

Identification of TEM-135 β -Lactamase in Penicillinase-Producing *Neisseria gonorrhoeae* Strains in Japan[∇]

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Ten penicillinase-producing *Neisseria gonorrhoeae* (PPNG) strains isolated from 2000 to 2008 were characterized by multilocus sequence typing, multiantigen sequence typing, and plasmid typing. Sequence analysis showed that 8 strains contained a TEM-1 β -lactamase gene. However, two other genetically distinct PPNG strains, isolated in 2004 and 2008, each contained a TEM-135 β -lactamase on different plasmids, a Toronto/Rio type R plasmid and an Asia type R plasmid, suggesting independent origins of these PPNG strains.

Antibiotic-resistant *Neisseria gonorrhoeae* is a major public health concern (15). An essential element in gonococcal-infection control is the availability of effective antimicrobial therapy. However, *N. gonorrhoeae* has developed resistance to multiple classes of antimicrobials. In Japan, the prevalence of fluoroquinolone-resistant *N. gonorrhoeae* strains is over 80% (12), and *N. gonorrhoeae* strains with reduced sensitivity and with resistance to cefixime (CFM) have emerged and spread nationwide (5, 7). In contrast to the high prevalence of *N. gonorrhoeae* strains with chromosomal β -lactam resistance genes, the prevalence of penicillinase-producing *N. gonorrhoeae* (PPNG) strains with a β -lactamase gene carried on a plasmid is relatively low in Japan. However, the prevalence of PPNG strains in other countries in Asia is high (16). To study the epidemiology of *N. gonorrhoeae*, nucleotide sequence-based typing methods, like multilocus sequence typing (MLST) and multiantigen sequence typing (MAST), are useful tools, since the analyses yield highly reproducible and easy-to-compare data from different laboratories.

Among the 719 *N. gonorrhoeae* strains isolated from January 2000 to December 2008 in the Nakano Sogo Hospital in Japan, 10 strains (1.4%) were found to be penicillinase-producing *N. gonorrhoeae* (PPNG) by the nitrocefin test (data not shown). The MICs of penicillin (PEN), cefixime (CFM), and ceftriaxone (CRO) were determined by the agar dilution method (6), suggesting that the strains were highly resistant to penicillin but not to cephalosporins (Table 1). This low prevalence was consistent with other reports (14, 16).

MLST and MAST (3, 4) were used to characterize these PPNG strains. As shown in Table 1, both MLST and MAST divided the 10 PPNG strains into 7 types, with 4 (NGON 00-002, NGON 00-027, NGON 04-025, and NGON 08-003) of the PPNG strains having unique sequence types (ST) by both MLST and MAST. However, three pairs of strains (NGON 05-042 and NGON 06-041, NGON 08-041 and NGON08-046,

and NGON 08-043 and NGON 08-044) had identical sequence types by MLST and by MAST (Table 1). Although we have no information linking the patients from whom each pair of strains was isolated, transmission of PPNG strains might be considered in these cases.

Plasmids of the PPNG strains carrying the β -lactamase gene (*bla*) have been typed based on plasmid size, since deletion mutants have been reported previously (9). To investigate plasmid diversity in the PPNG strains in this study, plasmid DNAs were purified using QIAprep Spin miniprep kits (Qiagen). To estimate β -lactamase plasmid size, we amplified the complete DNA of each plasmid by long PCR using *LA Taq* polymerase (TaKaRa) and primers *bla*-IR, 5'-TCGTGGTGTACAGCTC GTCC, and *bla*-IF, 5'-CTGCAGCAATGGCAACAACGTTG, which anneal to nucleotides 7426 to 7404 and 1 to 23, respectively, of the 7,426-bp pJD4 plasmid (Fig. 1A) (9). The PCR products were incubated for 2 min at 96°C followed by 30 cycles of 10 s at 96°C, 10 s at 63°C, and 8 min at 72°C. As shown in Fig. 1B, analysis of the amplified plasmid DNAs in a 1% agarose gel showed three plasmid sizes: 5.2, 5.6, and 7.4 kb. By use of a multiplex PCR method for plasmid typing (10), the 5.2-, 5.6-, and 7.4-kb plasmids were identified as Toronto/Rio, Africa, and Asia type R plasmids, respectively (Fig. 1A and C).

