Penicillinase-Producing Plasmid Types in *Neisseria gonorrhoeae* Clinical Isolates from Australia

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Penicillinase-producing *Neisseria gonorrhoeae* (PPNG) carrying the *bla*_{TEM-135} gene is of particular concern, as it is considered a stepping stone toward resistance to extended-spectrum cephalosporins. Here, we sought to characterize plasmid types and the occurrence of the *bla*_{TEM-135} gene for *N. gonorrhoeae* clinical isolates from Australia. We found that *bla*_{TEM-135} was prevalent in Australian PPNG and was detected on all three major plasmid types.

High-level resistance to penicillin in *Neisseria gonorrhoeae* is conferred by plasmid-mediated penicillinase production. To date, seven different penicillinase-harboring gonococcal plasmid types have been described and comprise three main plasmid types (Asian, African, and Rio/Toronto plasmids) and four less common variants (Nimes, New Zealand, Johannesburg, and Australian plasmids) (1–7). There are two different penicillinase genes that may be carried by these plasmids, including the classical *bla*_{TEM-1} gene and the more recently described gonococcal *bla*_{TEM-135} gene (8). The *bla*_{TEM-135} gene is of concern as it is considered to be an intermediate between a penicillinase and an extended-spectrum β -lactamase (9) and is therefore considered a potential future threat to the effectiveness of *N. gonorrhoeae* treatment. The *bla*_{TEM-135} gene was first described in an isolate from Thailand (8) and has since been reported in various countries, including Canada (10), China (11), Japan (12), and Switzerland (13). However, there are now more recent data showing that *bla*_{TEM-135} has been present in gonococci since at least 1984, suggesting that the gene is not, as originally thought, a recent evolutionary event driven by selective pressure through the use of extended-spectrum cephalosporins (14).

Data on the rate at which penicillinase-producing *N. gonorrhoeae* (PPNG) isolates carry the *bla*_{TEM-135} gene are limited, ranging from 9.4% (9/96 PPNG isolates) in Thailand (9) and 20% (2/10 PPNG isolates) in Japan (12) to 58% (66/114 PPNG isolates) in China (11). These studies also highlighted that *bla*_{TEM-135} may occur in distinct genetic backgrounds and suggested that the gene has continued to be shared among gonococci via horizontal genetic exchange, having been found on the Asian and Rio/Toronto plasmid types and across various multilocus sequence types (MLST) and *N. gonorrhoeae* multiantigen sequence types (NG-MAST) (8, 11, 12, 14).

In this study, we sought to characterize plasmid types and the occurrence of the *bla*_{TEM-135} gene in *N. gonorrhoeae* clinical isolates from Australia. A total of 351 PPNG clinical isolates were included in this study (Table 1). These were all PPNG isolates identified as part of a broader investigation of *N. gonorrhoeae* isolates ($n = 2,455$) that were collected from throughout Australia in

the first half of the year 2012 (7, 15). The isolates were initially determined to be PPNG on the basis of acidometric and nitrocefin disc testing and subsequently by real-time PCR (7). The DNA used for the real-time PCR testing was stored at -80°C until further investigation. Here, the DNA from these PPNG isolates was tested by a plasmid typing method that enables differentiation of the three main plasmid types, Asian, African, and Rio/Toronto, via conventional PCR and agarose gel electrophoresis detection (16). Plasmid types were assigned for 327 isolates (Table 1), and no amplification products were observed for the remaining 24 isolates; these 24 isolates were excluded from further analysis. Overall, the African plasmid type was most common, comprising 237/327 isolates (72.5%), followed by the Rio/Toronto ($n = 47$; 14.4%) and the Asian ($n = 43$; 13.1%) plasmids. (It should be noted that the 47 Rio/Toronto plasmids included a single isolate with the recently described “Australian plasmid”—a variant of the Rio/Toronto plasmid that we described in our preliminary investigations [7]. The plasmid typing method does not differentiate such variants.)

