# Selection of a *gyrA* Mutation and Treatment Failure with Gatifloxacin in a Patient with *Streptococcus pneumoniae* with a Preexisting *parC* Mutation

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An 81-year-old woman had pneumonia caused by *Streptococcus pneumoniae* (levofloxacin Etest minimum inhibitory concentration [MIC] 1.5 µg/ml) and was treated with intravenous gatifloxacin 200 mg/day. After 3 days of therapy, repeat sputum cultures were positive for *S. pneumoniae*, which was resistant to levofloxacin (Etest MIC > 32 µg/ml). The isolate obtained before therapy showed a preexisting *parC* mutation of aspartic acid-83 to asparagine (Asp83→Asn), and the isolate obtained during therapy showed an acquired *gyrA* mutation from serine-81 to phenylalanine (Ser81→Phe) and a second *parC* mutation from lysine-137 to Asn (Lys137→Asn). Both isolates were the same strain, as determined with pulsed-field gel electrophoresis. This case demonstrates the potential for resistance to emerge during 8-methoxy fluoroquinolone therapy for fluoroquinolone-susceptible *S. pneumoniae* with a preexisting *parC* mutation. Additional clinical failures with a fluoroquinolone may occur unless these first-step *parC* mutants can be identified to assist clinicians in selecting appropriate antimicrobial therapy.

**Key Words:** gatifloxacin, fluoroquinolone, *Streptococcus pneumoniae*, resistance.
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*Streptococcus pneumoniae* is the most common bacterial pathogen in community-acquired pneumonia, but the pneumococcus may also be an etiologic agent in hospital-acquired pneu-monia in both intensive care and other settings.[1, 2] Respiratory fluoroquinolones are recommended and frequently prescribed for treatment of these infections when *S. pneumoniae* is either suspected or documented.[3, 4] The prevalence of high-level fluoroquinolone resistance in *S. pneumoniae* is less than 2% in the United States and in Canada.[5, 6] Fluoroquinolone resistance in *S. pneumoniae* results from target mutations in the quinolone resistance–determining regions of *parC* and *gyrA*, which encode for topoisomerase IV and DNA gyrase, respectively.[7] Low-level resistance results after a first-step mutation occurs in one of these target genes, and high-level resistance results after a subsequent mutation occurs in the other target gene. Isolates with a single, first-step *parC* mutation are frequently susceptible to fluoroquinolones because the minimum inhibitory

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concentrations (MICs) are at or below susceptibility breakpoints.[8] However, a first-step *parC* mutation increases the likelihood of subsequent mutations in *gyrA*, resulting in high-level resistance.[9, 10]

Published reports of respiratory tract infections caused by fluoroquinolone-resistant pneumococci and resulting in clinical therapeutic failure were recently reviewed. Treatment failures and emergence of resistance in *S. pneumoniae* have been reported with ciprofloxacin and levofloxacin but not with gatifloxacin or moxifloxacin.[11] An estimated 21% of pneumococcal isolates in the United States harbor a first-step *parC* mutation.[5] With the increasing prevalence of first-step mutants, resistance and treatment failures may also occur with the 8-methoxy fluoroquinolones.

We describe the emergence of a *gyrA* mutation and treatment failure with gatifloxacin in a patient with an *S. pneumoniae* isolate with a preexisting *parC* mutation. We then sought to determine the genetic relatedness of the *S. pneumoniae* isolate that had been obtained from this patient before she had started gatifloxacin therapy and the isolate obtained while she was receiving the drug.

## Case Report

An 81-year-old woman was admitted to the hospital after having a subarachnoid hemorrhage. Her medical history was significant for hypertension, atrial fibrillation, pacemaker placement, transient ischemic attacks, and breast cancer. On admission, her temperature was 36.6°C, and her white blood cell count was 5.6 x $10^3$/mm$^3$ (normal range 4.5–11.5 x $10^3$/mm$^3$). She underwent right frontal ventriculostomy on hospital day 1 and coil obliteration of the aneurysm on hospital day 2.

On hospital day 8, the patient became febrile with a temperature of 38.8°C, and her white blood cell count increased to 13.9 x $10^3$/mm$^3$. On physical examination, expiratory crackles were heard bilaterally, and a moderate amount of thick, yellow secretions was suctioned because of her inability to cough or clear the secretions. She had increasing respiratory distress and required intubation and mechanical ventilation. Chest radiography revealed hazy opacities over the lung bases consistent with bilateral pleural effusions.

