Mosaic Penicillin-Binding Protein 2 in *Neisseria gonorrhoeae* Isolates Collected in 2008 in San Francisco, California

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Using a real-time PCR assay specific for a mosaic *penA* allele that has been associated with oral cephalosporin resistance in Asia, 54 available *Neisseria gonorrhoeae* isolates collected in San Francisco, CA, from January to October 2008 were analyzed. Five isolates tested positive for the mosaic *penA* gene by real-time PCR. DNA sequencing revealed two mosaic *penA* alleles (SF-A and SF-B). Isolates with SF-A and SF-B alleles possessed elevated MICs for the oral cephalosporins cefpodoxime and cefixime.

Isolates with decreased susceptibility to third-generation cephalosporins, particularly oral cephalosporins, have emerged in Asia, Australia, and elsewhere (1, 2, 4, 5, 7, 11, 12). Initial reports linked this decreased susceptibility to oral cephalosporins to an altered, mosaic penicillin-binding protein 2 (PBP2) encoded by the *penA* gene characterized by multiple genetic changes with segments that are nearly identical to the homologous regions of the *penA* genes of related commensal *Neisseria* species (2, 3). Recently, a real-time PCR assay has been developed for detection of this mosaic *penA* gene (9). We used this real-time PCR assay to determine whether the mosaic *penA* allele is present in clinical isolates of *Neisseria gonorrhoeae* in San Francisco, CA.

Available for testing were 54 *Neisseria gonorrhoeae* isolates collected during the period of January to October 2008 from male patients with symptomatic urethritis. Of these, five isolates (SM-1, SM-2, SM-3, SM-4, and SM-5) were found to be reactive by real-time PCR for the mosaic *penA* gene. An assortment of 100 *N. gonorrhoeae* isolates collected in San Francisco from 2002 to 2006 were also analyzed with the same real-time PCR assay, and none of those specimens were found to be reactive.

In order to confirm the presence of a mosaic *penA* allele in the five real-time PCR-reactive isolates, the *penA* genes of these isolates were analyzed by DNA sequencing. The primers used for the amplification and sequencing of the *penA* genes are shown in Table 1. As shown in Fig. 1, two distinct *penA* alleles were found in the five PCR-positive isolates. These two alleles were designated “SF-A” (from three isolates, SM-1, SM-2, and SM-3) and “SF-B” (from two isolates, SM-4 and SM-5). We compared these two novel *penA* alleles (SF-A and SF-B) to both a wild-type *penA* allele (GenBank accession no. M32091) and the mosaic *penA* allele associated with oral cephalosporin resistance in Asia and Australia (GenBank accession no. AB071984) (2). Neither of the San Francisco mosaic alleles was found to contain all of the mutations associated with the previously described mosaic *penA* alleles. The translated amino acid sequence of SF-A is identical to that of the mosaic *penA* allele for the first 549 amino acid residues. From amino acid 550 to the end of the translated sequence, the SF-A allele is identical to the reference wild-type allele. The translated amino acid sequence of SF-B possesses greater dissimilarity to the mosaic *penA* allele than SF-A. Although it contains many of the mosaic-associated mutations, SF-B lacks codons encoding amino acids 279, 285, 288, and 291 associated with the mosaic *penA* allele. Additionally, SF-B lacks all of the codons specific to the mosaic *penA* allele, from codon 388 to the end of the translated amino acid sequence. Interestingly, SF-B possessed unique amino acid residues distinct from those encoded by the wild type, SF-A, and the mosaic *penA* allele (GenBank accession no. AB071984) at residues 35, 42, 70, 230, and 515.

