New Ceftriaxone- and Multidrug-Resistant *Neisseria gonorrhoeae* Strain with a Novel Mosaic penA Gene Isolated in Japan

Shu-ichi Nakayama, Ken Shimuta, Kei-ichi Furubayashi, Takuya Kawahata, Magnus Unemo, Makoto Ohnishi

Department of Bacteriology I, National Institute of Infectious Diseases, Tokyo, Japan; Sonezaki Furubayashi Clinic, Osaka, Japan; Osaka Prefectural Institute of Public Health, Osaka, Japan; WHO Collaborating Centre for Gonorrhoea and other STIs, Department of Laboratory Medicine, Microbiology, Örebro University Hospital, Örebro, Sweden

We have characterized in detail a new ceftriaxone- and multidrug-resistant *Neisseria gonorrhoeae* strain (FC428) isolated in Japan in 2015. FC428 differed from previous ceftriaxone-resistant strains and contained a novel mosaic penA allele encoding a new mosaic penicillin-binding protein 2 (PBP 2). However, the resistance-determining 3’-terminal region of penA was almost identical to the regions of two previously reported ceftriaxone-resistant strains from Australia and Japan, indicating that both ceftriaxone-resistant strains and conserved ceftriaxone resistance-determining PBP 2 regions might spread.

Ceftriaxone is the last remaining option for empirical first-line antimicrobial monotherapy of gonorrhoea, but the evolving resistance in *Neisseria gonorrhoeae* threatens its use, i.e., in monotherapy and in the dual-therapy regimens together with azithromycin (1–3). Four different ceftriaxone-resistant *N. gonorrhoeae* strains previously isolated in Japan (H041 [4] and GU140106 [5]), France (6) and Spain (F89 [7]), and Australia (A8806 [8]) have been characterized in detail. None of these strains have spread widely nationally or internationally. However, once a ceftriaxone-resistant gonococcal strain spreads, gonorrhea control will become exceedingly difficult. Consequently, dual antimicrobial therapy, mainly ceftriaxone and azithromycin, is now recommended in Europe, the United States, Canada, and Australia, which hopefully will mitigate the development of antimicrobial resistance (AMR) and, at a minimum, the spread of AMR strains (1). It is a great concern that frequently occurring intraspecies and interspecies DNA transfer among *Neisseria* spp. continues to develop new mosaic penA alleles encoding novel penicillin-binding protein 2 (PBP 2), which is the main lethal target for all β-lactam antimicrobials, resulting in ceftriaxone resistance in *N. gonorrhoeae* (1–3, 9, 10).

We report a new ceftriaxone- and multidrug-resistant *N. gonorrhoeae* strain (FC428) isolated in January 2015 in Osaka, Japan, from a male urethritis patient, who was successfully treated with 2 g of spectinomycin intramuscularly at his first attendance at a sexually transmitted disease (STD) clinic. The patient was in his twenties. No information regarding the sexual orientation of the patient or sexual contacts were available.

FC428 was cultured on modified Thayer-Martin medium and species verified using Gonocheck-II (TCS Biosciences Ltd., Buckingham, United Kingdom) and the HN-20 rapid system identification test (Nissui, Tokyo, Japan). A nitrocefin test (Thermo Ingam, United Kingdom) and the HN-20 rapid system identified the species verified using Gonochek-II (TCS Biosciences Ltd., Buckingham, United Kingdom). A nitrocefin test (Thermo Ingam, United Kingdom) and the HN-20 rapid system identified the species verified using Gonochek-II (TCS Biosciences Ltd., Buckingham, United Kingdom). A nitrocefin test (Thermo Ingam, United Kingdom) and the HN-20 rapid system identified the species verified using Gonochek-II (TCS Biosciences Ltd., Buckingham, United Kingdom). A nitrocefin test (Thermo Ingam, United Kingdom) and the HN-20 rapid system identified the species verified using Gonochek-II (TCS Biosciences Ltd., Buckingham, United Kingdom).

