Fluoroquinolone Resistance in *Streptococcus pyogenes*

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Streptococcus pyogenes **isolated from blood and urine samples obtained from a 78-year-old woman was tested for susceptibility, and fluoroquinolone resistance (minimum inhibitory concentration of levofloxacin, 16 μg/mL) was found. DNA amplification and sequencing revealed a serine^{81}→tyrosine substitution in *gyrA* and 2 substitutions in *parC:* serine^{79}→phenylalanine and alanine^{121}→valine. This is the second report of a clinical isolate of *S. pyogenes* with high-level fluoroquinolone resistance.**

In this era of increasing antimicrobial resistance, it is remarkable that *Streptococcus pyogenes* has retained its susceptibility to penicillin. There has been concern about the emergence of macrolide resistance in this organism, with high rates reported in a number of countries, including Finland [1], Italy [2, 3], Taiwan [4], and Spain [3, 5]. Surveillance data from the United States suggest a low level of macrolide resistance among *S. pyogenes* (<3%) [6], but a clonal outbreak of infection with an erythromycin-resistant strain in Pittsburgh was recently documented [7]. Only 1 strain of *S. pyogenes* with high-level fluoroquinolone resistance has been reported previously [8]. We report the recovery of a second clinical isolate of *S. pyogenes* with mutations in the *gyrA* and *parC* genes conferring resistance to multiple fluoroquinolones.

Case report. A 78-year-old woman with a history of chronic obstructive pulmonary disease and osteoporosis was hospitalized for pneumonia and vertebral compression fractures in February 2002. She was discharged after 1 week, and she completed a regimen of oral levofloxacin therapy (250 mg q.d. for 2 weeks) at home 1 week later. One week after finishing therapy, she was hospitalized for upper gastrointestinal bleeding of uncertain etiology and a partial colonic obstruction. She had been taking narcotics to control her back pain. An endoscopic examination performed 4 months earlier (November 2001) had revealed gastritis, esophagitis, and severe diverticulosis. She was treated empirically with ranitidine, bowel rest, enemas, and transfusions.

The patient developed a nonproductive cough on day 6 of hospitalization. A chest radiograph showed small bilateral pleural effusions. On day 9 of hospitalization, she became febrile (temperature, 38.8°C) and had guaiac-positive emesis. A chest radiograph showed increasing pleural effusions and focal, asymmetric, bilateral infiltrates. Laboratory studies revealed leukocytosis (20,000 leukocytes/mm^3; leukocyte count 3 days earlier, 8300 leukocytes/mm^3) and abnormal urinalysis findings (trace leukocyte esterase positive with 18–22 WBCs per high-power field). A CT scan revealed mild diverticulitis. Initially, the patient intravenously received cefotaxime and metronidazole. *S. pyogenes* was recovered from 2 blood cultures and in moderate amounts from a urine culture (50,000–100,000 cfu/mL). Susceptibility testing was not performed because of the organism's predictable susceptibility to β-lactams. A change in therapy to oral ciprofloxacin and metronidazole was made on day 12 of hospitalization, and this antimicrobial therapy was continued through day 19. The change to ciprofloxacin and metronidazole was directed toward diverticulitis because the patient had remained afebrile and did not appear septic during the initial 3 days of intravenously administered therapy.

The patient's WBC count decreased but subsequently increased. A urine culture and 1 of 2 blood culture sets performed on day 23 of hospitalization yielded *Staphylococcus epidermidis* and *Enterococcus faecium*. The patient received a 10-day course of intravenously administered vancomycin. Chest radiography performed on day 30 for hypoxia revealed resolution of the infiltrates and pleural effusions. The patient developed a transient, low-grade fever on day 37 of hospitalization, and both sets of blood cultures were negative. The patient's condition improved, and she was discharged to a skilled nursing facility on day 63. Later, as part of the Emerging Infections and Epidemiology of Iowa Organisms surveillance program, the isolate of *S. pyogenes* from the blood culture was submitted to the University of Iowa (Iowa City) for susceptibility testing. The isolate was found to be resistant to levofloxacin (MIC, 16 μg/mL).

Methods. MICs of penicillin, ampicillin, ceftriaxone, erythromycin, clindamycin, tetracycline, vancomycin, quinu-

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pristin-dalfopristin, linezolid, chloramphenicol, ciprofloxacin, levofloxacin, gatifloxacin, and moxifloxacin were determined by a microdilution method in Mueller-Hinton broth supplemented with 3% lysed horse blood, in accordance with the guidelines of the National Committee for Clinical Laboratory Standards (NCCLS) [9]. Resistance was defined according to NCCLS MIC interpretive standards [10]. Quality control was performed by testing *S. pneumoniae* ATCC 49619 (American Type Culture Collection).

The isolate was sent to the World Health Organization Collaborating Center for Reference and Research on Streptococci at the University of Minnesota in Minneapolis for serological classification. Lancefield group, serum opacity factor production, M-type antigen, and T-protein typing pattern were determined.

