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

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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 gonorrhea, 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 Gonochek-II (TCS Biosciences Ltd., Buckingham, United Kingdom) and the HN-20 rapid system identification test (Nissui, Tokyo, Japan). A nitrocefin test (Thermo Scientific Yokohama, Japan) showed that FC428 was a penicillinase-producing *N. gonorrhoeae* (PPNG) strain. According to Etest (bioMérieux, Marcy l'Etoile, France), FC428 had a ceftriaxone MIC of 0.5 μg/ml, i.e., was resistant according to EUCAST breakpoints (www.eucast.org) and nonsusceptible according to CLSI breakpoints (www.clsi.org), which only state susceptible or nonsusceptible. FC428 was additionally resistant to cefixime (MIC, 1 μg/ml), benzylpenicillin (MIC, >32 μg/ml), and ciprofloxacin (MIC, >32 μg/ml) but susceptible to spectinomycin (MIC, 8 μg/ml) and azithromycin (MIC, 0.25 μg/ml) (www.clsi.org and www.eucast.org) (Table 1).

Multilocus sequence typing (MLST) and *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) were performed as previously described (11–13). FC428 belonged to MLST sequence type 1903 (ST1903) and NG-MAST ST3435, which differ from the previously described ceftriaxone-resistant gonococcal strains (4–8). For example, MLST ST1903 differs by two and three loci from ST7363 and ST1901, respectively (Table 1). In our laboratory, among all Japanese gonococcal isolates examined using MLST (n = 1,327) and NG-MAST (n = 1,476), no ST1903 or ST3435 (*porB1053* or *tbpB21*) strain, respectively, has been found. However, MLST ST1903 strains have been identified, for example, in 2005 to 2007 in Thailand among PPNG strains (14). Notably, FC428 is also a PPNG with $bla_{\text{TEM-135}}$. Regarding NG-MAST, *porB1053* has not been found in our collection of Japanese gonococcal isolates, although *tbpB21* is the fifth most prevalent allele (111 [7.5%] of 1,476 isolates).

The *penA* allele of FC428 ($penA_{\text{FC428}}$) was sequenced as previously described (13) to investigate the relatedness with the *penA* alleles of the previously reported ceftriaxone-resistant gonococcal strains (H041, GU140106, F89, and A8806 [4–8]). The deduced amino acid sequence of PBP 2 in FC428 elucidated a shared trait with the ceftriaxone-resistant strains GU140106 (5) and A8806 (8). As in GU140106 (5) and A8806 (8), the mosaic PBP 2 of FC428 possessed two (A311V and T483S) of the three (A311V, A316P, and T483S) critical amino acid substitutions resulting in the ceftriaxone resistance in H041 (4, 15). Transformation of PCR-amplified full-length $penA_{\text{FC428}}$, performed as previously described, to the ceftriaxone-susceptible *N. gonorrhoeae* strain

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TABLE 1 Comparison of characterized ceftriaxone-resistant *Neisseria gonorrhoeae* strains

Characteristic	FC428	GU140106 (5)	A8806 (8)	F89 (6, 7)	H041 (4)
Location, yr of isolation[a]	Japan, 2015	Japan, 2014	Australia, 2013	France and Spain, 2010	Japan, 2009
MIC (μg/ml)					
Ceftriaxone	0.5	0.5	0.5	1–2	2–4
Azithromycin	0.25	0.5	0.25	1	1
Spectinomycin	8	32	≤64	16	16
MLST[b]	1903	7363	7363	1901	7363
NG-MAST	3435	6543	4015	1407	4240
porB	1053	3854	1059	908	2594
tbpB	21	10	10	110	10
Key alternations in PBP2	A311V, T483S	A311V, T483S	A311V, T483S	A501P in mosaic PBP 2 XXXIV	A311V, T316P, T483S[c]

[a] MLST, multilocus sequence typing; NG-MAST, *N. gonorrhoeae multiantigen* sequence typing.
[b] MLST ST1903 (*abcZ126*, *adk39*, *aroE67*, *fumC157*, *gdh148*, *pdhC153*, and *pgm65*) differs with two and three loci from ST7363 (*abcZ59*, *adk39*, *aroE67*, *fumC78*, *gdh148*, *pdhC153*, and *pgm65*) and ST1901 (*abcZ109*, *adk39*, *aroE170*, *fumC111*, *gdh148*, *pdhC153*, and *pgm65*) (differences are underlined), respectively.
[c] Key amino acid alternations causing the ceftriaxone resistance (15).

