Resistance to Multiple Fluoroquinolones in a Clinical Isolate of *Streptococcus pyogenes*: Identification of gyrA and parC and Specification of Point Mutations Associated with Resistance

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A strain of *Streptococcus pyogenes* resistant to multiple fluoroquinolones was isolated from the blood of an immunocompromised patient. Resistance to fluoroquinolones in *S. pyogenes* has not been previously studied. Compared to 10 sensitive strains of *S. pyogenes*, the fluoroquinolone-resistant clinical isolate of *S. pyogenes* presented point mutations in gyrA, predicting that serine-81 was changed to phenylalanine and that methionine-99 was changed to leucine, and in parC, predicting that serine-79 was changed to tyrosine. The mechanism of fluoroquinolone resistance in this isolate of *S. pyogenes* appears to be analogous to previously reported mechanisms for *Streptococcus pneumoniae*.

Development of penicillin resistance in *Streptococcus pneumoniae* has prompted a search for alternative effective therapy for infections caused by this organism (4, 12, 13). Newer fluoroquinolones have demonstrated excellent activity against penicillin-sensitive and penicillin-resistant *S. pneumoniae* strains. However, with the increasing use of fluoroquinolones, there have been reports of emergence of *S. pneumoniae* isolates with resistance to this class of antibiotics (3, 7, 8, 12, 16). In contrast to *S. pneumoniae*, *Streptococcus pyogenes* remains uniformly sensitive to penicillin despite intensive exposure to the agent, and penicillin remains the drug of choice for infections caused by *S. pyogenes* (10). For this reason, susceptibility testing of *S. pyogenes* isolates is not routinely performed. Resistance to fluoroquinolones among *S. pyogenes* isolates has not been reported previously, though slightly increased MICs of sparfloxacin (10) and ciprofloxacin and levofloxacin (2) have been described elsewhere. We report here a clinical strain of *S. pyogenes* (NIH-R01-GAS) isolated from an immunocompromised patient who had received repeated antibiotic treatment including levofloxacin for various infections. This isolate was found to be highly resistant to several fluoroquinolones, and analysis of gyrA and parC gene sequences from the isolate indicated that point mutations along the quinolone resistance-determining regions (QRDRs) were the probable mechanism for its resistance.

**Case history.** The patient was an eighteen-year-old black male with hyper-immunoglobulin E recurrent infection (Job’s syndrome) diagnosed at age four who has been previously described (6). He had had multiple recurrent pulmonary and sinus infections requiring multiple courses of long-term therapy and prophylactic antibiotics. One month prior to admission, he had extensive bilateral inguinal crease infections. Empiric therapy with levofloxacin, 500 mg orally daily, was initiated. Wound cultures subsequently grew *S. pyogenes*. One month later, while still on levofloxacin, he complained of headaches, fever (40.1°C), and purulent drainage from his right ear and nose. Blood cultures at this time grew *S. pyogenes* resistant to levofloxacin. He was admitted for 10 days for intravenous administration of vancomycin, and other antibiotics were discontinued. An echocardiogram was negative for any vegetation, and ophthalmic examination revealed no Roth spots. Blood cultures taken after completion of vancomycin therapy were negative.

All isolates of *S. pyogenes* (ATCC 700294, 12384, and 12344; the fluoroquinolone-resistant blood isolate; and seven fluoroquinolone-sensitive clinical isolates from a community hospital) were initially grown on 5% sheep blood plates (Remel, Lenexa, Kans.) in the presence of 5% CO2 at 35°C. The original isolate from the patient’s wound cultures was unavailable for further investigation. Antimicrobial susceptibility was determined by a frozen microdilution MicroStrep panel (Dade Behring, Inc., West Sacramento, Calif.), the Etest (AB Biodisk, Solna, Sweden), or the Kirby-Bauer (KB) disk (Becton Dickinson, Cockeysville, Md.) diffusion methods following manufacturers’ or NCCLS recommendations (5). Interpretation of susceptibility was made according to NCCLS standards whenever available (5). Quality control for all methods of susceptibility testing was performed using *S. pneumoniae* strain ATCC 49619, and results were within acceptable limits.

Mutational alterations in the QRDRs of gyrA and parC of NIH-R01-GAS were investigated by PCR using Ready-To-Go PCR Beads (Pharmacia Biotech, Piscataway, N.J.) with chromosomal DNA as template and subsequent DNA sequencing (Perkin-Elmer, Applied Biosystems, Foster City, Calif.). For amplification of a 614-bp fragment of gyrA containing the QRDR, a pair of primers (5’ GCAAGATCGAAATTTAATTGCAAGATCGAAATTTAATT, nucleotides 1 to 24, and 5’ CAGTCTGG, nucleotides 595 to 616) was used. For the amplification of the QRDR of parC of *S. pyogenes*, primers 5’ AGTCTAATGACACTTGACACTTGACACTTGACACTTGACACTTGACACT (nucleotides 1 to 24, and 5’ AGCCTGCGGAAATACCGAGAAG, nucleotides 500 to 520, were used to amplify a 520-bp fragment.

The levofloxacin-resistant isolate NIH-R01-GAS was sensitive to other antibiotics in the MicroStrep panel (azithromycin, ceftiraxone, chloramphenicol, clindamycin, penicillin, tetracycline, and vancomycin) according to NCCLS criteria (5). Susceptibility testing by Etest, however, demonstrated that the...
NIH-R01-GAS isolate was resistant to trovafloxacin, levofloxacin, and grepafloxacin as defined by NCCLS criteria (5) (Table 1). High MICs of ciprofloxacin, sparfloxacin, and norfloxacin were also found, and there were no zones of inhibition around the KB disks for enrofloxacin, lomefloxacin, and ofloxacin (Table 1). A MIC of 1.0 mg/ml suggested that the isolate was sensitive to clinafloxacin. Low MICs (<2.0 mg/ml) and/or large KB disk zone sizes (>19 mm) for all fluoroquinolones tested, indicative of sensitivity, were found for the three ATCC strains and the seven additional clinical isolates that were tested.