For DNA amplification, the isolate was subcultured on Columbia sheep blood agar (BBL) overnight in 5% CO_2 at 35°C. A lysate was made by suspending 2–3 colonies in 100 μL of distilled water and was denatured for 15 min at 95°C in a thermal cycler (GeneAmp PCR System 9700; Applied Biosystems). The primers described by Yan et al. [8] were used to amplify a 614-bp region of *gyrA* and a 520-bp region of *parC*. The amplification reaction was performed in a 50-μL final volume with use of PCR Supermix (Invitrogen). The thermal cycling profile for *gyrA* and *parC* consisted of 40 cycles of amplification (denaturation at 94°C for 15 s, annealing at 52°C for 45 s, and extension at 72°C for 45 s). Automated sequencing was performed in accordance with the manufacturer's instructions (Applied Biosystems). DNA sequences were compared with published GenBank sequences for *gyrA* (AF220945) and *parC* (AF220946) [8].

Results. The MICs were in the susceptible range for all of the agents tested except the fluoroquinolones, as follows: penicillin (MIC, ≤0.06 μg/mL), ampicillin (MIC, ≤0.25 μg/mL), ceftriaxone (MIC, ≤0.03 μg/mL), erythromycin (MIC, ≤0.25 μg/mL), clindamycin (MIC, ≤0.25 μg/mL), tetracycline (MIC, ≤0.25 μg/mL), vancomycin (MIC, 0.5 μg/mL), quinupristin-dalfopristin (MIC, ≤0.12 μg/mL), linezolid (1 μg/mL), chloramphenicol (4 μg/mL), ciprofloxacin (32 μg/mL), levofloxacin (16 μg/mL), gatifloxacin (4 μg/mL), and moxifloxacin (2 μg/mL). The serological classification was confirmed to be Lancefield group A, and the M-protein serotype was 12. Streptococcal serum opacity factor was not detected, and there was no agglutination with T-protein antisera (nontypeable).

DNA amplification and sequencing revealed mutations in the quinolone resistance–determining regions (QRDR) of the *gyrA* and *parC* genes. A serine^{81}→tyrosine substitution was found in *gyrA*. There were 2 substitutions in *parC:* serine^{79}→phenylalanine and alanine^{121}→valine.

Discussion. The source of this patient's *S. pyogenes* bacteremia is uncertain. Burkert and Watanakunakorn [11] reviewed 18 publications that described multiple cases (15–293 patients per report) of group A streptococcal bacteremia. The percentage of cases of *S. pyogenes* bacteremia with no identifiable source ranged from 0% to 41%, and the percentage of cases associated with pneumonia had a range of 0%–30% [11]. An association between pleural effusions and *S. pyogenes* pneumonia has been noted [11, 12]. The infiltrates and pleural effusions seen on this patient's chest radiograph implicate the lower respiratory tract as the probable source. The frequency of bacteremia in patients with *S. pyogenes* pneumonia is 10%–15% [13].

Another possible source of this patient's bacteremia is the gastrointestinal tract. *S. pyogenes* may be included in bowel flora [14] and may explain why the anus is the most common asymptomatic carriage site among health care workers [15]. Pharyngeal, skin, and vaginal carriage have also been implicated in nosocomial infections [15]. This patient had upper gastrointestinal bleeding and diverticulitis; however, intra-abdominal infections due to *S. pyogenes* are rare [11]. Although *S. pyogenes* is not typically associated with urinary tract infections, the patient's positive urine culture points to the urinary tract as another potential source.

The M-protein serotype of this isolate (M type 12) is 1 of 5 frequently associated with invasive disease: M types 1, 3, 11, 12, and 28 [16]. However, M type 12 isolates causing invasive infection are typically T-protein type 12 rather than nontypeable, as this isolate was [17, 18].

There are no NCCLS streptococcal breakpoints for ciprofloxacin. For *S. pneumoniae,* an MIC of ciprofloxacin of ≥4 μg/mL has been generally accepted as resistant [19–22], but an MIC of ≥2 μg/mL was used to define resistance for *S. pyogenes* in the following surveillance studies. Blondeau et al. [23] reported slightly elevated MICs of ciprofloxacin (2–3 μg/mL; determined by use of Etest [AB BIODISK]) among 0.6% of 1003 Canadian *S. pyogenes* isolates recovered during 1996. Blondeau et al. [24] also found increased MICs of ciprofloxacin (2 to ≥4 μg/mL) among 22% of 60 *S. pyogenes* isolates recovered during 1993–1994, but they later questioned the "suitability" of the Microscan MIC Plus Type 2 panel that had been used to determine the MICs [23]. Barry et al. [25] noted that 8% of 764 *S. pyogenes* isolates from the United States had MICs of ciprofloxacin of ≥2 μg/mL; 2 isolates had off-scale MICs of >4 μg/mL. Baquero et al. [5] also reported a small portion of isolates (<10%) with MICs of ciprofloxacin of 2–4 μg/mL among 786 pharyngeal isolates of *S. pyogenes* from Spain.