NG9807 (4, 13) verified that the $penA_{FC428}$ 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 $penA_{FC428}$ was also further compared to *penA* of the GU140106 ($penA_{GU140106}$) and A8806 ($penA_{A8806}$) strains, which had a ceftriaxone MIC (0.5 μg/ml) identical to that of FC428 (Table 1). The 5′-terminal region of $penA_{FC428}$ (nucleotide positions 1 to 293) was identical to the corresponding regions of $penA_{GU140106}$ and $penA_{A8806}$. The 3′-terminal region of $penA_{FC428}$ was also nearly identical (99.9%; only one nucleotide mismatch was found at position 1296) to the corresponding regions of $penA_{GU140106}$ and $penA_{A8806}$ (positions 919 to 1749 and 907 to 1749, respectively). However, the central region of $penA_{FC428}$ showed substantially lower nucleotide sequence similarity with both $penA_{GU140106}$ and $penA_{A8806}$ (83.7% and 93.5%, respectively). On the contrary, the central region of $penA_{FC428}$ was identical to the corresponding region of *penA* of the penicillin- and cephalosporin-susceptible *N. gonorrhoeae* strain FA1090 ($penA_{FA1090}$). In fact, the entire 5′-terminal half of $penA_{FC428}$ (nucleotide positions 1 to 904) was identical to the corresponding region of $penA_{FA1090}$, while the 3′-terminal half was completely different (Fig. 1). All this indicates that the new mosaic $penA_{FC428}$ and the ceftriaxone resistance of FC428 evolved due to acquisition of the identical ceftriaxone resistance-determining 3′-terminal 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 ceftriaxone-resistant strains and conserved ceftriaxone resistance-determining PBP 2 regions might spread. Taking advantage of the unique and characteristic construct of $penA_{FC428}$, the develop-

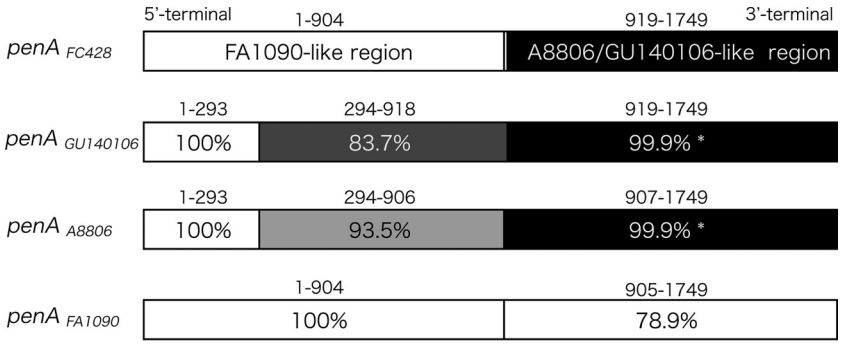

FIG 1 Mosaic *penA* genes of the ceftriaxone-resistant *Neisseria gonorrhoeae* strains FC428 (present study), GU140106 (5), A8806 (8), and the ceftriaxone-susceptible genome-sequenced gonococcal strain FA1090. Sequence similarities of $penA_{FC428}$ (GenBank accession no. LC113953), $penA_{GU140106}$ (GenBank accession no. LC056026), $penA_{A8806}$ (D. M. Whiley, personal communication), and $penA_{FA1090}$ (GenBank accession no. AE004969) are shown by rectangles. The regions conserved among ceftriaxone-resistant strains are shown by black rectangles, and unique regions of each strain are indicated by gray rectangles. The $penA_{FA1090}$ and *penA* regions in the ceftriaxone-resistant strains similar to $penA_{FA1090}$ are shown by white rectangles. * indicates one nucleotide difference at position 1296 of *penA*: G in $penA_{FC428}$, and A in $penA_{GU140106}$ and $penA_{A8806}$.

ment of a PCR specific for $penA_{FC428}$ 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 *N. gonorrhoeae*. 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 *Neisseria* species.

In conclusion, a new ceftriaxone- and multidrug-resistant *N. gonorrhoeae* 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 <1 g of ceftriaxone for the treatment of gonorrhea 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.

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