The gyrA and parC genes of *S. pneumoniae*, encoding DNA gyrase A and topoisomerase IV subunit C, respectively, have been well characterized elsewhere (1, 9, 11). The genome of *S. pyogenes* ATCC 700294 is currently being sequenced at the University of Oklahoma (*Streptococcus pyogenes* Genome Project). For defining gyrA in *S. pyogenes*, the nucleic acid sequences of gyrA (1) and parC (11) from *S. pneumoniae* were used to search the *Streptococcus pyogenes* Genome Project Database. Based on homology with the counterpart gyrA genes of *S. pneumoniae*, *Staphylococcus aureus*, and *Escherichia coli*, the putative open reading frame of gyrA of *S. pyogenes* was defined as a gene of 2,487 bp, encoding a protein of 829 residues. The putative promoter of gyrA of *S. pyogenes* has a striking similarity to that of gyrA of *S. pneumoniae* (1). Extended putative −10 (TATGTTATAAT) (1) and −35 (CTG ATAA) regions were identified upstream of the start codon ATG. The deduced amino acid sequence of the gyrase subunit A of *S. pyogenes* demonstrated 79% identity with GyrA of *S. pneumoniae* (1). However, identity was 88% when the first 400 amino acids in the N-terminal region were compared based on the genes from these two species (data not shown). The open reading frame of parC of *S. pyogenes* is a gene of 2,460 bp encoding a protein of 820 residues. The deduced amino acid sequence of subunit C of topoisomerase IV had 82% identity among the first 620 residues between *S. pyogenes* and *S. pneumoniae* (data not shown).

A phylogenetic protein tree was constructed based on amino acid sequences available from GenBank using an unbalanced method provided by the computer program MegAlign (DNASTAR, Inc. Madison, Wis.). Both GyrA and ParC of *S. pyogenes*...
were most closely related to those of *S. pneumoniae* (Fig. 1). Based on the available data, gyrA of *S. pyogenes* is next most closely related to those of *S. aureus* and *Bacillus subtilis*; ParC is next most closely related to that of *Streptococcus mitis*. These data are in agreement with but expand the data from the phylogenetic comparisons of these two genes reported by Balas et al. (1).

All 10 fluoroquinolone-sensitive ATCC and clinical isolates demonstrated identical amino acid sequences for the QRDRs of both *gyrA* and *parC* (data not shown). In contrast, mutations were identified in both *gyrA* and *parC* in the isolate NIH-R012-GAS. Specifically, two point mutations within the QRDR were identified in *gyrA*, with codon TCT (Ser-81, location designation for *S. pyogenes* ATCC 700294) being replaced by TTT (Phe) and ATG (Met-99) being replaced by CTG (Leu). Only a single point mutation was found in the QRDR of *parC*, in which TAC (Tyr) replaced the codon TCC (Ser-79). Resistance to fluoroquinolones usually results from mutations in the QRDRs of either *gyrA* or *parC*, or both genes, particularly at the highly conserved residues Ser-83 and Asp-87 (positions refer to those of *E. coli*) (14, 18). Munoz and De La Campa (11) demonstrated that most ciprofloxacin-resistant *S. pneumoniae* strains in their study had alterations at Ser-79 (analogous to Ser-83 of *E. coli* or Ser-81 of *S. pyogenes*), and the amino acid replacing the serine residue was either phenylalanine or tyrosine. This observation has also been reported by other investigators studying fluoroquinolone resistance in *S. pneumoniae* (9, 12). Therefore, the quinolone-resistant isolate of *S. pyogenes* has developed mutational alterations of key topoisomerases analogous to those reported for quinolone resistance of *S. pneumoniae*.

Quinolone resistance in *S. pneumoniae* arises through mutations of *parC* (and/or *parE*) before changes in *gyrA* occur, suggesting that topoisomerase IV is the primary target for the fluoroquinolones in this organism (8, 12). In the quinolone-resistant isolate of *S. pyogenes* in this study, mutations were identified in both *gyrA* and *parC*, which may explain its high level of resistance to fluoroquinolones. Because the resistant strain in the current study presented mutations at both sites at the time of isolation, we cannot determine the sequence of genetic transition from quinolone sensitive to resistant for these two target genes. The resistant isolate in this study demonstrated no sensitivity to all available fluoroquinolones tested, except to clinafloxacin. The superior activity of clinafloxacin has also been previously observed for *S. pneumoniae* (9, 13). Clinafloxacin is a novel C-8-substituted fluoroquinolone and is highly active against *S. pneumoniae* (17). Pan and Fisher have demonstrated that, in *S. pneumoniae*, neither gyrA nor parC quinolone-resistance-conferring mutants alone confer increased resistance to clinafloxacin (13). Laboratory experiments have shown that four consecutive mutational steps are required to induce significant resistance to clinafloxacin, while only two steps are required to achieve the same level of resistance for ciprofloxacin and three steps are required for sparfloxacin resistance (13). Compared to other tested quinolones, the mutations identified in the *gyrA* and *parC* genes of the clinical isolate of *S. pyogenes* had less effect on the activity of clinafloxacin, suggesting a potential clinical advantage for clinafloxacin.

Nucleotide sequence accession number. The DNA sequences obtained from this study were submitted to GenBank under accession no. AF220945, AF220946, AF222013, and AF223159.

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