

Outbreak of Cefozopran (Penicillin, Oral Cephems, and Aztreonam)-Resistant *Neisseria gonorrhoeae* in Japan

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We have previously reported that the *Neisseria gonorrhoeae* isolates from clinical failure cases treated with cefdinir and aztreonam, β -lactams exhibited high MICs. These resistant isolates were clearly separated from the isolates exhibiting a low level of resistance to β -lactams as shown by the MIC distribution of cefozopran. Restriction fragment length polymorphism DNA typing revealed that the outbreak of cefozopran-resistant isolates in Kitakyushu, Japan, occurred as a result of clonal spread.

As a result of the absence of strains of *Neisseria gonorrhoeae* resistant to the expanded spectrum of cepheims, the National Committee for Clinical Laboratory Standards (NCCLS) (8) has not defined the breakpoint MICs of expanded-spectrum cepheims such as cefixime (CFM), cefpodoxime (CPD), cefepime (FEP), etc. A previous study reported the incidence of clinical failures in gonococcal urethritis treated with cefdinir (CDR) or aztreonam (ATM) (1). For the *N. gonorrhoeae* isolates from such clinical failure cases, high-level MICs of CDR, ATM, and other β -lactams were observed. In order to investigate the prevalence of these resistant isolates in Kitakyushu, Japan, we examined 54 *N. gonorrhoeae* isolates from different cases occurring during 1999 for susceptibility to a variety of antimicrobial agents. Forty of 54 strains were isolated from male patients with gonococcal urethritis, while the remaining isolates were from female patients with gonococcal cervicitis. Identification of *N. gonorrhoeae* and testing for production of β -lactamase were performed by ID-test-HN-20 Rapid (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) using colonies cultured on Thayer-Martin Agar Base, Modified (Nissui Pharmaceutical Co.). The MICs of various antimicrobials were determined by the twofold serial agar dilution method on BBL GC Agar Base (Becton Dickinson and Co., Cockeysville, Md) with 1% BBL IsoVitalX enrichment (Becton Dickinson Europe, Meylan, France) according to the guidelines of the NCCLS (8). The antimicrobial agents used in this study were purchased from or provided by the corresponding companies.

The MIC distribution of cefozopran (CZO) for *N. gonorrhoeae* isolates was divided into two groups. The MICs for the high-level resistance group (8 to 16 $\mu\text{g/ml}$) were more than 16 times greater than the MICs for the susceptible and low-level resistance groups ($<0.5 \mu\text{g/ml}$). The MICs of CZO were cor-

related with those of CDR, CPD, cefpirome (CPI), FEP, ATM, cefuroxime, cefotiam, ceftizoxime (ZOX), CFM, and cefcapene (CPN). The MICs of CZO correlated poorly with those of penicillin (PEN), cefmetazole, flomoxef, and cefodizime (CDZ) despite the fact that all 17 CZO-resistant isolates for these four agents belonged to the high-level MIC group. CFM, CDZ and ceftriaxone (CRO) exhibited lower MICs but the resistant isolates belonged to the group with reduced susceptibility to these three agents. These new resistant strains were clearly divided into two groups by the MIC distribution of CZO, with all of the CZO-resistant isolates exhibiting either resistance or a reduced susceptibility to all β -lactams tested. Clinical failures caused by these resistant isolates occurred in three patients treated with CDR or ATM (Table 1). For these 17 isolates, high-level MICs for penicillins, narrow- and expanded-spectrum cephalosporins, cephamycins, the majority of broad-spectrum oral cephalosporins, and aztreonam were observed, together with a reduced susceptibility to most broad-spectrum parenteral cephalosporins. In the measurement of MIC and the disk diffusion test, these resistant isolates can be identified easily with CZO. CZO has been rarely used in gonococcal infection, but we consider that it may well be profitable to use CZO to define this resistant organism. We therefore called these isolates CZO-resistant *N. gonorrhoeae* (CZRNG). These strains did not produce β -lactamase. CZRNG accounted for 32% (17/54) of all isolates tested. The MICs of CDZ, CRO, and spectinomycin (SPT) showed that the isolates were susceptible, while CFM, cefotaxime (CTX), and ZOX retained good activity. To other β -lactams, the isolates were resistant or had a reduced susceptibility. All of the 17 CZRNG isolates were resistant to ciprofloxacin (CIP), with MICs of this drug ranging between 0.125 and 64 $\mu\text{g/ml}$. Six of 17 isolates exhibited high resistance to ciprofloxacin (MICs $> 2 \mu\text{g/ml}$). All 17 isolates were resistant to tetracycline (MIC, 1 to 4 $\mu\text{g/ml}$). The MICs of minocycline (MIN) for 15 of the 17 isolates were greater than 0.5 $\mu\text{g/ml}$. The MIC of erythromycin for one isolate was 0.125 $\mu\text{g/ml}$, while MICs for the other isolates had values of 4 to 16 $\mu\text{g/ml}$. Thus, 17 CZRNG isolates were multiresistant strains.

