AAC Accepts, published online ahead of print on 22 June 2009
Antimicrob. Agents Chemother. doi:10.1128/AAC.00406-09




AAC00406-09 Version 2

# Identification of Mosaic *Neisseria gonorrhoeae* Penicillin-Binding Protein 2 — 2008, San Francisco, California

Running title: *N. gonorrhoeae* mosaic PBP2 in San Francisco

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Body Word Count: 1,014

**Abstract:**

**Abstract Word Count: 74**

Using a real-time PCR assay specific for a mosaic *penA* allele that has been associated with oral cephalosporin resistance in Asia, fifty-four available *Neisseria gonorrhoeae* isolates collected in San Francisco from January–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 MIC 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) coded 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 (8). 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.

Fifty-four *Neisseria gonorrhoeae* isolates collected during January–October 2008 from male patients with symptomatic urethritis were available for testing. Of these, 5 isolates were found to be reactive by real-time PCR for the mosaic *penA* gene (SM-1, SM-2, SM-3, SM-4 and SM-5). An assortment of 100 *N. gonorrhoeae* isolates from 2002–2006 collected in San Francisco 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 2. As shown in Figure 1, two distinct *penA* alleles were found in the five PCR-positive isolates. These two alleles were designated “SF-A” (3 isolates:, SM-1, SM-2, and SM-3) and “SF-B” (2 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 # M32091) and the mosaic *penA* allele associated with oral

cephalosporin resistance in Asia and Australia, AB071984 (2). Neither of the San Francisco mosaic alleles was found to contain all of the mutations associated with AB071984. The translated amino acid sequence of SF-A is identical to that of AB071984 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 AB071984 than SF-A. Although containing many of the mosaic-associated mutations, SF-B lacks AB071984-associated codons at amino acids 279, 285, 288 and 291. Additionally, SF-B lacks all of the AB071984-specific codons from codon 388 to the end of the translated amino acid sequence. Interestingly, SF-B possessed unique amino acid residues distinct from wild type, SF-A and 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 (GISP) for ceftriaxone. Isolates SM-1 and SM-2 each possessed a ceftriaxone MIC of 0.06 ug/mL, and isolate SM-3 had a ceftriaxone MIC of 0.03 µg/mL (Table 1). SM-4 and SM-5 each possessed ceftriaxone MICs of ≤0.008 µg/mL. All five isolates were evaluated with regard to their susceptibilities to the oral third-generation cephalosporins, cefixime and cefpodoxime, using agar dilution (protocol available at http://www.cdc.gov/std/gisp/protocol2006_web_version_rev12_2007.pdf Accessed 05/01/2008). The five *penA* mosaic isolates were compared with two isolates from San Francisco determined by real-time PCR and *penA* sequencing to possess non-mosaic *penA* alleles (SW-1 and SW-2). Isolates with the SF-A *penA* allele (SM-1, SM-2 and SM-3) had MIC values for both cefixime and cefpodoxime that were notably higher than the values for strains found containing wild-type

*penA* alleles. SF-B-containing isolates possessed modestly elevated MIC values to cefpodoxime, while possessing little or no elevation in MIC to cefixime compared with strains with non-mosaic *penA* alleles. Further investigation of the five isolates with mosaic *penA* alleles included multi-antigen sequence typing of these five strains (NG-MAST) using a previously published method (6) Four of the five isolates possessed the NG-MAST sequence type 1407 (Table 1). SM-2 possessed sequence type 1513.

These data demonstrate the presence of two previously undescribed *penA* alleles (SF-A and SF-B) within *Neisseria gonorrhea* 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 impending development and spread of isolates in the United States resistant to cephalosporins, particularly oral third-generation cephalosporins.

Of the two newly described alleles, SF-A most resembles the previously described mosaic allele associated with strains resistant to oral third-generation cephalosporin. SF-A also has 99% similarity with *penA* allele from an *N. gonorrhoeae* isolate with cefuroxime (a second generation cephalosporin) resistance (Gen Bank # DQ335216, unpublished submission, JE Corkill). Osaka et al identified three amino acid alterations important for oral cephalosporin resistance, I312M, V316T, and G545S among cephalosporin resistant isolates in Japan (9). 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 cephalosporin MICs than isolates with the SF-A allele. Interestingly,

isolates with the either the SF-A or the SF-B alleles had elevated cefpodoxime 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 cephalosporin MICs were elevated. These data support previous reports demonstrating the importance of these codons in cephalosporin resistance.(9)

These results raise several questions for future study including determining 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.

**Acknowledgments:**

This study is funded in part by U.S. Public Health Service T32 Grant AI007641-06A2.

**Figure Legends:**

Figure 1: Comparison of *penA* gene sequences for specimens with mosaic *penA* alleles detected in this study (SF-A and SF-B) to wild type sequence (GenBank #M32091) and a previously described mosaic *penA* allele (GenBank #AB071984).

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Table 2. Oligonucleotides utilized for amplification and sequencing of *penA* gene

| PCR Primers: |
|---|
| Fragment 1 of *penA* gene: |
| 5'-GCATCAGGATAATAATAACGAGAAG-3' * |
| 5'-TGTAAGGCAGGGTATTGAAT-3' |
| Fragment 2 of *penA* gene: |
| 5'-TCGGGCAATACCTTTATGGTGGAACAT-3' ** |
| 5'-CAGCCAAAGGGGTTAACTTGCTGAAC-3' ** |
| **Sequencing primers:** |
| 5'-GCATCAGGATAATAATAACGAGAAG-3' * |
| 5'-TGTAAGGCAGGGTATTGAAT-3' |
| 5'-AACCTTCCTGACCTTTGCCGTC-3' † |
| 5'-AAAACGCCATTACCCGAAGGG-3' † |
| 5'-CAGCCAAAGGGGTTAACTTGCTGAAC-3' ** |
| 5'-AATTGAGCCTGCTGCAATTGGC-3' † |
| 5'-GTTGGATGCCCGTACTGGG-3'. †† |

* Reference 10
** Reference 2
† Reference 3
†† Reference 8

Table 1. Description of *Neisseria gonorrhoeae* isolates with mosaic *penA* alleles in San Francisco, CA, 2008

| Isolate | Agar Dilution | | | Mosaic PCR[a] | *penA* allele | NG-MAST ST[b] |
|---|---|---|---|---|---|---|
| | Cro (µg/mL) | Cfm (µg/mL) | Cpd (µg/mL) | | | |
| SM-1 | 0.060 | 0.25 | 1.0 | (+) | SF-A | 1407 |
| SM-2 | 0.060 | 0.125 | 0.5 | (+) | SF-A | 1513 |
| SM-3 | 0.030 | 0.25 | 1.0 | (+) | SF-A | 1407 |
| SM-4 | ≤0.008 | ≤0.008 | 0.06 | (+) | SF-B | 1407 |
| SM-5 | ≤0.008 | 0.015 | 0.25 | (+) | SF-B | 1407 |
| SW-1 | ≤0.008 | ≤0.008 | 0.015 | (–) | NM | 242* |
| SW-2 | ≤0.008 | 0.015 | 0.03 | (–) | NM | 2992 |

NM: Non-mosaic *penA* sequence, Cro: ceftriaxone, Cfm: cefixime, Cpd: cefpodoxime, NG-MAST: *Neisseria gonorrhoeae* multiantigen sequence typing, ST: sequence type

[a] reactivity with real-time PCR (8)

[b] sequence type (determined by NG-MAST (6))

* isolate not previously identified by NG-MAST database; 99% similarity to ST242

**Figure 1.**