Conjunctivitis Caused by *Neisseria gonorrhoeae* Isolates with Reduced Cephalosporin Susceptibility and Multidrug Resistance

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We report two cases of conjunctivitis caused by *Neisseria gonorrhoeae* with reduced cephalosporin susceptibility. Patients showed no response to cefmenoxime eye drops and intravenous ceftriaxone administration. The patients' condition improved after the addition of oral minocycline. The isolates contained the mosaic *penA* for reduction of β -lactam susceptibility.

CASE REPORTS

Case 1. A 31-year-old man visited a private clinic and presented with conjunctival injection and discharge in his right eye. A diagnosis of acute conjunctivitis was made, and he was treated with a combination of 1.5% levofloxacin (LVFX) and 0.1% beta-methasone eye drops 4 times per day. However, his symptoms had worsened 2 days later, and he was referred to our hospital. Slit-lamp biomicroscopy revealed severe purulent discharge, conjunctival injection, and eyelid edema in the right eye (Fig. 1A). However, he did not have conjunctival papillae or follicles or corneal epithelial damage. Direct microscopy and bacterial culture of the discharge were performed. Direct microscopy demonstrated the presence of Gram-negative diplococci, and the culture reports confirmed the presence of *Neisseria gonorrhoeae*. The patient did not have other gonococcal infection, such as pharyngitis or urethritis. We considered conjunctivitis caused by *N. gonorrhoeae* and began treatment with topical 0.5% cefmenoxime (CMX) every hour and intravenous administration of ceftriaxone (CTRX) (1 g/day) for 3 days. Although the amount of discharge was slightly decreased 1 week after initiating the therapy, conjunctival injection was still active (Fig. 1B). We added oral minocycline (MINO; 200 mg/day) for 2 weeks after obtaining drug susceptibility testing results, and the conjunctivitis resolved within 7 days (Fig. 1C).

Case 2. A 28-year-old woman visited a local hospital and presented with conjunctival injection and discharge in the right eye. She was treated with a combination of 1.5% LVFX and 0.1% fluorometholone eye drops 4 times per day. However, the amount of discharge increased, and she was referred to our hospital. Slit-lamp biomicroscopy revealed severe purulent discharge and eyelid edema. The patient's condition was similar to the condition shown in Fig. 1A. Microbiological tests revealed the presence of *Neisseria gonorrhoeae*. She did not have other gonococcal infection. We started treatment with topical 0.5% CMX every hour and intravenous administration of CTRX (1 g/day) for 3 days. As conjunctival infection was still active 3 days later, oral MINO (200 mg/day) was administered for 1 week. Conjunctival inflammation subsided within 5 days after the addition of MINO.

Antimicrobial susceptibility testing. Three *N. gonorrhoeae* conjunctivitis isolates (EC358, EC359, and EC985) were obtained between 2003 and 2013 at Ehime University Hospital. These isolates, along with two other isolates (EC1025 from case 1 and EC1050 from case 2) were used. The ID Test HN-20 Rapid system confirmed identification of *N. gonorrhoeae*. Antimicrobial suscep-

tibility testing was performed using the Etest as described elsewhere (1). Etest strips containing CTRX, cefuroxime (CXM), cefalotin (CET), benzylpenicillin (PCG), and MINO, along with various other antimicrobial agents, including routine ophthalmic agents such as LVFX, gatifloxacin (GFLX), moxifloxacin (MFLX), chloramphenicol (CP), tobramycin (TOB), erythromycin (EM), and azithromycin (AZM), were purchased from AB bioMérieux. The MIC was determined according to the manufacturer's instructions. After incubation, an ellipse appeared on the MIC value scale (in μ g/ml), where the concentration of the antibiotic tested inhibited bacterial growth. All results were interpreted using cut-off values for susceptibility and resistance according to the Clinical and Laboratory Standards Institute (CLSI; M100-S22) and The European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org). No organization has established cut-off values for CET, MINO, CP, TOB, and EM. MICs of the drugs tested are summarized in Table 1. The MICs of various antimicrobials, except TOB and MINO, were higher for EC985, EC1025, and EC1050 than for EC358 and EC359. EC985, EC1025, and EC1050 showed decreased susceptibility to cephalosporins, fluoroquinolones, and macrolides. The MICs of MINO against EC358, EC359, EC1025, and EC1050 were similar.