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TABLE 1. Clinical data of 17 cefozopran-resistant *N. gonorrhoeae* isolates^a

Strain no.	Hospital ID ^b	Age (yr) and sex of patient ^c	First treatment			Final treatment		
			Agents ^d	Regimen ^e	Clinical outcome	Agent(s)	Regimen	Clinical outcome
SNG27	Ue	27, M	CDR	100 mg × 3, p.o., 3 days	Failure	CRO SPX	1 g i.v., single dose 100 mg, 3 days	Cure
SNG28	Ya	24, M	SPT DOX	2 g i.m., single dose 100 mg × 2, p.o., 7 days	Cure			
SNG32	Ar	19, F	CM	Suppository, 2 times	Not tested			
SNG33	UO	29, M	AZT	1 g i.v., single dose	Failure	SPT	2 g i.m., single dose	Cure
SNG46	Ya	21, M	SPT DOX	2 g i.m., single dose 100 mg × 2, p.o., 7 days	Cure			
SNG50	Ni	19, M	CDR	100 mg × 3, p.o., 3 days	Failure	SPT	2 g i.m., single dose	NF
SNG52	An	22, F	AMO	375 mg × 4, p.o., 7 days	Failure	CDZ	1 g i.v., single dose	Cure
SNG53	Kr	24, M	CRO	1 g i.v., single dose	Cure			
SNG54	AY	42, M	LVX	100 mg × 3, p.o.	Failure	CDZ	1 g i.v., single dose	Cure
SNG57	BM	36, M	Unknown					
SNG65	Ni	19, M	CDR	100 mg × 3, 3 days	NF ^f			
SNG70	BM	33, M	Unknown					
SNG74	Ni	30, M	SPT MIN	2 g i.m., single dose 100 mg × 2, 7 days	Cure			
SNG75	Ni	27, M	SPT MIN	2 g i.m., single dose 100 mg × 2, p.o., 7 days	Cure			
SNG76	Ni	29, M	CDZ	1 g i.v., single dose	Cure			
SNG79	Ni	26, M	CDZ	1 g i.v., single dose	Cure			
SNG81	An	19, M	CDZ	1 g i.v., single dose	Cure			

^a Including isolates from clinical failure cases treated with CDR and AZT. Data for SNG27 and SNG33 have been previously reported (1).

^b Blind names of hospitals.

^c M, male; F, female.

^d CDR, cefdinir; DOX, doxycycline; MIN, minocycline; AMO, amoxicillin; SPX, sparfloxacin.

^e p.o., orally; i.m., intramuscularly; i.v., intravenously.

^f NF, not followed.