The 327 isolates were then investigated for the *bla*_{TEM-135} allele using a previously described allele-specific PCR method (9). The results of this testing showed that 90 (27.5%) of the 327 PPNG isolates harbored *bla*_{TEM-135} and comprised all 47 (100%) of the Rio/Toronto plasmids, 23/43 (53.5%) of the Asian plasmids, and 20/237 (8.4%) of the African plasmids. These 90 isolates were further genotyped by single nucleotide polymorphism (SNP)-based MLST (17), and 23 different sequence types (STs) were ob-

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TABLE 1 Summary of plasmid types and *bla*_{TEM-135} gene results for 327 PPNG isolates

Plasmid type	<i>bla</i> _{TEM-135}	No. of isolates from ^a :						Total no. of isolates
		NSW	VIC	QLD	WA	SA	NT	
African	Negative	94	77	14	23	8	1	217
African	Positive	2	15	3	0	0	0	20
Asian	Positive	3	15	2	2	1	0	23
Asian	Negative	5	5	4	5	1	0	20
Rio/Toronto	Positive	17	16	6	6	2 ^b	0	47
Total no. of isolates		121	128	29	36	12	1	327

^a NSW, New South Wales; VIC, Victoria; QLD, Queensland; WA, Western Australia; SA, South Australia; NT, Northern Territory.^b One of these isolates represented the novel Australian plasmid, a variant of the Rio/Toronto plasmid type (7).

served for 89 isolates (one isolate could not be typed by the SNP MLST method) (Table 2). All the isolates with the African plasmid harboring *bla*_{TEM-135} ($n = 20$) were a single ST (ST1600; Table 2), whereas the Asian and Rio/Toronto plasmids harboring *bla*_{TEM-135} comprised 5 and 17 different STs, respectively. Of note was that six of the plasmid type/ST combinations observed in our population had previously been observed in studies of *bla*_{TEM-135} in Japan and Thailand, which is consistent with international transmission of these

strains. Of further interest was that the Rio/Toronto plasmids carried only the *bla*_{TEM-135} gene and not the *bla*_{TEM-1} gene. This adds further weight to the suggestion by Muhammad et al. (14) that the Rio/Toronto plasmids are the origin of *bla*_{TEM-135} in gonococci.

In summary, the *bla*_{TEM-135} gene was commonplace in this sample of Australian PPNG isolates, was detected on all three major plasmid types, was consistent with previous studies, and was found across genetically distinct clonal groups. These data provide further evidence of the broad distribution and ongoing spread of the *bla*_{TEM-135} gene among gonococci.

TABLE 2 Summary of MLST and plasmid types for the 90 *bla*_{TEM-135}-positive isolates

Plasmid type	MLST ^a	No. of isolates	Comment
African	1600	20	
Asian	1902, 8131 ^b	9	Previously observed in Japan (12)
	7822	7	
	7360	4	
	7365	1	
	1901, 6814, 9365 ^b	1	Previously observed in Thailand (8)
	NA ^c	1	
Rio/Toronto	8143	10	Previously observed in Thailand (8)
	8784	8 ^e	Previously observed in Thailand (8)
	8130	7	Previously observed in Thailand (8)
	8136	3	Previously observed in Thailand (8)
	8778	3	Previously observed in Thailand (8)
	New1 ^d	3	Previously observed in Japan (12)
	New2 ^d	2	
	New3 ^d	2	
	1587	1	
	1597	1	
	7829	1	
	7363, 7832 ^b	1	
	New4 ^d	1	
	New5 ^d	1	
	New6 ^d	1	
	New7 ^d	1	
	New8 ^d	1	

^a Single nucleotide polymorphism (SNP)-based MLST (17).^b These MLST could not be distinguished by SNP-based MLST.^c NA, not applicable; this isolate could not be typed by SNP-based MLST.^d "New" indicates that STs could not be assigned for these SNP profiles and were considered to represent previously unrecognized STs.^e One of these isolates represented the novel Australian plasmid, a variant of the Rio/Toronto plasmid type (7).

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