Blood and urine cultures were obtained on hospital day 8, and a sputum culture was obtained on day 9. The blood cultures were negative, but the urine culture was positive for *Escherichia coli*, which was fluoroquinolone susceptible. Intravenous gatifloxacin 200 mg/day was started to treat the urinary tract infection. At that time, the patient's estimated creatinine clearance was approximately 50 ml/minute.

The sputum culture subsequently grew *S. pneumoniae*, which was resistant to penicillin (Etest MIC 2 µg/ml) and susceptible to ceftriaxone (Etest MIC 1 µg/ml) and levofloxacin (Etest MIC 1.5 µg/ml). Gatifloxacin was continued at the same dosage as before, but the patient's clinical condition did not improve. Her maximum temperature reached 40.8°C on hospital day 12, and her white blood cell count increased to 17.0 x $10^3$/mm$^3$ on day 13. After three doses of gatifloxacin were given, the sputum culture was repeated, and piperacillin-tazobactam was added to the patient's regimen.

On hospital day 13, purulent discharge was noted at the site of the right subclavian catheter; vancomycin was begun, and the subclavian catheter was replaced. The sputum culture was then reported to be positive for methicillin-resistant *Staphylococcus aureus* and *S. pneumoniae*. This pneumococcal isolate was resistant to penicillin (Etest MIC 2 µg/ml), intermediate to ceftriaxone (Etest MIC 1.5 µg/ml), and resistant to levofloxacin (Etest MIC > 32 µg/ml). Gatifloxacin was discontinued on hospital day 16. The patient became afebrile on day 17, and her white blood cell count returned to normal on day 20. She completed a 14-day course of vancomycin, and *S. pneumoniae* was not isolated from any cultures for the remainder of her hospital stay.

## Methods

The genetic relatedness of the *S. pneumoniae* isolates obtained before and during gatifloxacin therapy was examined in duplicate by using pulsed-field gel electrophoresis, as previously described.[12] The DNA banding patterns were digitized for analysis with Molecular Analyst Fingerprinting Plus software, version 1.12 (Bio-Rad Laboratories, Hercules, CA), and a dendrogram was calculated by applying the unweighted pair-group method with arithmetic means.[13, 14] Strains were considered to be the same type on pulsed-field gel electrophoresis if their patterns differed by three bands or fewer.[15]

The capsular serotype of the two isolates was determined by conducting the quellung reaction with type-specific antisera (Statens Serum Institut, Copenhagen, Denmark) according to the

**Table 1. In Vitro Activity and Susceptibility of *Streptococcus pneumoniae* Isolates Tested with Broth Microdilution at Two Laboratories**

| | Minimum Inhibitory Concentration (µg/ml), Susceptibility[a] | | | |
|---|---|---|---|---|
| | Isolate Obtained Before Therapy | | Isolate Obtained During Therapy | |
| Drug | Laboratory 1 | Laboratory 2 | Laboratory 1 | Laboratory 2 |
| Penicillin | 4, R | 4, R | 4, R | 4, R |
| Ceftriaxone | 1, S | 1, S | 1, S | 2, I |
| Vancomycin | 0.25, S | Not tested | 0.25, S | Not tested |
| Clindamycin | 16, R | > 32, R | 16, R | > 32, R |
| Azithromycin | 128, R | > 32, R | 128, R | > 32, R |
| Ciprofloxacin | 2, I | 4, R | 16, R | 16, R |
| Levofloxacin | 2, S | 2, S | 8, R | 8, R |
| Gatifloxacin | 0.5, S | 0.5, S | 2, I | 2, I |
| Moxifloxacin | 0.25, S | 0.25, S | 2, I | 2, I |
| Gemifloxacin | 0.03, S | 0.03, S | 0.25, I | 0.25, I |

R = resistant; S = susceptible; I = intermediate.

[a]Clinical and Laboratory Standards Institute breakpoints for susceptible, intermediate, and resistant, respectively, were levofloxacin ≤ 2, 4, and ≥ 8 µg/ml; gatifloxacin and moxifloxacin ≤1, 2, and ≥ 4 µg/ml; and gemifloxacin ≤ 0.12, 0.25, and ≥ 0.5 µg/ml.[18]

Laboratory 1 was at the Health Sciences Centre, Winnipeg, Manitoba, Canada, and laboratory 2 was at Purdue University, Indianapolis, Indiana.

