THE NEISSERIA GONORRHOEA 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) worldwide, but has 13 additional mutations compared to the mosaic penA gene from the previously studied Ceph 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, T316F, 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 FBP 2 shows that A311V and T316F 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 spell the end of ceftriaxone as an effective treatment for gonococcal infections.

**PT020** PHENOTYPIC AND GENETIC CHARACTERIZATION OF THE FIRST THREE CASES OF EXTENDED-SPECTRUM CEPHALOSPORIN RESISTANT NEISSERIA GONORRHOEA 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