We were unable to find any reports of high-level fluoroquinolone resistance in *S. pyogenes* other than the invasive isolate obtained from an immunocompromised male patient with hyper-IgE syndrome and purulent drainage from his ear and nose [8]. One month earlier, that patient had received levofloxacin therapy (500 mg q.d.) for *S. pyogenes* cellulitis [8]. A

Table 1. Data regarding mutations in the quinolone resistance-determining regions (QRDRs) and susceptibility for 2 fluoroquinolone-resistant isolates of *Streptococcus pyogenes*.

	Mutations in the QRDR		MIC,[a] μg/mL			
Study	*parC*	*gyrA*	Cpfx	Gtfx	Lvfx	Mox
Present report	Ser[79]→Phe and Ala[121]→Val	Ser[81]→Tyr	32	4[b]	16[b]	2[c]
Yan et al. [8]	Ser[79]→Tyr	Ser[81]→Phe and Met[99]→Leu	>32	—	>32[b]	—

NOTE. Ala, alanine; Cpfx, ciprofloxacin; Gtfx, gatifloxacin; Leu, leucine; Lvfx, Levofloxacin; Met, methionine; Mox, moxifloxacin; Phe, phenylalanine; Ser, serine; Tyr, tyrosine; Val, valine.

[a] Determined by Etest (AB BIODISK) for [8]; broth microdilution for current report.

[b] Resistant, as determined on the basis of National Committee for Clinical Laboratory Standards (NCCLS) interpretive criteria for *Streptococcus* species other than *Streptococcus pneumoniae* [10].

[c] Intermediate resistance, as determined on the basis of NCCLS interpretive criteria for *S. pneumoniae* [10].

comparison of the current and previously reported isolates' MICs of fluoroquinolone and *gyrA* and *parC* mutations is presented in table 1.

Both isolates had mutations in the *gyrA* and *parC* genes, which have been identified in isolates of *S. pneumoniae* with high-level fluoroquinolone resistance [20, 21, 26, 27]. Two of the 3 specific QRDR mutations identified in each *S. pyogenes* isolate are considered to be classic alterations in pneumococci: *gyrA* (serine[81]→tyrosine or phenylalanine) and *parC* (serine[79]→tyrosine or phenylalanine) [20, 21, 27]. An analysis of 49 *S. pneumoniae* isolates with QRDR mutations demonstrated that most isolates with *parC* and *gyrA* mutations were not susceptible to ciprofloxacin, levofloxacin, moxifloxacin, or gatifloxacin, whereas isolates with *parC* mutations alone were only ciprofloxacin nonsusceptible [20]. The susceptibility profiles of the 2 fluoroquinolone-resistant *S. pyogenes* isolates (table 1) suggest a pattern of *gyrA* and *parC* mutation expression similar to that documented in pneumococci [20]. Once an isolate develops a 2-step mutation, all fluoroquinolones should be considered ineffective, regardless of the MIC.

Of the 2 fluoroquinolones tested with enhanced activity against gram-positive bacteria (moxifloxacin and gatifloxacin), moxifloxacin had the greatest activity against this patient's isolate (MIC, 2 μg/mL). The isolate would still be considered nonsusceptible (intermediate resistance) according to the NCCLS breakpoints for *S. pneumoniae* [10]. Of 197 isolates of *S. pyogenes* recovered from adults with pharyngitis in Mexico, where antimicrobials are available without a prescription, 90% had MICs of moxifloxacin of ≤0.25 μg/mL, and the highest MIC was 0.5 μg/mL [28].

It is fortunate that this patient recovered despite receiving only 3 days of therapy with an agent (cefotaxime) that demonstrated good in vitro activity. Routine susceptibility testing is not recommended for *S. pyogenes* and is usually only performed as part of a surveillance program, as was the case for this isolate. By the time that the results of susceptibility testing of this isolate were known, therapy decisions had been made (2 weeks earlier), and there was no evidence of persistent *S. pyogenes* infection. Although most fluoroquinolones have good in vitro activity against *S. pyogenes*, a β-lactam (or a macrolide, for patients who are allergic to penicillin) is considered the primary therapy of choice [13]. Given the uniform activity of β-lactams against *S. pyogenes,* this case provides a reason to avoid the use of fluoroquinolones as empirical therapy for infections that are likely to have been caused by this organism.

The recovery of a second isolate of *S. pyogenes* with high-level fluoroquinolone resistance is another reminder of the inevitable outcome of indiscriminate use of antimicrobials. Ciprofloxacin use has increased in the United States during the past decade from 7.5 million prescriptions in 1990 to 12.9 million prescriptions in 1998 [22]. Although we can only document a single course of fluoroquinolone therapy before recovery of the resistant isolate from our patient, additional exposure may have occurred because of her history of chronic obstructive pulmonary disease. The patient's community-acquired pneumonia is an accepted indication for fluoroquinolone therapy, but inappropriate use must be avoided to minimize the selective pressure for the expansion of what is currently a small population of fluoroquinolone-resistant streptococci.

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