manufacturer's instructions. Quinolone resistance–determining regions of *parC* and *gyrA* were sequenced in both directions with a kit (ABI Prism BigDye Terminator; PE Applied Biosystems, Mississauga, Ontario, Canada) and the primers described previously.[16] Sequences were obtained by using an ABI Prism 310 sequencer (PE Applied Biosystems) and analyzed by using Sequence Navigator (PE Applied Biosystems).[12]

The MICs of 10 antimicrobial agents were determined in triplicate for both pneumococcal isolates by means of broth microdilution.[17] Testing of MICs was performed independently at the Health Sciences Centre, Winnipeg, Manitoba, Canada, and at Purdue University, Indianapolis, Indiana, and the modal MIC was recorded. The isolates were categorized as susceptible, intermediate, or resistant by using Clinical and Laboratory Standards Institute (CLSI) breakpoints.[18] Ciprofloxacin resistance was defined as an MIC of 4 µg/ml or higher.[19] For quality control, *S. pneumoniae* ATCC 49619 was used.

## Results

The isolates obtained before and during therapy differed by one band on pulsed-field gel electrophoresis; therefore, they were the same strain of *S. pneumoniae*. Capsular serotyping revealed that both strains were serotype 23F. Sequencing of DNA demonstrated that the initial isolate had a preexisting *parC* mutation from aspartic acid-83 to asparagine (Asp83→Asn). According to current CLSI breakpoints, this isolate was susceptible to all respiratory fluoroquinolones. The MIC for ciprofloxacin was 2–4 µg/ml, varying 2-fold between laboratories (Table 1). The isolate obtained after 3 days of gatifloxacin treatment acquired a *gyrA* mutation from serine-81 to phenylalanine (Ser81→Phe) and a second *parC* mutation from lysine-137 to Asn (Lys137→Asn). The MICs for fluoroquinolone for this isolate were 4–8-fold higher than those for the pretherapy isolate, and this isolate was highly resistant to levofloxacin and intermediate to gatifloxacin, moxifloxacin, and gemifloxacin (Table 1).

## Discussion

Respiratory fluoroquinolones are frequently prescribed to treat infections involving *S. pneumoniae* because of their convenience and low prevalence of resistance. In the United States, resistance to levofloxacin, gatifloxacin, moxifloxacin, and gemifloxacin was 0.7%, 0.7%, 0.2%, and 0.2%, respectively, among 1817 *S. pneumoniae* isolates collected in 2002–2003.[5] Fluoroquinolone-resistant isolates usually possess mutations in both *parC* and *gyrA*, but pneumococcal isolates that harbor first-step *parC* mutations alone are difficult to detect during susceptibility testing because their MICs are frequently below susceptibility breakpoints. Pneumococci are susceptible to levofloxacin if the MIC is 2 µg/ml or lower, yet 59–71% of isolates with levofloxacin MICs of 2 µg/ml harbor

*parC* mutations.[8, 20] Unfortunately, the prevalence of first-step *parC* mutants is increasing in the United States. An estimated 21% of *S. pneumoniae* isolates collected in 2002–2003 harbored mutations in *parC* compared with 4.7% of isolates with *parC* and/or *gyrA* mutations collected in 1997–1998.[5]

In vitro, animal, and human data have demonstrated that a first-step *parC* mutation in *S. pneumoniae* substantially increases the probability of a second mutation in *gyrA*, resulting in high-level fluoroquinolone resistance.[9, 10, 21–25] Researchers reported a treatment failure with levofloxacin in a patient with pneumococcal pneumonia in whom the initial isolate had a Ser79→Phe mutation in *parC*.[25] After 3 days of therapy, the isolate had acquired a Ser81→Phe mutation in *gyrA* and was resistant to all fluoroquinolones tested. Despite the increased potency of the 8-methoxy fluoroquinolones against *S. pneumoniae*, a *parC* mutation facilitated the enrichment of high-level resistant mutants in rabbits treated with gatifloxacin or moxifloxacin.[23, 24] Therefore, treatment failure and emergence of resistance with these two agents is a great concern in patients with infections caused by pneumococci that harbor *parC* mutations.

To our knowledge, we describe the first known treatment failure and emergence of reduced fluoroquinolone susceptibility in *S. pneumoniae* during gatifloxacin therapy. A preexisting *parC* mutation (Asp83→Asn) in the pretherapy isolate likely facilitated the acquisition of a *gyrA* mutation (Ser81→Phe) and a second *parC* mutation (Lys137→Asn). In addition, the patient was treated with gatifloxacin 200 mg/day for a urinary tract infection due to *E. coli*. This dosage was one half of the U.S. Food and Drug Administration–approved dosage for the treatment of pneumococcal pneumonia in patients with creatinine clearances greater than 40 ml/minute. At the time of therapy, the patient's creatinine clearance was estimated to be 50 ml/minute, but the dosage of gatifloxacin was not increased when the sputum cultures were reported to be positive for *S. pneumoniae*. Therefore, underdosing of gatifloxacin might also have played an important role in selection of the *gyrA* mutant.

