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Interspecies Recombination in Type II Topoisomerase Genes Is Not a Major Cause of Fluoroquinolone Resistance in Invasive *Streptococcus pneumoniae* Isolates in the United States

Mathias W. R. Pletz,[1,2]* Lesley McGee,[1,2] Bernard Beall,[2] Cynthia G. Whitney,[2] and Keith P. Klugman[1,2]

Department of International Health, Rollins School of Public Health, and Division of Infectious Diseases, School of Medicine, Emory University,[1] and Division of Bacterial and Mycotic Diseases, National Center for Infectious Diseases, Centers for Disease Control and Prevention,[2] Atlanta, Georgia

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Mutations in the topoisomerase type II enzymes account for fluoroquinolone resistance in *Streptococcus pneumoniae*. These mutations can arise spontaneously or be transferred by intraspecies or interspecies recombination, primarily with viridans streptococci. We analyzed the nucleotide sequences of the quinolone resistance-determining regions of 49 invasive levofloxacin-resistant pneumococcal isolates and did not find any evidence for interspecies recombination.

Because of an increase in the incidence of macrolide and beta-lactam resistance among pneumococcal strains, fluoroquinolones are now included among the choices for first-line therapy in clinical guidelines for treatment of pneumonia (8). Resistance to fluoroquinolones in pneumococci is caused by efflux and/or by mutations in the quinolone resistance-determining regions (QRDR) of the genes coding for type II topoisomerase enzymes: DNA gyrase and topoisomerase IV. Antimicrobial resistance-conferring mutations can arise spontaneously or be transferred by the integration of foreign genetic material.

In contrast to many other bacteria, *S. pneumoniae* is naturally transformable in vivo, and it has been demonstrated that the alterations in the penicillin-binding proteins (PBP) conferring beta-lactam resistance are frequently a result of genetic exchange with commensal viridans streptococci of the oral flora (3, 5).

Viridans streptococci are rarely pathogenic in an immune-competent host but exhibit high rates of resistance to different classes of antibiotics even in healthy adults (7). They can be considered a pool for resistance genes, and their genetic relatedness to pneumococci increases the likelihood of successful interspecies recombination or transformation.

Except for some foci, fluoroquinolone resistance rates are still low (1 to 3%) among pneumococci (10) but can be as high as 23% in viridans streptococci (11). There is concern that the uptake of DNA pieces from viridans streptococci containing the QRDR mutations might increase the prevalence of fluoroquinolone resistance in pneumococci. Spontaneous mutations conferring fluoroquinolone resistance in pneumococci are relatively rare, with frequencies of 10^{-7} to 10^{-8} (9). In vitro studies demonstrated that transformation of fluoroquinolone resistance from viridans streptococci to pneumococci is much more likely than spontaneous mutation (6). However, to date only a few studies have been published addressing the frequency of horizontal transfer of fluoroquinolone resistance determinants between pneumococci and viridans streptococci in clinical isolates (1, 2).

The aim of this study was to determine the proportion of horizontal transfer of fluoroquinolone resistance determinants in 49 of 50 recently described invasive, levofloxacin-resistant (MIC $\geq$ 8 mg/liter) pneumococcal isolates (10).

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The isolates were collected during 1998 to 2002 by the Centers for Disease Control and Prevention's (CDC) Emerging Infections Program Network, through its Active Bacterial Core surveillance (ABCs). QRDRs of *gyrA*, *gyrB*, *parC*, and *parE* were amplified and sequenced as described previously (10). The National Center for Biotechnology Information Blast program (www.ncbi.nlm.nih.gov/BLAST/bl2seq/bl2.html) was used for comparison of the QRDR nucleotide sequences to the DNA sequences of wild-type *S. pneumoniae* R6 (gi15902044). With the exception of one isolate, all strains exhibited at least one mutation known to confer fluoroquinolone resistance. Compared to R6, all isolates showed relatively little nucleotide variation of $\leq$1% (the nucleotide changes conferring resistance were subtracted) over the entire length of the sequences (*gyrA*, 345 bp; *gyrB*, 402 bp; *parC*, 330 bp; *parE*, 258 bp). This is consistent with the intraspecies genomic variation described for *S. pneumoniae* (5). Considering the high degree of similarity between the QRDR of pneumococci and viridans streptococci (4), the integration of small DNA pieces might not result in an obvious increase of nucleotide variation. Therefore, all QRDR sequences and the R6 reference sequence were aligned using Megalign (DNAStar). The alignment was carefully screened for nucleotide changes, particularly in the flanking regions of the fluoroquinolone resistance-conferring mutations.

* Corresponding author. Mailing address: Department of International Health, Rollins School of Public Health, Emory University, 1518 Clifton Rd., Atlanta, GA 30322. Phone: (404) 727-3984. Fax: (404) 712-8419. E-mail: mpletz@sph.emory.edu.

We did not find any nucleotide diversity in flanking regions of the mutations or any mosaic pattern in the QRDRs of any of the four genes. This was also true for two isolates with rare mutations in *gyrB* (isolates 349:D435N and 344:G486N). In a recently published paper, Balsalobre et al. describe five ciprofloxacin-resistant pneumococcal isolates that exhibited evidence for a viridans streptococcal origin of the resistance-conferring mutations (1). They describe an N91D amino acid exchange in *parC* and an S114G exchange in *gyrA* that can be caused by interspecific exchange with viridans streptococci. However, we did not see these mutations in our isolates.

In conclusion, we did not find any evidence for interspecies recombination in the QRDR of 49 levofloxacin-resistant invasive pneumococcal isolates. This means that, in our studied population, only spontaneous mutations and subsequent clonal spread (10) give rise to levofloxacin resistance.

One limitation of our study is the unknown frequency of fluoroquinolone-resistant viridans streptococci in the population covered by the CDC′s ABCs study. If this frequency is very low, then there would be only a limited source of resistance-conferring DNA. However, our results are consistent with those of a Canadian study (2) which identified interspecies recombination only in 1 out of 71 ciprofloxacin-resistant pneumococcal isolates. A Spanish group found a larger proportion (5 out of 46 ciprofloxacin-resistant isolates) (1). Whereas the first analysis included primarily invasive and some respiratory isolates, the latter study described respiratory isolates only. Because we investigated only invasive isolates, the origin of our isolates may account for the lack of interspecies recombination observed here. Interspecies recombination takes place in the nasopharynx, which is colonized by both pneumococci and viridans streptococci. One could speculate that the antecedent nasopharyngeal colonization period for invasive strains is shorter than that of respiratory isolates. Therefore, there may be less time for interspecies recombination in invasive isolates.

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