Although the molecular sizes of *N. gonorrhoeae* R plasmids are diverse, plasmids carrying β -lactamases are genetically related and carry a TEM-1 type *bla* gene, *bla*_{TEM-1} (12). To confirm the conservation of *bla*_{TEM-1}, the *bla* genes of the 10 PPNG isolates were analyzed by DNA sequencing (8). The primers used for amplification and sequencing were *bla*-F, 5'-CGCTCATGAGACAATAACCCTGG, and *bla*-R, 5'-GGGTCTGACGCTCAGTGGAAACG. The PCR products were incubated for 2 min at 96°C followed by 30 cycles of 10 s at 96°C, 10 s at 60°C, and 1 min at 72°C. Nucleotide sequencing was carried out as described previously (8). As shown in Table 1, two distinct *bla*_{TEM} alleles were found: 8 PPNG strains contained *bla*_{TEM-1}, and the other 2 strains (NGON 04-025 and NGON 08-003) contained *bla*_{TEM-135}, a TEM allele originally identified in *Salmonella enterica* serovar Typhimurium (11). These alleles, *bla*_{TEM-1} and *bla*_{TEM-135}, had one base difference, which resulted in a single amino acid

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TABLE 1. Penicillinase-producing *Neisseria gonorrhoeae* strains isolated in Tokyo from 2000 to 2008

Strain	Time of isolation	Sex of patient ^a	Age (yr) of patient	Specimen type ^b	MLST type	MAST type	Plasmid type	<i>bla</i> type	MIC ($\mu\text{g/ml}$)		
									PEN	CFM	CRO
NGON 00-002	January 2000	M	26	UD	ST-1590	ST-270	Africa	TEM-1	16	0.032	0.016
NGON 00-027	June 2000	M	42	U	ST-1921	ST-1817	Asia	TEM-1	>64	0.008	0.008
NGON 04-025	April 2004	M	27	U	ST-1597	ST-1549	Toronto/Rio	TEM-135	>64	0.004	\leq 0.004
NGON 05-042	August 2005	F	30	VD	ST-1588	ST-4012	Africa	TEM-1	64	0.25	0.064
NGON 06-041	October 2006	M	56	U	ST-1588	ST-4012	Africa	TEM-1	32	0.25	0.032
NGON 08-003	January 2008	F	31	VD	ST-7823	ST-4013	Asia	TEM-135	>64	0.032	0.032
NGON 08-041	September 2008	M	52	U	ST-1584	ST-1478	Africa	TEM-1	64	0.008	0.004
NGON 08-043	September 2008	M	31	U	ST-7823	ST-1288	Asia	TEM-1	>64	0.064	0.064
NGON 08-044	September 2008	F	25	VD	ST-7823	ST-1288	Asia	TEM-1	>64	0.064	0.064
NGON 08-046	October 2008	F	59	VD	ST-1584	ST-1478	Africa	TEM-1	64	0.25	0.032

^a M, male; F, female.

^b U, urine; VD, vaginal discharge; UD, urethral discharge.

substitution, M182T (residue numbering follows that of Ambler et al. [1]).

Interestingly, the two PPNG strains with *bla*_{TEM-135} were genetically different: the sequence types of strain NGON 04-025 were MLST ST-1597 and MAST ST-1549, and those of strain NGON 08-003 were MLST ST-7823 and MAST ST-4013 (Table 1). The plasmids carried by strains NGON 04-025 and NGON 08-003 were also distinct: the plasmid for the former was a Toronto/Rio type, and that for the latter was an Asia type. Taken together, these findings suggest that *bla*_{TEM-135} may have been introduced independently into these two *N. gonorrhoeae* strains or may have emerged by a point mutation in each. Recently, Srifeungfung et al. (13) reported that a PPNG strain isolated in Thailand contained a *bla*_{TEM-135} allele. PPNG strains containing *bla*_{TEM-135} might be widespread in Asian countries, although further study is needed to determine the prevalence.