We therefore performed restriction fragment length polymorphism (RFLP) analysis as described by Struelens et al. (10) using a CHEFF Mapper pulsed-field gel electrophoresis system (Nippon Bio-Rad Laboratories, Tokyo, Japan). Figure 1

and Table 2 present the results of the macrorestriction genomic DNA analysis by *SpeI* carried out with 17 CZRNG strains. The 17 strains were isolated over a period of 8 months (May to December) from 17 cases at nine hospitals that were

TABLE 2. DNA typing patterns and antimicrobial susceptibilities of 17 cefozopran resistant *N. gonorrhoeae* isolates

Strain no.	RFLP pattern	MIC ^a (μg/ml)														
		PEN	CXM	CMZ	CTX	ZOX	CDZ	CZO	CRO	CFM	CPD	CDR	CIP	MIN	TET	SPT
SNG27 ^b	A	4	16	32	1	1	0.25	16	0.125	0.25	4	1	0.5	1	4	16
SNG28	B	2	8	16	1	2	0.25	16	0.125	0.25	4	1	0.25	0.5	2	16
SNG32	B	8	16	32	0.5	0.5	0.125	16	0.125	0.25	4	1	0.25	0.5	4	16
SNG33 ^b	B	4	16	16	1	1	0.125	16	0.125	0.25	4	1	4	1	4	16
SNG46	A	4	8	16	1	2	0.25	16	0.125	0.5	4	1	0.25	1	2	32
SNG50	B	4	16	16	0.5	2	0.125	16	0.125	0.5	4	1	2	1	4	32
SNG52	A	4	16	16	0.5	1	0.125	16	0.125	0.25	4	1	2	1	4	32
SNG53	B	4	16	32	1	1	0.25	16	0.125	0.25	4	1	64	1	4	32
SNG54	A	4	16	32	2	1	0.25	16	0.25	0.5	4	1	0.5	1	2	32
SNG57	A	4	16	32	1	1	0.125	16	0.125	0.25	4	1	64	1	4	32
SNG65	A	4	16	16	1	1	0.25	16	0.125	0.25	4	1	2	0.5	4	32
SNG70	A	1	2	2	0.125	0.5	0.015	8	0.031	0.125	0.5	1	0.125	0.125	1	32
SNG74	A	4	8	8	1	1	0.25	16	0.063	0.25	2	1	0.125	0.5	2	32
SNG75	B	2	8	8	0.5	1	0.063	8	0.063	0.125	1	1	0.125	0.25	1	32
SNG76	A	2	16	16	0.5	1	0.063	8	0.031	0.125	2	1	0.25	0.5	2	32
SNG79	A	4	16	32	1	1	0.125	16	0.125	0.25	2	1	0.5	0.5	4	32
SNG81	A	4	16	32	1	2	0.25	16	0.125	0.25	2	1	0.5	1	4	32
ATCC49226		0.25	1	1	2	0.063	0.015	0.031	0.125	0.015	0.063	0.031	0.004	0.5	1	32

^a Abbreviations: PEN, benzylpenicillin; CXM, cefuroxime; CMZ, cefmetazole; CTX, cefotaxime; ZOX, ceftizoxime; CDZ, cefodizime; CZO, cefozopran; CRO, ceftriaxone; CFM, cefixime; CPD, cefpodoxime; CIP, ciprofloxacin; TET, tetracycline; SPT, spectinomycin.

^b MICs and patient data for this isolate have been previously reported (1).

