

CONCISE COMMUNICATION

Fluoroquinolone-Resistant *Streptococcus pneumoniae* Associated with Levofloxacin Therapy

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Fluoroquinolone-resistant cultures of *Streptococcus pneumoniae* were isolated from 2 patients who were treated for pneumonia with levofloxacin. Nucleotide sequence analysis of bacterial DNA showed that the isolates contained mutations in both *parC* (DNA topoisomerase IV) and *gyrA* (DNA gyrase), which were shown previously to confer fluoroquinolone resistance. With the resistant isolates, the MICs for ciprofloxacin, gatifloxacin, grepafloxacin, levofloxacin, and trovafloxacin were above the maximal serum drug concentrations reported for standard dosage regimens. In contrast, the MICs for gemifloxacin and moxifloxacin were below the maximal serum concentrations. Increased effectiveness at blocking the growth of resistant mutants should make gemifloxacin and moxifloxacin less likely to allow the enrichment of mutants within susceptible populations. Additional resistance mutations in the isolates were readily obtained by plating on gemifloxacin- or moxifloxacin-containing agar. Thus, neither compound is expected to halt further accumulation of resistance mutations once mutant enrichment has been initiated by less potent derivatives.

Within the last decade, *Streptococcus pneumoniae*, a common cause of respiratory tract infection, has exhibited a striking increase in resistance to agents that traditionally cleared infection easily [1]. Clinicians turned to 2 fluoroquinolones, ciprofloxacin and levofloxacin, for treatment; as a result, fluoroquinolone resistance in isolates of *S. pneumoniae* is increasing [2]. It is not clear whether resistance is due only to the use of ciprofloxacin, the less potent of the two compounds, or whether both compounds contribute to the enrichment of mutants.

We argued previously that fluoroquinolone treatment will enrich resistant mutant portions of bacterial populations if the relevant tissue concentration of the agent is not high enough to require the bacteria to have ≥ 2 concurrent resistance mutations for growth [3]. Such a concentration (termed the mutant prevention concentration [MPC]) can be approximated in vitro by determining the point at which no mutant can be recovered from $>10^{10}$ cells [4]. For levofloxacin, the MPC is near or above the maximal serum concentration, when tested against clinical isolates of *S. pneumoniae* [5]. These data are consistent with

the development of fluoroquinolone resistance during treatment with levofloxacin [6].

The present work describes 2 recent clinical cases in which levofloxacin failed to eradicate respiratory tract infections from which fluoroquinolone-resistant *S. pneumoniae* were isolated. Both isolates contained *parC* (DNA topoisomerase IV) and *gyrA* (DNA gyrase) mutations associated with fluoroquinolone resistance. For levofloxacin and several other fluoroquinolones, the MIC with the mutants was above serum drug concentrations reported previously for standard doses. However, for gemifloxacin and moxifloxacin, the MIC was below serum concentration, making these 2 agents less likely to enrich mutants within susceptible *S. pneumoniae* populations.

Materials and Methods

Two levofloxacin-resistant *S. pneumoniae* isolates were obtained from the Clinical Microbiology Laboratory (CML), New York Hospital Medical Center of Queens (Flushing, NY). Procedures of the National Committee for Clinical Laboratory Standards were used by the CML to assess susceptibility by use of Kirby-Bauer disk diffusion methods, except that the MIC of penicillin was determined by E-test methodology. The MICs of levofloxacin, gatifloxacin, gemifloxacin, ciprofloxacin, moxifloxacin, trovafloxacin, and grepafloxacin were determined by E-test methodology (AB Biodisk) in the Infectious Disease Research Laboratory (New York Hospital Medical Center of Queens) by protocols specified by the manufacturer.

A gemifloxacin-resistant mutant, strain 70-G, was obtained from isolate 70 after 8 passages of bacteria applied as a liquid culture (10^8 cells/mL) to a chocolate Mueller-Hinton agar plate in the pres-

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ence of a gemifloxacin E-test strip. For each passage, ~15–20 colonies with the highest MIC (located within the zone of inhibition) were selected, were pooled, were expanded in Mueller-Hinton broth, and were reappplied to a fresh plate. After 8 passages, an isolate with a MIC of 32 $\mu\text{g}/\text{mL}$ was obtained. Three moxifloxacin-resistant mutants (strains 70-1Ma, 70-1Mb, and 70-1Mc) were obtained from strain 70 by selection on brain-heart infusion agar supplemented with 10% sheep blood and 10 $\mu\text{g}/\text{mL}$ moxifloxacin. We used 2 of these mutants (strains 70-1Ma and 70-1Mb) for a second round of selection at 60 $\mu\text{g}/\text{mL}$ moxifloxacin that produced strains 70-1Ma-2M and 70-1Mb-2M.

