Emergence of Levofloxacin-Resistant Pneumococci in Immunocompromised Adults after Therapy for Community-Acquired Pneumonia

Kevin B. Anderson,1 James S. Tan,1 Thomas M. File, Jr.,1 Joseph R. DiPersio,1 Barbara M. Willey,2 and Donald E. Low2

1Summa Health System, Akron, Ohio; and 2Toronto Medical Laboratories/Mount Sinai Hospital Department of Microbiology, University of Toronto, Toronto, Ontario, Canada

We describe 4 patients infected with levofloxacin-resistant pneumococci after therapy for community-acquired pneumonia (CAP). The 4 patients had 15 episodes of CAP; Streptococcus pneumoniae was isolated from blood or sputum samples obtained during 14 of the episodes. The underlying medical condition was Bruton agammaglobulinemia in 3 patients and chronic lymphoid leukemia in the other. The initial episode of CAP in each patient was due to a levofloxacin-susceptible strain. One of 4 reinfections and 5 of 6 relapses were due to levofloxacin-resistant strains. All of these strains had amino acid substitutions in the quinolone-resistance–determining region of the genes parC and gyrA. The time between episodes of pneumonia varied from 1 to 4 months. In immunocompromised patients with suspected or proven pneumococcal infection, it may be prudent not to use fluoroquinolone monotherapy empirically when the patient has a history of fluoroquinolone therapy in at least the past 4 months.

The emergence of Streptococcus pneumoniae resistant to the β-lactam and macrolide antimicrobial drugs has raised concerns regarding the use of these agents for the empirical treatment of community-acquired pneumonia (CAP) [1]. Fluoroquinolones with increased activity against S. pneumoniae, such as levofloxacin, moxifloxacin, and gatifloxacin, are now being recommended and used for the treatment of patients with CAP who are at risk for infection with multidrug-resistant strains [1–6]. Although the prevalence of levofloxacin-resistant S. pneumoniae is increasing in many parts of the world, the rate of such resistance is <2% in the United States [7–13]. Resistance to the fluoroquinolones can emerge de novo during treatment and, in some cases, can be associated with clinical failures [14–20]. We describe multiple episodes of CAP in 4 immunocompromised patients due to levofloxacin-susceptible pneumococci. There were 6 subsequent episodes due to levofloxacin-resistant pneumococci.

MATERIALS AND METHODS

Definitions. A recurrent disease is defined as a second episode of pneumonia with a similar serotype and similar PFGE type that occurred ≥1 week after a previous episode. A relapse is defined as a second episode of pneumonia with the same serotype/PFGE type that occurred within a week after a previous episode. Reinfection was defined as 2 episodes of pneumonia caused by 2 strains of different serotypes and/or PFGE types.

Patients. The clinical records of 4 patients who...
were assessed at an area hospital in Akron, Ohio, during the period of June 2000 through January 2002 were included in these case reports.

**Microbiologic susceptibility and typing.** In vitro susceptibility testing was performed by broth microdilution testing according to NCCLS guidelines [21, 22]. Susceptibility interpretive criteria used were those published by the NCCLS [23]. All isolates were serotyped by the National Centre for Streptococcus (Edmonton, Alberta, Canada). Isolates were typed by PFGE after digestion with Smal by means of methods described by Murray et al. [24]. Strains were considered to be of the same PFGE type if the patterns had \( \leq 3 \) bands’ difference [25]. However, if there was a new band or new bands (\( \leq 3 \) bands) present, the isolate was noted. Amplification of the parC and gyrA genes and DNA sequencing were performed as described elsewhere [26].

**Confidentiality.** All hospital personnel are required to sign a confidentiality agreement as conditions for employment.

## RESULTS

**Patient A.** A patient with asthma and Bruton agammaglobulinemia was admitted to the hospital on 30 June 2000. CAP was diagnosed. He was treated with a single dose of intravenous IgG (IVIG) and intravenous levofloxacin (500 mg q.d.). Culture of a sputum specimen obtained at admission grew *S. pneumoniae* serotype 6A that was susceptible to levofloxacin (table 1). The patient improved and was discharged home 2 days later receiving oral levofloxacin therapy (500 mg q.d.) to complete 14 days.

