ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2010, p. 554–556 Vol. 54, No. 1
0066-4804/10/$12.00 doi:10.1128/AAC.01108-09
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Characteristics and Population Dynamics of Mosaic *penA* Allele-Containing *Neisseria gonorrhoeae* Isolates Collected in Sydney, Australia, in 2007-2008▽

John W. Tapsall,* Sanghamitra Ray, and Athena Limnios

Neisseria Reference Laboratory, Microbiology Department, South Eastern Area Laboratory Services, The Prince of Wales Hospital, Randwick, Sydney, Australia

Received 5 August 2009/Returned for modification 18 October 2009/Accepted 5 November 2009

Eighteen hundred *Neisseria gonorrhoeae* isolates collected in Sydney, Australia, in 2007 and 2008 were examined for mosaic *penA* alleles that mediated cephalosporin resistance, and the genotypes of the isolates were evaluated. In 2008, there were substantial increases in numbers (from 15 to 85) and proportions (from 1.5 to 10.3%) of mosaic-containing gonococci and major shifts in genotypic patterns, with 10 new genotypes representing 74 of the 85 mosaic-containing isolates and genotypes detected between 2001 and 2005 having disappeared. Enhanced surveillance of gonococcal resistance to cephalosporins is necessary.

Neisseria gonorrhoeae isolates collected following failed treatment for gonorrhea with oral extended-spectrum cephalosporins (ESC) possessed a mosaic *penA* gene that was associated with increased MICs of these antibiotics (1, 30). Penicillin-binding protein 2 (PBP2), encoded by *penA*, is the major target site for ESC in gonococci, and altered PBP2, when accompanied by polymorphisms in *mtrR* and *porB1b*, has a demonstrated role in gonococcal resistance to ESC (4, 7, 31). A small number of subtypes of *N. gonorrhoeae* with a mosaic PBP2 spread widely in Japan (4), but considerable sequence type (ST) heterogeneity in geographically and temporally diverse populations of mosaic PBP2-containing gonococci (mPBP2GC) was recorded following the dissemination of these subtypes in the Asia-Pacific region (20, 26). Small numbers of mPBP2GC with limited ST distribution were found in San Francisco (12), and "cefixime-resistant" gonococci from Taiwan (27) were of the same ST, 835, as mPBP2GC from Hong Kong responsible for treatment failures with ceftibuten (8).

We examined gonococci from the Neisseria Reference Laboratory (NRL) in Sydney, Australia (6), isolated in 2007 and 2008 for the presence of a mosaic PBP2 (24) and compared the findings with our earlier data on mPBP2GC (20, 24, 26). MICs of ceftriaxone, penicillin, and ciprofloxacin (17) for these isolates, *N. gonorrhoeae* multiantigen STs (NG-MASTs) (10), and auxotypes (5, 6) of the isolates were determined. Control strains (22) included the mPBP2GC strain WHO K and a Hong Kong isolate of ST835 (8), kindly provided by Janice Lo.

A sequential sample comprising the last 60 NRL isolates from 2007 and the first 60 isolates from 2008 contained no mPBP2GC from 2007 but six in the 2008 strains that were of NG-MASTs 3158 ($n = 4$) and 1407 ($n = 2$) (Table 1). The mPBP2GC were found exclusively among isolates with an extended phenotype defined by chromosomally mediated resistance to penicillin and ciprofloxacin (17), nonsusceptibility to ceftriaxone (defined here by an MIC of 0.016 μg/ml or more), and proline auxotrophy (CMRP/CipR/CefNS/Pro$^-$). mPBP2GC from earlier studies (20, 24) and both the control isolates also had this phenotype.

All gonococci of the CMRP/CipR/CefNS/Pro$^-$ phenotype identified among the *N. gonorrhoeae* isolates received by the NRL in 2007 ($n = 978$) and 2008 ($n = 835$) were then examined for the presence of a mosaic PBP2, and the NG-MASTs were determined. In 2007, there were 64 nonduplicate strains (6.5% of all isolates) with this phenotype, of which 15 (1.5% of all isolates) contained a mosaic PBP2 and were of STs 1407 ($n = 9$), 3158 ($n = 4$), 3380 ($n = 1$), and 3505 ($n = 1$) (Table 1). In 2008, a total of 114 gonococci (13.5%), including those examined in the initial sample, had the nominated phenotype, of which 85 nonduplicate strains (10.3%) contained a mosaic PBP2 allele. These strains comprised 10 STs: 1407 ($n = 35$), 3149 ($n = 26$), and 3158 ($n = 13$); 3159, 3161, 3168, and 3294 (n, 2 strains each); and 2955, 3380, and 3381 (n, 1 strain each). The STs of gonococci that had the extended phenotype but lacked a mosaic PBP2 were distinct from the STs of the mPBP2GC.

These longitudinal data provided significant insights into the population dynamics of mPBP2GC. Substantial increases in the numbers and diversity of mPBP2GC isolated in Sydney emerged during the study period, consistent with earlier reports regarding the spread of mPBP2GC (4) and quinolone-resistant *N. gonorrhoeae* (15, 18). Changes in mPBP2GC STs in 2007 and 2008 that saw the loss of ST835 and the emergence of STs 1407, 3149, and 3158 (Table 1) were also typical of the dynamics of the spread of gonococcal subtypes (6, 9, 13, 15, 23). Rapid increases in resistant gonococci may also occur through their spread within sexual networks (2, 15, 18).