Nucleotide sequences for the quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* were determined as described elsewhere [5]. Primers for amplification and sequence determination of *gyrB* and *parE* QRDRs were as follows: SP-*gyrB*.fwd, 5'-AGA TGT TCG CGA AGG ATT AAC-3'; SP-*gyrB*.rev, 5'-GAT AAA CTC ACG ACG AGG CT-3'; SP-*gyrB*.seq, 5'-CTG CCA AGC GTG CGC GTG AAG-3'; SP-*parE*.fwd, 5'-CGG CCG TTC TTT CTA TCT TAG TTC-3'; SP-*parE*.rev, 5'-AGA GGT GGG AGG GCA ATA TAG AC-3'; and SP-*parE*.seq, 5'-TGG GGA ATT AGC TTC TAA CCT CAT C-3'.

Results

Patient histories. The first patient, a 50-year-old man, had a history of chronic obstructive pulmonary disease. He reported shortness of breath, cough, and increased sputum production for 1 week. He denied fever, chills, or recent antibiotic use. He underwent endotracheal intubation in the emergency room because of severe dyspnea and hypoxia. At physical examination, he was afebrile and had decreased breath sounds. Laboratory results were significant for a peripheral leukocyte count of 4500 cells/mm³, with 76% segmented neutrophils and 19% band forms. A chest radiograph showed hyperexpanded lungs without focal infiltrates. Intravenous corticosteroids were given, in addition to metronidazole and piperacillin-tazobactam. On the

second hospital day, his temperature was 38.4°C, which returned to normal on subsequent days. On the third day, intravenous levofloxacin therapy at 500 mg daily was initiated; metronidazole and piperacillin-tazobactam were discontinued on day 5. Tracheostomy was performed on day 8. After 5 days of intravenous levofloxacin, several sputum samples showed many neutrophils, by Gram's stain.

Cultures yielded *S. pneumoniae* that exhibited intermediate resistance to penicillin (MIC, 0.25 $\mu\text{g}/\text{mL}$) and resistance to levofloxacin (MIC, >32 $\mu\text{g}/\text{mL}$) by E-test methodology. The isolate, designated number 69, was susceptible to ampicillin-sulbactam, trimethoprim-sulfamethoxazole (TMP-SMZ), rifampin, clindamycin, erythromycin, chloramphenicol, tetracycline, and vancomycin, by Kirby-Bauer methods. The *S. pneumoniae* serotype was categorized as 9V by the Centers for Disease Control and Prevention (CDC). *Klebsiella pneumoniae*, which is resistant to ampicillin only, also was isolated. Blood and urine cultures remained negative. Oral TMP-SMZ was administered on day 13 of hospitalization, and levofloxacin was withdrawn. Gradual improvement in clinical status was seen within 72 h; this treatment was continued for 2 weeks.

The second patient was an 84-year-old man with a history of chronic obstructive pulmonary disease, hypertension, and cigarette smoking. Before admission, he had progressive shortness of breath for 5 days following an upper respiratory tract infection that had been treated with oral azithromycin 1 month earlier. Two sputum cultures had shown normal pharyngeal flora, and the patient was given oral levofloxacin for an unknown period. When seen in the emergency room, he was afebrile and had a respiratory rate of 40 breaths/min, with oxygen saturation of 79%. The peripheral leukocyte count was 8000 cells/mm³, with 78% segmented neutrophils.

The patient was admitted to the intensive care unit and required endotracheal intubation with ventilatory assistance on

Table 1. Susceptibility of *Streptococcus pneumoniae* to fluoroquinolones.

Compound	MIC, $\mu\text{g}/\text{mL}$ ^a			Daily dose, mg ^b	C _{max} ^c , $\mu\text{g}/\text{mL}$	T _{1/2} , h ^d	Reference ^e
	Strain 69	Strain 70	Strain 70-G				
Ciprofloxacin	>32	>32	>32	750	3.0	4	[7]
Gatifloxacin	12.0	8.0	>32	400	4.2	8	Internet ^f
Gemifloxacin	0.38	0.38	32	320	1.2–1.5	6.6	[8, 9]
Grepafloxacin	>32	>32	>32	600	<2.7 ^g	14	[10]
Levofloxacin	>32	>32	>32	500	5.7	8	Internet ^h
Moxifloxacin	4.0	3.0	>32	400	4.5	12	[11]
Trovafoxacin	>32	>32	>32	300	3.1	12	Internet ⁱ

^a Determined by E-test.

^b Recommended multiple, once-daily doses for *S. pneumoniae*.

^c Maximal serum concentration determined at steady state after multiple, once-daily doses at level recommended for *S. pneumoniae*, except for grepafloxacin (see footnote g).