The TEM type β -lactamase genes, which are widely distributed in Gram-negative bacteria, are diverse in sequence and in substrate spectrum. Some types of TEM β -lactamases can hydrolyze extended-spectrum cephalosporins with an oxyimino side chain, including ceftriaxone, which is still an effective antibiotic for *N. gonorrhoeae*. The diverse substrate spectra of TEM β -lactamases are due to mutations in the *bla*_{TEM} gene that alter the amino acid configuration around the β -lactamase active site. Since bacteria with *bla*_{TEM-135} have a restricted β -lactamase substrate spectrum, as reported in a previous study (10) and also in this study (Table 1), the selective pressure for emergence of *N. gonorrhoeae bla*_{TEM-135} is not known. It is noteworthy that there are other TEM β -lactamases with extended substrate spectra that may have arisen as a single point mutation in *bla*_{TEM-1} or *bla*_{TEM-135}, e.g., *bla*_{TEM-29} and *bla*_{TEM-20} (2). Since point mutations in *bla*_{TEM-1} and *bla*_{TEM-135} could lead to emergence of *N. gonorrhoeae* β -lactamases with extended substrate spectra, the antibiotic resistance profiles of PPNG strains should be monitored, especially in areas of high PPNG prevalence.

Nucleotide sequence accession number. The sequence data for the *bla*_{TEM-135} gene have been assigned DDBJ accession number AB551787.

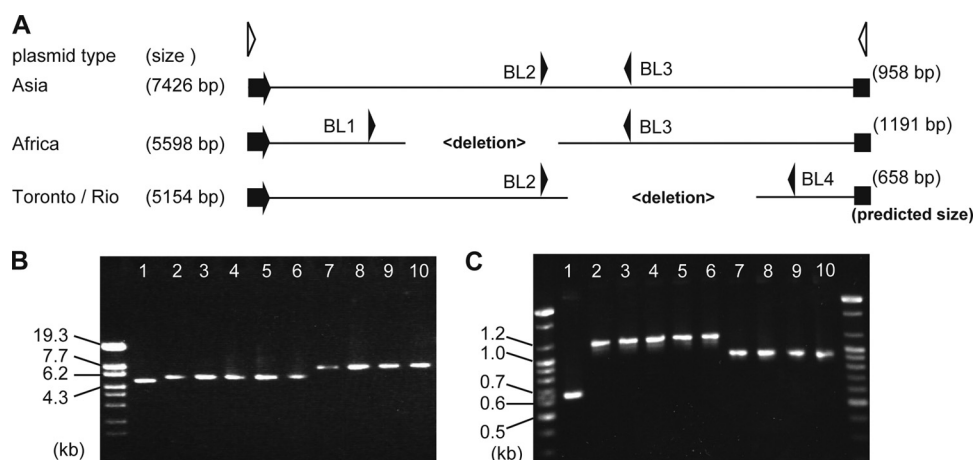


FIG. 1. Typing of plasmids carrying β -lactamases from *Neisseria gonorrhoeae* strains. (A) Schematics of Asia, Africa, and Toronto/Rio type plasmids. Each β -lactamase gene is shown by an arrowhead. The annealing sites of the primers used in this study for plasmid size determination (white arrowheads) and for plasmid type determination (black arrowheads) are shown. (B) Products of whole-plasmid PCR amplification, separated on a 1% agarose gel. (C) Products of multiplex PCR, separated on a 2% agarose gel. The size marker lanes contain StyI-digested lambda DNA (Toyobo) (B) or a 100-bp DNA ladder (Bioneer) (C). Lane 1, NGON 04-025; lane 2, NGON 00-002; lane 3, NGON 05-042; lane 4, NGON 06-041; lane 5, NGON 08-041; lane 6, NGON 08-046; lane 7, NGON 00-027; lane 8, NGON 08-003; lane 9, NGON 08-043; lane 10, NGON 08-044.

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