Genetic characterization. For molecular epidemiological examination, strains were genotyped by multilocus sequence typing (MLST) (2). PCR and sequencing of resistance determinants, i.e., the *penA*, *mtrR*, *ponA*, *porB1b* (*penB*), *gyrA*, and *parC* genes, were performed as described elsewhere (3–5). The genetic characteristics of isolates are summarized in Table 2. The sequence type (ST) of EC985 and EC1025 by MLST was ST1901, which was the same as that of a multidrug-resistant gonococcal clone that has spread worldwide (6–9). The ST of EC1050 was ST7363, which was the same as that of cefixime-resistant isolates circulating in Japan (2). EC985, EC1025, and EC1050 were found to have *penA* mosaic

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FIG 1 Case 1. (A) Photograph of the eye before treatment with CTRX and CMX, showing the massive conjunctival infection and severe purulent discharge; (B) photograph of the eye 1 week after treatment with CTRX and CMX, showing conjunctival infection; (C) photograph of the eye 7 days after addition of MINO, showing normal appearance of the conjunctival sac.

allele X, which has correlated with reduced susceptibility to cephalosporins, including CTRX, in Japan (7–10). We investigated mutations in the *gyrA* and *parC* genes, which confer fluoroquinolone resistance. EC358 and EC359 strains contained two amino acid substitutions, Ser91→Phe and Asp95→Asn mutations, within the GyrA protein, while EC985 and EC1050 had an Ser87→Arg mutation and EC1025 had Ser87→Arg and Ser88→Pro mutations within the ParC protein along with two mutations within the GyrA protein. Sequencing of other resistance determinants showed that all strains contained the identical *mtrR* and *penB* alterations, i.e., an A deletion in the inverted repeat of the *mtrR* promoter and two consecutive amino acid alterations in *penB*. In addition, they also showed alteration of amino acid 421 in *penA*.

N. gonorrhoeae, which is the Gram-negative coccus responsible for sexually transmitted infection, rarely causes acute conjunctivitis. Transmission to the eye could be by contact with infected urine or genital secretions. The incidence rates of gonococcal conjunctivitis increase during spring and summer (11). This is a potentially devastating ocular infection, because *N. gonorrhoeae* can cause severe ulcerative keratitis, which may rapidly progress to corneal perforation (11–14). Kawashima et al. reported that the durations between conjunctivitis and corneal perforation were from 9 to 11 days in 5 cases of gonococcal keratoconjunctivitis (12). Although it is critical to obtain an accurate diagnosis and start treatment as early as possible, it is difficult to diagnose in cases that do not show systemic involvement, as in the present cases. As neither patient 1 nor 2 had systemic gonococcal infection and the infection routes in these cases were not known, diagnosis was delayed. Parenteral and topical antibiotics are necessary to

treat gonococcal conjunctivitis. A parenteral cephalosporin, such as CTRX, is recommended as a first-line treatment for gonococcal infection. Along with parenteral cephalosporins, topical antibiotics are generally added for treatment of conjunctivitis. As the commercial cephalosporin available for ophthalmic solution in Japan is CMX, which is a third-generation cephalosporin antibiotic, CMX eye drops are generally chosen for gonococcal conjunctivitis. Isolates from patients 1 and 2 contained the identical *penA* mosaic allele X and showed reduced susceptibility to cephalosporin, including first-generation (CXM), second-generation (CET), and third-generation (CTRX) agents. The global spread of gonococcal strains with decreased susceptibility to cephalosporins, including CTRX, from Asia to Europe is now a major public health concern (6, 8, 9). Patients 1 and 2 did not respond promptly to parenteral CTRX and CMX eye drops. Reduced *in vitro* susceptibility could be related to treatment failure. CTRX-resistant *N. gonorrhoeae*, which had a high MIC of CTRX (2 µg/ml), was isolated from female commercial sex workers in Japan (15). As STs of isolates from patients 1 and 2 by MLST were the same as those of a cephalosporin-resistant gonococcal clone that is spreading worldwide, the clonal spread of resistant *N. gonorrhoeae* is a matter of concern. Due to the emergence of CTRX-resistant strains in Japan, it is critical to consider several possibilities for treatment of gonococcal infection. To treat gonococcal conjunctivitis, it is necessary to use systemic antibiotics or antibiotic eye drops that can penetrate into the ocular tissue. In our cases, inflammation of the conjunctival sac decreased promptly after addition of oral MINO. The MICs of MINO against isolates from patients 1 and 2 were 0.125 and 0.25 µg/ml, respectively, which were similar to those against isolates in 2003. Although the isolates tested had *penB* mutations that reduce porin permeability of the outer membrane to hydrophilic antibiotics, such as tetracycline, susceptibility to