Ceftriaxone MICs for mPBP2GC ranged between 0.016 and 0.06 μg/ml (Tables 1 and 2). In 2007 and 2008, ceftriaxone MICs for non-mPBP2GC ranged between 0.004 and 0.12 μg/ml (data not shown). Almost all the mPBP2GC in the

* Corresponding author. Mailing address: WHO Collaborating Centre for STD, Microbiology Department, The Prince of Wales Hospital, Randwick, Sydney 2031, Australia. Phone: 61293829079. Fax: 61293984275. E-mail: j.tapsall@unsw.edu.au.

▽ Published ahead of print on 16 November 2009.

TABLE 1. Comparison of mosaic PBP2-containing *N. gonorrhoeae* strains found in Sydney, Australia, in 2007 and 2008 with those detected in 2001 to 2005[a]

Yr of isolation	No. of isolates with mosaic PBP2/total no. of isolates (%) in:		Total no. (%) of isolates with mosaic PBP2	NG-MAST	No. of isolates with indicated ST	Ceftriaxone MIC (μg/ml)
	Sequential sample	Selected sample				
2001–2005		13 (NA)	13 (NA)	835	4	0.06
				1414	3	0.06
				1424	2	0.06
				2453	2	0.06
				326	1	0.06
				1677	1	0.06
2007	0/60 (0)	15 (1.5)	15 (1.5)	1407	9	0.016–0.06
				3158	4	0.016–0.03
				3380	1	0.03
				3505	1	0.06
2008	6/60 (10)	79 (73)	85 (10.3)	1407	35	0.016–0.06
				3149	26	0.016–0.06
				3158	13	0.016–0.03
				3159	2	0.03
				3161	2	0.03
				3168	2	0.03
				3294	2	0.016
				2955	1	0.03
				3381	1	0.016
				3380	1	0.03

[a] Data for isolates from 2001 to 2005 are from reference 20. A sequential sample of 60 nonduplicate isolates collected in 2007 and 2008 and a selected sample of isolates with an extended phenotype collected in 2007 and 2008 were examined for the presence of mPBP2GC. STs were determined by NG-MAST genotyping (10). All isolates listed in the table had the Pro[minus] (proline-requiring) auxotype. NA, not applicable.

present study, as those in other studies, were resistant to penicillin and ciprofloxacin (7, 8, 19, 26). The highest ceftriaxone MICs associated with a mosaic PBP2 allele in *N. gonorrhoeae* were previously reported to occur in the presence of other gene polymorphisms, including some that are as yet undetermined (31). Ceftriaxone MICs may remain relatively unaltered in the presence of a mosaic PBP2 alone (4, 12), and other mosaic or "partial mosaic" PBP2 lesions confer little or no increase in ceftriaxone MICs (14, 25). Additionally, non-mosaic-based *penA* alterations, e.g., the widely distributed A501V mutation, also confer a wide range of increases in MICs of ceftriaxone (11, 26). Further, the *in vitro* effects of the mosaic PBP2 allele on susceptibilities to oral ESC and injectable ceftriaxone differ (31). The clinical relevance of increased MICs of ceftriaxone is at present unclear, and better correlates of clinical outcomes and *in vitro* susceptibility to ESC are therefore urgently required (19, 29). However, these considerations also need to account for the significant differences in ceftriaxone doses in current standard regimens (16) and for confounding effects on treatment outcomes of infections at different anatomical sites (21, 28).

TABLE 2. Distribution of ceftriaxone MICs for 100 mosaic PBP2-containing *N. gonorrhoeae* isolates collected in Sydney, Australia, in 2007 and 2008

Yr	No. of isolates for which ceftriaxone MIC (μg/ml) was:			Total
	0.016	0.03	0.06	
2007	2	9	4	15
2008	20	61	4	85
Total	22	70	8	100

Altered susceptibility of gonococci to ESC is now recognized as a major concern which calls for enhanced surveillance of gonococcal resistance (3, 19, 28, 30). The findings reported here in regard to the local spread of one important genetic change associated with decreased ceftriaxone susceptibility reinforce these recommendations. However, *in vitro* detection of the phenomenon remains problematic (19). Molecular systems proposed for the direct detection of the mosaic PBP2 allele in clinical samples (12) are necessarily restricted to this nonexclusive mechanism and thus of limited clinical application. Careful ongoing appraisals of assay specificity and sensitivity would be required because *penA* mosaicisms are derived from commensal *Neisseria* spp. and continually change. The finding here that all mPBP2GC were of a particular phenotype is unlikely to be of practical assistance in their detection because of the ubiquity of the various phenotypic markers involved. About 25% of all NRL isolates in 2007 and 2008 were proline auxotrophs (data not shown). Thus, the current interim recommendation (19) that phenotypic examination of isolates for ESC resistance by MIC determination or some other form of validated susceptibility testing, e.g., disc screening, using recommended controls (22) would thus seem to be appropriate at present to ensure high surveillance standards and to enable reliable application of any findings to the optimization of treatment regimens for gonorrhea.

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