^d Serum half-life determined as in footnote c.

^e References for pharmacokinetic parameters.

^f US prescribing information for Tequin (gatifloxacin) available at <http://www.tequin.com/>.

^g No steady-state pharmacokinetic profile for grepafloxacin at 600-mg, multiple, once-daily dose was available; the C_{max} value listed was obtained with a dose of 800 mg.

^h US prescribing information for Levaquin (levofloxacin) available at <http://www.levaquin.com/>.

ⁱ US prescribing information for Trovan (trovafoxacin) available at <http://www.pfizer.com/hml/pi/si/trovanpi.html>.

day 2. He was treated with intravenous levofloxacin (500 mg once/day), corticosteroid therapy, and nebulized bronchodilators. Cultures from specimens taken at admission yielded methicillin-susceptible *Staphylococcus aureus* and *S. pneumoniae* (isolate 70). The latter was resistant to levofloxacin, erythromycin, tetracycline, and TMP-SMZ and was susceptible to clindamycin, as determined by Kirby-Bauer susceptibility testing. Isolate 70 exhibited an intermediate level of resistance to penicillin (MIC, 0.75 $\mu\text{g}/\text{mL}$) and resistance to levofloxacin (MIC, >32 $\mu\text{g}/\text{mL}$), by E-test methodology. The serotype was categorized as 23F by the CDC. Levofloxacin treatment was discontinued when the susceptibility results were available. The initial chest radiograph showed no parenchymal disease, but new left lower lobe infiltrates appeared on day 7. Intravenous clindamycin and ceftazidime were given for 11 days. The patient improved and was discharged from the hospital.

Characterization of fluoroquinolone-resistant isolates. Examination of *S. pneumoniae* isolates 69 and 70 confirmed low susceptibility to several fluoroquinolones: maximal serum concentrations were significantly below the observed MIC for levofloxacin, ciprofloxacin, gatifloxacin, grepafloxacin, and trovafloxacin (table 1). Changes in the predicted amino acid sequence of the ParC and GyrA proteins (table 2) were as expected from previous work [5, 6]. These amino acid changes explain the low

level of susceptibility observed. The data in table 1 also show that gemifloxacin and moxifloxacin are significantly more potent against isolates 69 and 70 than is gatifloxacin, a C-8-methoxy fluoroquinolone that has been indicated for use against *S. pneumoniae*. These data suggest that resistance is more likely to develop with gatifloxacin than with the other two compounds.

Selection of additional topoisomerase mutations. Because the MIC for gemifloxacin with *gyrA parC* double mutants is low (table 1), the suggestion has been made that this quinolone would be useful against mutants generated by prior treatment with ciprofloxacin or levofloxacin [12]. As a test of this idea, we determined whether a double mutant of *S. pneumoniae* can become less susceptible to gemifloxacin. Passages of strain 70 were done in the presence of gemifloxacin (see Materials and Methods), and a mutant (strain 70-G) was obtained that was resistant to 32 $\mu\text{g}/\text{mL}$ gemifloxacin. Changes in the nucleic acid sequence and predicted amino acid sequence of the ParC and GyrA proteins (table 2) explained the low level of susceptibility observed. Changes in ParE also were observed (table 2). Reserpine, an inhibitor of fluoroquinolone efflux in *S. pneumoniae* [6], had no effect on the MIC for strain 70-G when added to agar at a concentration of 10 $\mu\text{g}/\text{mL}$. This result was consistent with the decreased susceptibility being due to the quinolone target mutations in *parC* and *gyrA*. When strain 70 was chal-

Table 2. Nucleotide sequence and amino acid sequence changes associated with fluoroquinolone resistance.

Strain ^a	Changes to QRDR ^b		
	parC	parE	gyrA
69	TCT→TAT (S79 to Y) AAG→AAT (K137 to N) ^c	ATC→GTC (I460 to V)	TCC→TTC (S81 to F)
70	GAT→GGT (D83 to G) AAG→AAT (K137 to N) ^c	None	GAA→AAA (E85 to K)
70-G	GAT→GGT (D83 to G) AAG→AAT (K137 to N) ^c TCT→TTT (S79 to F)	ATC→GTC (I460 to V) GAA→AAA (E474 to K)	GAA→AAA (E85 to K) TCC→TAC (S81 to Y)
70-1Ma	GAT→GGT (D83 to G) AAG→AAT (K137 to N) ^c	None	GAA→AAA (E85 to K) TCC→TTC (S81 to F)
70-1Mb	GAT→GGT (D83 to G) AAG→AAT (K137 to N) ^c	None	GAA→AAA (E85 to K) TCC→TAC (S81 to F)
70-1Mc	GAT→GGT (D83 to G) AAG→AAT (K137 to N) ^c	None	GAA→AAA (E85 to K) TCC→TTC (S81 to Y)
70-1Ma-2M	GAT→GGT (D83 to G) AAG→AAT (K137 to N) ^c TCT→TTT (S79 to F)	None	GAA→AAA (E85 to K) TCC→TTC (S81 to F)
70-1Mb-2M	GAT→GGT (D83 to G) AAG→AAT (K137 to N) ^c TCT→TAT (S79 to Y)	None	GAA→AAA (E85 to K) TCC→TTC (S81 to F)

NOTE. No strains had changes in quinolone resistance-determining region (QRDR) in *gyrB*.