The susceptibilities of the five real-time PCR-positive isolates to certain third-generation cephalosporins were evaluated. Results of agar dilution susceptibility testing were available through the Gonococcal Isolate Surveillance Program for ceftriaxone. Isolates SM-1 and SM-2 each possessed a ceftriaxone MIC of 0.06 μg/ml, and isolate SM-3 had a ceftriaxone MIC of 0.03 μg/ml (Table 2). SM-4 and SM-5 each possessed a ceftriaxone MIC of ≤0.008 μg/ml. All five isolates were evaluated with regard to their susceptibilities to the oral third-generation cephalosporins, cefixime and cefpodoxime, by using agar dilution (protocol available at http://www.cdc.gov/std/gisp/protocol2006_web_version_rev12_2007.pdf). The five *penA* mosaic isolates were compared with two isolates from San Francisco that were determined by real-time PCR and *penA* sequencing to possess nonmosaic *penA* alleles (SW-1 and SW-2). Isolates with the SF-A *penA* allele (SM-1, SM-2 and SM-3) had MICs for both cefixime and cefpodoxime that were notably higher than those for strains found containing nonmosaic *penA* alleles. SF-B-containing isolates possessed modestly elevated MICs to cefpodoxime, while possessing little or no elevation in MICs to cefixime compared with strains with nonmosaic *penA* alleles. Further investigation of the five isolates with...
mosaic penA alleles included N. gonorrhoeae multiantigen sequence typing (NG-MAST) of these five strains using a previously published method (6). Four of the five isolates possessed the NG-MAST sequence type 1407 (Table 2). SM-2 possessed sequence type 1513.

These data demonstrate the presence of two previously un-described penA alleles (SF-A and SF-B) within N. gonorrhoeae associated with elevated cephalosporin MICs in San Francisco. These alleles resemble the mosaic penA alleles previously associated with cephalosporin resistance in Asia and Australia (2, 5, 11). Although the exact relationship between the mosaic penA and the development of decreased susceptibility to cephalosporins is not completely understood, these findings are concerning because they might indicate the impending development and spread of isolates in the United States that are associated with cephalosporin resistance.

<table>
<thead>
<tr>
<th>Type of primer</th>
<th>PCR primer sequence</th>
<th>Reference</th>
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<tbody>
<tr>
<td>Fragment 1 of penA</td>
<td>5'-GCATCAGGATATAAATAACGGAAG-3' 5'-TGCAGCCGGTGGAATTAATTAGTA-3'</td>
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<tr>
<td>Fragment 2 of penA</td>
<td>5'-TCGGCAGATACCTTTATGGTGGAACT-3' 5'-CACGGGCTCACTCTGGCTGAC-3'</td>
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<td>Sequencing penA</td>
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<td>8 3 3 2 3</td>
</tr>
</tbody>
</table>

**FIG. 1.** Comparison of translated amino acid sequences for specimens with mosaic penA alleles detected in this study (SF-A and SF-B) to the wild-type sequence (GenBank accession no. M32091) and a previously described mosaic penA allele (GenBank accession no. AB071984).
resistant to cefalosporins, particularly oral third-generation cefalosporins.

Of the two newly described alleles, SF-A most resembles the previously described mosaic allele associated with strains resistant to oral third-generation cefalosporin. SF-A also has 99% similarity to the penA allele from an N. gonorrhoeae isolate with cefuroxime (a second-generation cefalosporin) resistance (GenBank accession no. DQ335216) (J. E. Corkill, unpublished data). Takahata et al. identified three amino acid alterations important for oral cefalosporin resistance, I312M, V316T, and G545S, among cephalosporin-resistant isolates in Japan (10). SF-A and SF-B both have I312M and V316T, but only SF-A has G545S. Both isolates bearing the SF-B penA allele were found to have lower cefalosporin MICs than isolates with the SF-A allele. Interestingly, isolates with either the SF-A or the SF-B alleles had elevated cefepoxide MICs, while SF-A had a higher MIC for cefixime than SF-B. Both the penA allele type and the presence of I312M, V316T, and G545S appeared to correlate with the degree to which cefalosporin MICs were elevated. These data support previous reports demonstrating the importance of these codons in cefalosporin resistance (10).

These results raise several questions for future study, including the determination of whether isolates with SF-A or SF-B penA alleles are associated with treatment failure. The primary method of N. gonorrhoeae detection in our laboratory includes nucleic acid amplification testing, which does not involve the collection of viable organisms for isolation. We are currently working to develop an assay that will allow us to screen such specimens for the presence of mosaic penA alleles in an effort to more carefully define the prevalence of these alleles in our setting and identify patients for close follow-up after treatment.

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REFERENCES