NG9807 (4, 13) verified that the penA<sub>FC428</sub> allele caused the ceftriaxone resistance, i.e., the ceftriaxone MIC of the recipient NG9807 increased 32-fold (from 0.016 μg/ml to 0.5 μg/ml). The mtrR and penB resistance determinants, which also increase the MICs of ceftriaxone, were determined in both of these strains, as previously described (4, 13). Both FC428 and the recipient NG9807 contained these resistance determinants, i.e., a single-nucleotide (A) deletion in the promoter region of mtrR and a G120K alteration in PorB1b. However, while FC428 possessed a wild-type sequence of MtrR and an A121D alteration in PorB1b, NG9807 had a G45D alteration in MtrR and an A121N alteration in PorB1b. The penA<sub>FC428</sub> was also further compared to penA of the GU140106 (penA<sub>GU140106</sub>) and A8806 (penA<sub>A8806</sub>) strains, which all harbor the mainly identical ceftriaxone resistance-determining penA<sub>FA1090</sub> allele caused the ceftri-axone resistance (15). On the contrary, the central region of penA<sub>FC428</sub> was identical to the corresponding region of penA of the porB (positions 919 to 1749) and tbpB (positions 919 to 1244) genes of the ceftriaxone-resistant Neisseria gonorrhoeae strain FA1090. In fact, the entire 5’-terminal half of penA<sub>FC428</sub> (nucleotide positions 1 to 904) was identical to the corresponding region of penA encoding PBP 2, which caused the ceftriaxone resistance in the recently reported ceftriaxone-resistant strains from Japan (5) and Australia (8). Accordingly, FC428, GU140106 (5), and A8806 (8) represent different N. gonorrhoeae strains (according to MLST, NG-MAST, and complete penA sequencing), but they all harbor the mainly identical ceftriaxone resistance-determining 3’-terminal region of penA. This indicates that both ceftri-axone-resistant strains and conserved ceftriaxone resistance-determining PBP 2 regions might spread. Taking advantage of the unique and characteristic construct of penA<sub>FC428</sub>, the develop-
ment of a PCR specific for penA<sub>FC428</sub> and utilization of this PCR for rapid molecular screening of ceftriaxone-resistant strains (among cultured strains and samples for nucleic acid amplification tests) might be valuable. A comprehensive investigation of the origin of this unique penA allele might also elucidate the existence of a genetic source or reservoir of these ceftriaxone resistance-determining PBP 2 regions, which repeatedly donate these and other PBP 2 regions to different lineages of <i>Neisseria gonorrhoeae</i>. The origin of the ceftriaxone resistance-determining PBP 2 region in FC428 has not been possible to identify; however, the genetic source is most likely some commensal <i>Neisseria</i> species.

In conclusion, a new ceftriaxone- and multidrug-resistant <i>Neisseria gonorrhoeae</i> strain (FC428) isolated in Japan in 2015 has now been characterized. Our results indicate that both ceftriaxone-resistant strains and conserved ceftriaxone resistance-determining PBP 2 regions might spread. According to pharmacodynamic analyses (16), using <i>&lt;1 g</i> of ceftriaxone for the treatment of gonorrhoea caused by strains, like FC428 (ceftriaxone MIC, 0.5 µg/ml), is unlikely to clear the infection. Accordingly, dual antimicrobial therapy (ceftriaxone plus azithromycin [1]) might be crucial to introduce in additional regions globally. Continuous strengthened AMR surveillance in the Osaka/Kyoto area of Japan and other regions worldwide is essential.

**Nucleotide sequence accession number.** The complete nucleotide sequence of the penA gene of FC428 has been deposited in DDBJ under accession no. LC113953.

**ACKNOWLEDGMENTS**

We thank Mitsufumi Fujiwara, Shuichi Hida, Hiroshi Kameoka, Mikio Itoh, and Ryouji Yasumoto for the gonorrhea and gonococcal surveillance in Kyoto and Osaka.

This work was partly supported by the Research Program on Emerging and Re-emerging Infectious Diseases, Japan Agency for Medical Research and Development.

**FUNDING INFORMATION**

This work, including the efforts of Makoto Ohnishi, was funded by Japan Agency for Medical Research and Development (15fk0108014h0001).

**REFERENCES**