^a Strains 69 and 70 are clinical isolates. Strain 70-G was obtained from strain 70 by multiple passages in presence of gemifloxacin. Strains 70-1Ma, 70-1Mb, and 70-1Mc were obtained from strain 70 by selection at 10 $\mu\text{g}/\text{mL}$ moxifloxacin; strains 70-1Ma-2M and 70-1Mb-2M were obtained from strains 70-1Ma and 70-1Mb, respectively, by selection at 60 $\mu\text{g}/\text{mL}$ moxifloxacin.

^b Codon changes are indicated, followed by the expected amino acid changes in parentheses in the QRDR. Underlined changes occurred during laboratory selection setup. Amino acid abbreviations: D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; K, lysine; N, asparagine; S, serine; Y, tyrosine.

^c Whether this allele reduces susceptibility to fluoroquinolones has not been established.

lenged stepwise with moxifloxacin, additional resistance alleles were obtained, first in *gyrA* (strains 70-1Ma, 70-1Mb, and 70-1Mc; table 2) and then in *parC* (strains 70-1Ma-2M and 70-1Mb-2M; table 2).

Discussion

Fluoroquinolone-resistant *S. pneumoniae* was isolated from 2 patients who were treated with levofloxacin. Mutations found in the bacteria were consistent with gyrase- and topoisomerase IV-mediated resistance. Although it is likely that mutants in both cultures were enriched by levofloxacin treatment, bacterial samples were unavailable before levofloxacin administration; consequently, we could not exclude the possibility that the patients were colonized by *S. pneumoniae* that had been selected previously for resistance to ciprofloxacin (target mutations for the 2 drugs are identical and would not distinguish between the 2 as selecting agents). Additional clinical cases are being sought with available pretreatment samples to test more rigorously the idea that levofloxacin resistance develops during treatment.

Two new fluoroquinolones, gemifloxacin and moxifloxacin, were particularly effective against the resistant mutants. With isolates 69 and 70, the MICs of both agents were below attainable serum levels for part of the dosing period (table 1). During that time, these double mutants would require a third mutation for growth, which suggests that use of these agents against fully susceptible strains might significantly slow the development of fluoroquinolone-resistant *S. pneumoniae*. However, additional resistance alleles that lower the susceptibility to gemifloxacin and moxifloxacin can be obtained in mutant isolates of *S. pneumoniae* (table 2). Thus, it will be difficult to preserve the efficacy of gemifloxacin and moxifloxacin if resistance enriched by earlier fluoroquinolone generations continues to increase in prevalence.

Gemifloxacin exhibited a lower MIC, but pharmacokinetic data indicate that the difference in effectiveness may not be large (table 1). For example, moxifloxacin achieves a serum concentration that is 3–3.7 times that of gemifloxacin with recommended doses, the half-life of moxifloxacin is about twice that of gemifloxacin, and the serum protein binding of gemifloxacin is about 1.3 times that of moxifloxacin [9, 13]. Thus, the 10-fold difference in MIC may require a correction of 8–9-fold when effectiveness is considered.

Moxifloxacin was examined previously for the ability to restrict the selection of resistant mutants with ~100 clinical isolates. A provisional MPC value was below serum levels for this compound with 90% of the isolates [5] but not so for levofloxacin. These data suggested that resistance would develop less often with moxifloxacin than with levofloxacin. Subsequent work with the same set of strains indicated that gemifloxacin has a provisional MPC₉₀ of 0.5–1 µg/mL, whereas the value for

moxifloxacin was 1–2 µg/mL (J. Blondeau, G. Hansen, and K. Drlica, unpublished data). Both are below maximal serum concentration (table 1).

We emphasize that it is not known how in vitro characteristics, such as those described above, translate into the enrichment of resistant mutants within human patients, since no tests have been done. Empirical correlations between treatment failure and pharmacokinetic parameters, such as the ratio of the maximal serum concentration to MIC and the area under the time-concentration curve to MIC, have been obtained [14, 15]. However, the general relevance of these parameters to fluoroquinolone resistance is unclear, because the inoculum size may not have been large enough to assure the presence of the topoisomerase mutants normally associated with resistance.

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