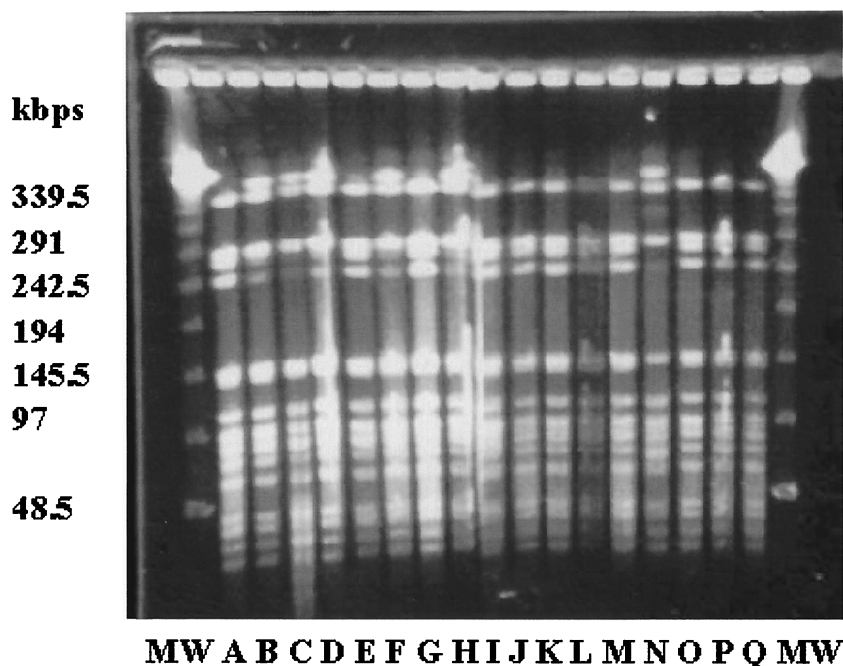


FIG. 1. The *SpeI* restriction patterns of chromosomal DNAs from 17 cefazopran-resistant *Neisseria gonorrhoeae* isolates. MW, λ DNA ladders; A, SNG27; B, SNG28; C, SNG32; D, SNG33; E, SNG46; F, SNG50; G, SNG52; H, SNG53; I, SNG54; J, SNG57; K, SNG65; L, SNG70; M, SNG74; N, SNG75; O, SNG76; P, SNG79; Q, SNG81.

scattered within a radius of 40 km. Although the 17 CZRNG strains were isolated from multiple patients, RFLP analysis clearly divided the isolates into only two groups. The RFLP patterns by *SpeI* of the two groups differed by only one band. Molecular analysis software (Molecular Analyst Fingerprinting Plus; Nippon Bio-Rad Laboratories) indicated that the two groups were greater than 90% similar. The 17 CZRNG isolates underwent further RFLP typing by *NheI* (5), which produced identical RFLP patterns for all of the 17 CZRNG isolates. These results indicate that the outbreak of CZRNG isolates in Kitakyushu, Japan, occurred as a result of clonal spread.

The CZRNG strain was first isolated at a hospital in Kitakyushu, Japan, in May, 1999. After that, the frequency of patients suffering from CZRNG rapidly increased such that the ratio of CZRNG among *N. gonorrhoeae* isolates reached 45.5% (5/11) during the month of December 1999. To our surprise, the genomic RFLP analysis revealed that the outbreak of CZRNG isolates occurred due to clonal spread. In Japan, most patients suffering from *N. gonorrhoeae* are treated with oral antimicrobials such as penicillins, broad-spectrum cepheims, fluoroquinolones, and tetracyclines. Therefore, the emergence of CZRNG isolates resistant to cepheims, fluoroquinolones, and tetracyclines is of serious concern. In the cases of gonococcal infection caused by CZRNG, the administration of 1 g of CRO, 1 g of CDZ, or 2 g of SPT was effective (Table 1). In Japan, CRO has not been recognized for use in gonococcal infections, and we have therefore treated patients with gonococcal infections with CDZ or SPT. Although SPT-resistant isolates were not isolated in this study, there have been reports of SPT-resistant *N. gonorrhoeae* isolates in other areas (6, 7). We therefore

recommend that CDZ be used as the first-line treatment for gonococcal infection.

It has been reported that the mechanisms of low-level resistance to β -lactams, including cepheims, involved the mutation of the porin encoded at the *penB* locus (4) and the decreased affinity of β -lactams to PBP-2 (2, 3, 9). Recently, we have discovered that many codons of the PBP-2 gene (*penA*) of the CZRNG isolates were different from those of the other susceptible isolates (data not shown). This area is under further investigation since this may be a potential resistance mechanism of CZRNG.

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