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Further Questions Regarding the Role of Mosaic *penA* Sequences in Conferring Reduced Susceptibility to Ceftriaxone in *Neisseria gonorrhoeae*▽

With treatment options for gonorrhea diminishing, the isolation of *Neisseria gonorrhoeae* strains with reduced susceptibilities to the newer cephalosporins represents a worrying trend that requires monitoring and investigation. Mutations in the *penA* gene encoding the penicillin-binding protein 2 (PBP2) are a major contributor to the emergence of chromosomally mediated resistance to β-lactam antibiotics in *N. gonorrhoeae*. Recent studies have identified mosaic-like structures of the PBP2 in clinical isolates of *N. gonorrhoeae* with decreased susceptibilities to both cefixime and ceftriaxone (1, 2, 4, 5). These mosaic sequences are thought to have evolved from recombination events involving *penA* gene sequences from several *Neisseria* species, including *N. perflava*, *N. sicca*, and *N. cinerea* (1, 3). Thus, the reduced susceptibility to the newer cephalosporins is attributed to the acquisition of genetic material from resistant commensal *Neisseria* spp. by originally susceptible gonococci.

We have identified eight clinical isolates of *N. gonorrhoeae* harboring mosaic PBP2 sequences as part of an ongoing study investigating reduced susceptibility to ceftriaxone in Australian gonococcal isolates (6). DNA sequencing of the complete *penA* genes from these isolates revealed that seven of these strains possessed PBP2 amino acid sequences (Fig. 1, mosaic A) that were identical to the mosaic PBP2 sequence previously described by Ito et al. (2). All seven strains had reduced susceptibility to ceftriaxone (MIC = 0.06 mg/liter). The remaining strain possessed a variation of the mosaic sequence (Fig. 1, mosaic B). Briefly, the first 430 amino acids of the mosaic B sequence in this strain were nearly identical to the GenBank reference sequence M32091, except for an additional Asp-345A codon, whereas the remaining amino acids were similar to those in the mosaic sequence described by Ito et al. but with three substitutions. Notably, the strain possessing this variant mosaic B sequence was fully susceptible to ceftriaxone (MIC ≤0.008 mg/liter).

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                    10         20         30         40         50         60
M32091       MLIKSEYKPR MLPKEEQVKK PMTSNGRISF VLMAMAVLFA CLIARGLYLQ TVTYNFLKEQ
Mosaic A(7)  .......... .......... .......... .......... .......... ..........
Mosaic B(1)  .......... .......... .......... .......... .......... ..........
                    70         80         90        100        110        120
M32091       GDNRIVRTQA LPATRGTVSD RNGAVLALSA PTESLFAVPK DMKEMPSAAQ LERLSELVDV
Mosaic A     .......... .......... .......... .......... E......... ..........
Mosaic B     .......... .......... .......... .......... .......... ..........
                   130        140        150        160        170        180
M32091       PVDVLRNKLE QKGKSFIWIK RQLDPKVAEE VKALGLENFV FEKELKRHYP MGNLFAHVIG
Mosaic A     .......... .......... .......... .........A .......... ..S.......
Mosaic B     .......... .......... .......... .......... .......... ..........
                   190        200        210        220        230        240
M32091       FTDIDGKGQE GLELSLEDSL YGEDGAEVVL RDRQGNIVDS LDSPRNKAPQ NGKDIILSLD
Mosaic A     .......... .......... HAGE...... ...E...... .......... ..........
Mosaic B     .......... .......... .......... .......... .......... ..........
                   250        260        270        280        290        300
M32091       QRIQTLAYEE LNKAVEYHQA KAGTVVVLDA RTGEILALAN TPAYDPNRPG RADSEQRRNR
Mosaic A     .......... .......... .......... ........V. ....E..K.. Q.........
Mosaic B     .......... .......... .......... .......... .......... ..........
                   310        320        330        340        350        360
M32091       AVTDMIEPGS AIKPFVIAKA LDAGKTDLNE RLNTQPYKIG PSPVR-DTHV YPSLDVRGIM
Mosaic A     .......... .M...T.... ..S..V.ATD TF..L..... SAT.Q-.... ..T.......
Mosaic B     .......... .......... .......... .......... .....D.... ..........
                   370        380        390        400        410        420
M32091       QKSSNVGTSK LSARFGAEEM YDFYHELGIG VRMHSGFPGE TAGLLRNWRR WRPIEQATMS
Mosaic A     .......... ...M.TPK.. .....D..V. .......... ......S... .QK.......
Mosaic B     .......... .......... .......... .......... .......... ..........
                   430        440        450        460        470        480
M32091       FGYGLQLSLL QLARAYTALT HDGVLLPLSF EKQAVAPQGK RIFKESTARE VRNLMVSVTE
Mosaic A     .......... .......V.. ...E...V.. .......K.. .VI.A...KK ..E.......
Mosaic B     .......... .......V.. ...E...V.. .......K.. .VI.A...KK ..E.......
                   490        500        510        520        530        540
M32091       PGGTGTAGAV DGFDVGAKTG TARKFVNGRY ADNKHVATFI GFAPAKNPRV IVAVTIDEPT
Mosaic A     A......... .......... ....L..... V.Y....... .......... ..........
Mosaic B     .......... .......... ....L..... V.Y....... .......... ..........
                   550        560        570        580
M32091       AHGYYGGVVA GPPFKKIMGG SLNILGISPT KPLT-AAAVK TPS
Mosaic A     .N...S...T ..V..QV... ......V... ....NV.... ...
Mosaic B     .N.......T ..V..LV... ......V... ....NV.... ...
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FIG. 1. Mosaic PBP2 amino acid sequences identified in the *N. gonorrhoeae* isolates in this study. The sequences are aligned with a GenBank *N. gonorrhoeae* sequence (accession number M32091). The number of isolates with each pattern is indicated in parentheses.

Takahata et al. recently demonstrated that particular amino acid substitutions in the mosaic PBP2 (G545S, I312M, V316T) were responsible for reduced susceptibility to cefixime in Japanese gonococci (4). All three substitutions were present in our seven strains with the mosaic A sequence but were absent in the variant mosaic B sequence, suggesting that they may also have a role in altered ceftriaxone susceptibility. However, Takahata et al. also found that these substitutions and several others, although impinging on cefixime MICs, did not greatly affect ceftriaxone susceptibility (4). This was also shown in transformation experiments conducted by Takahata et al. in which full-length mosaic *penA* sequences conferred only very minor reductions in ceftriaxone susceptibility to a susceptible strain and so could not fully explain the reduced susceptibility of the donor strain (4).

The demonstration that full susceptibility to ceftriaxone is possible in the presence of this variant mosaic B sequence has been, thus far, detected only in a single isolate. However, this finding adds weight to those of Takahata et al. (4) by suggesting that other mosaic-associated mutations located within the transpeptidase domain have but little impact on ceftriaxone susceptibility. This brings into question the extent of the contribution of mosaic sequences in conferring reduced susceptibility to ceftriaxone in *N. gonorrhoeae*. These findings would need to be confirmed by additional evidence such as the detection of additional ceftriaxone-susceptible gonococci with mosaic B or similar sequences and adjunctive evidence derived from transformation experiments. Other mechanisms that may confer reduced susceptibility to ceftriaxone, including mutations in the *ponA* (PBP1) gene (5), have been proposed, and alternatively, as yet undetected second-site mutations may also be responsible for reversion to the susceptibility patterns reported here. Overall, the data currently available suggest that the role mosaic *penA* sequences play in reduced susceptibility to ceftriaxone, as opposed to cefixime, needs careful and further examination.

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