TABLE 1 Antibiotic susceptibilities of *N. gonorrhoeae* strains

Strain	Isolate yr	MIC (µg/ml)											
		CTRX	LVFX	GFLX	MFLX	CXM	CET	PCG	CP	TOB	EM	AZM	MINO
E358	2003	0.008	2	0.25	0.5	0.032	1	0.25	1	8	0.032	0.032	0.125
E359	2003	0.008	2	0.25	0.5	0.064	2	0.25	1	8	0.032	0.032	0.125
E985	2009	0.125	8	4	2	4	16	4	4	8	1	0.25	0.5
E1025 (patient 1)	2011	0.125	8	2	2	4	16	2	4	4	1	0.25	0.125
E1050 (patient 2)	2012	0.125	8	2	2	2	8	0.5	4	8	0.5	0.125	0.25

TABLE 2 Genetic characterization of *N. gonorrhoeae* strains

Strain	Genetic analysis ^a						
	MLST	<i>penA</i> (allele)	<i>gyrA</i> mutation(s)	<i>parC</i> mutation(s)	<i>ponA</i> mutation	<i>penB</i> mutation(s)	<i>mtrR</i> mutation
EC358	ST7360	V	Ser91→Phe, Asp95→Asn	WT	Leu421→Pro	Gly101→Lys, Ala102→Asp	A-del
EC359	ST7360	V	Ser91→Phe, Asp95→Asn	WT	Leu421→Pro	Gly101→Lys, Ala102→Asp	A-del
EC985	ST1901	X (mosaic)	Ser91→Phe, Asp95→Asn	Ser87→Arg	Leu421→Pro	Gly101→Lys, Ala102→Asp	A-del
EC1025 (patient 1)	ST1901	X (mosaic)	Ser91→Phe, Asp96→Asn	Ser87→Arg, Ser88→Pro	Leu421→Pro	Gly101→Lys, Ala102→Asp	A-del
EC1050 (patient 2)	ST7363	X (mosaic)	Ser91→Phe, Asp97→Asn	Ser87→Arg	Leu421→Pro	Gly101→Lys, Ala102→Asn	A-del

^a MLST, multilocus sequencing typing; WT, wild type; A-del, A deletion in promoter region.

MINO was not markedly different among the isolates tested. MINO is known to penetrate into ocular tissue very well and has anti-inflammatory, antimetalloproteinase, and antiapoptotic properties (16, 17). Thus, MINO may be useful for treatment of gonococcal conjunctivitis. Although fluoroquinolone eye drops are increasingly used for treatment of bacterial conjunctivitis in general clinical practice, due to their drug stability and broad spectra, the gonococcal resistance level to fluoroquinolones is increasing in Japan (5, 18). Gonococcal isolates in 2003 had mutations within the GyrA protein, but isolates from our cases also had mutations within not only the GyrA protein but also the ParC protein and showed higher fluoroquinolone MICs. Consistent with the results of *in vitro* susceptibility testing, the LVFX eye drops initially administered were ineffective for treatment of conjunctivitis in our cases. However, the MICs of GFLX and MFLX (2 µg/ml) were lower than that of LVFX. It is likely that eye drops with high concentrations of GFLX (3 mg/ml) and MFLX (5 mg/ml) would have efficacy for gonococcal conjunctivitis rather than LVFX. The MICs of CP, EM, and AZM, which are available for ophthalmic solutions, against isolates from patients 1 and 2 were higher than those against isolates in 2003. However, AZM eye drops could be effective, because this drug can penetrate into conjunctival tissue and show a sustained high concentration exceeding the MIC (0.125 to 0.25 µg/ml) (19). For effective treatment of conjunctivitis caused by multidrug-resistant gonococcus, antibiotics should be chosen carefully with reference to the results of confirmatory susceptibility testing.

In conclusion, we encountered two cases of conjunctivitis caused by *N. gonorrhoeae* with reduced cephalosporin susceptibility. Ophthalmologists should diagnose gonococcal conjunctivitis as early as possible and refer patients for gonococcal antimicrobial susceptibility testing. Further epidemiological surveillance of *N. gonorrhoeae* isolated from conjunctivitis is necessary.

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