In this patient, gatifloxacin clearance was estimated from her creatinine clearance and body weight by using the following two equations: gatifloxacin clearance (L/hr) = 8.11 + [0.0629 • (creatinine clearance [ml/min] – 75.0)] from one study,[26] and gatifloxacin clearance (L/hr) = 8.43 + [0.036 • (creatinine clearance [ml/min] – 91.0)] + [0.073 • (weight [kg] – 82.5)], from another study.[27]

The patient's estimated clearance rate for gatifloxacin was approximately 6.5 L/hour, which corresponded to an area under the serum concentration–time curve (AUC) of 30.8 mg•hour/L for a 200-mg daily dose. If 20% protein binding is assumed, the free 24-hour ratio of this AUC:MIC is approximately 50 because the MIC for the initial isolate was 0.5 µg/ml. This ratio exceeds the recommended target of 33.7, which was associated with 100% microbiologic eradication of *S. pneumoniae* in patients with community-acquired respiratory tract infections.[28] However, the studies conducted to derive the target of 33.7 were reported before 2000, and patients were unlikely to have been infected with *parC* mutants. The target ratio for AUC:MIC needed to prevent *gyrA* mutations in isolates with preexisting *parC* mutations is unknown, but it is likely to be higher than any ratio that can be achieved clinically by using approved dosing regimens for the respiratory fluoroquinolones.

What can be done to minimize the potential for treatment failures and emerging resistance in pneumococcal infections caused by *parC* mutants? First, clinicians must recognize that *S. pneumoniae* is susceptible to fluoroquinolones according to current CLSI breakpoints, but MICs of 2 µg/ml for levofloxacin, 0.5 µg/ml for gatifloxacin, 0.25 µg/ml for moxifloxacin, and 0.06 µg/ml gemifloxacin are associated with *parC* mutations more than 50% of the time.[29] If a *parC* mutation is suspected based on MIC results, antimicrobial treatment options should include agents other than respiratory fluoroquinolones.

Second, fluoroquinolone breakpoints for *S. pneumoniae* could be changed to identify isolates that likely harbor *parC* mutations. Respective breakpoints for *S. pneumoniae* are as follows: levofloxacin—susceptibility ≤ 2 µg/ml, intermediate 4 µg/ml, and resistance ≥ 8 µg/ml; gatifloxacin and moxifloxacin—susceptibility ≤ 1 µg/ml, intermediate 2 µg/ml, and resistance ≥ 4 µg/ml; and gemifloxacin—susceptibility ≤ 0.12 µg/ml, intermediate 0.25 µg/ml, and resistance ≥ 0.5 µg/ml.[18] Investigators proposed the use of microbiologic breakpoints for fluoroquinolones and *S. pneumoniae*.[29] These breakpoints define resistance as the MIC at which more than 50% of isolates harbor mutations in the quinolone resistance–determining regions. Proposed microbiologic breakpoints for resistance are greater than 1 µg/ml for levofloxacin, greater

than 0.25 µg/ml for gatifloxacin, greater than 0.12 µg/ml for moxifloxacin, and greater than 0.03 µg/ml for gemifloxacin. These breakpoints were more sensitive than current CLSI breakpoints in identifying decreased susceptibility due to first-step mutations. Therefore, the incorporation of microbiologic breakpoints into susceptibility testing may help in identifying isolates with first-step mutations. It may also help limit the inappropriate use of fluoroquinolones to treat pneumococcal infections caused by first-step mutants and thus reduce the likelihood of treatment failure and the future development of high-level resistance.

## Conclusion

To our knowledge, this is the first clinical report of a treatment failure with an 8-methoxy fluoroquinolone (gatifloxacin) due to selection of a *gyrA* mutation in *S. pneumoniae* with a pre-existing *parC* mutation. The possibility of additional treatment failures with all respiratory fluoroquinolones is a great concern because the prevalence of first-step mutants has increased. Clinicians should have a high index of suspicion for first-step *parC* mutations in pneumococci, as based on MIC data. An antimicrobial agent other than a fluoroquinolone or a combination of a fluoroquinolone with another agent (e.g., β-lactam) should be used to treat infections caused by these organisms.

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