Four months later, on 10 November 2000, the patient was again admitted to the hospital and had CAP diagnosed. He had not received a fluoroquinolone since his prior admission. He was provided IVIG and intravenous levofloxacin (500 mg q.d.). Cultures of sputum samples obtained before therapy grew *S. pneumoniae* that was the same serotype (6A), and PFGE revealed an identical DNA pattern as the pneumococcus isolated during the first episode (table 1). However, this isolate was now resistant to levofloxacin (MIC, 16 \( \mu \)g/mL) and was found to have amino acid substitutions in the quinolone-resistance-determining region (QRDR) of *parC* and *gyrA* (table 1). He improved with receipt of levofloxacin therapy and was discharged home the next day receiving oral levofloxacin therapy (500 mg q.d.) to complete 14 days.

Approximately 1 month later, on 23 June 2001, the patient was again admitted to the hospital and received a diagnosis of CAP. Treatment was initiated with levofloxacin (500 mg iv) and azithromycin (500 mg iv). He was discharged the next day receiving oral amoxicillin–clavulanic acid (875 mg b.i.d. for 14 days). The pneumococcal isolates recovered during episodes 3 and 4 were both serotype 9N, had identical PFGE patterns, and were susceptible to levofloxacin.

On 14 September 2001, the patient was admitted to the hospital with signs and symptoms of CAP. He was initially treated with clindamycin (600 mg iv t.i.d.), oral cefixime (400 mg q.d.), and oral ciprofloxacin (500 mg b.i.d.). The *S. pneumoniae* isolated from his sputum sample was serotype 18C and was susceptible to levofloxacin. He improved and was discharged home 2 days later receiving oral levofloxacin (500 mg q.d.) for 14 days.

On 6 October 2001, the patient was admitted to the hospital and received a diagnosis of CAP. Treatment was initiated with intravenous azithromycin (500 mg q.d.). No pathogen was isolated. He improved and was discharged the next day receiving oral amoxicillin–clavulanic acid (875 mg b.i.d. for 13 more days).

On 22 December 2001, the patient was admitted to the hospital and received a diagnosis of CAP. Treatment was initiated with intravenous levofloxacin (500 mg). He improved and was discharged home 2 days later receiving oral levofloxacin (500 mg q.d.) to complete a 14-day course. After discharge, culture results revealed 2 different strains of *S. pneumoniae*, which were isolated from blood samples obtained at admission. One isolate was serotype 7A and was susceptible to levofloxacin. The other isolate was serotype 18C and was identical to the pneumococcus isolated from his sputum >3 months earlier during his fifth episode of CAP. The isolate was now resistant to levofloxacin and was found to have amino acid substitutions in the QRDR of *parC* and *gyrA* (table 1).

On 4 January 2002, the patient was again admitted to the hospital and received a diagnosis of CAP. He completed his 14-day course of oral levofloxacin from his previous discharge on 24 December 2001. In-hospital therapy was initiated with intravenous azithromycin (500 mg q.d.) and ampicillin (500 mg q6h). *S. pneumoniae* was isolated from his blood sample. It was the same serotype (18C with a 1-band difference on the PFGE pattern) as that isolated from his fifth and seventh episodes and illustrates a case of clinical failure of infection to clear due to levofloxacin-resistant *S. pneumoniae*. He improved and was discharged home 3 days later receiving amoxicillin–clavulanate (875 mg) to complete a 14-day course.

**Patient B.** This patient was a blood relative of patient A; he lived in the same town and had recurrent contact with patient A. He had asthma and Bruton agammaglobulinemia and was admitted to the hospital on 30 May 2001 with signs...
and symptoms of CAP. After a sputum sample was obtained for culture, he was treated with IVIG and intravenous levofloxacin (500 mg q.d.). He improved and was discharged the next day receiving oral levofloxacin (500 mg q.d.) to complete a 14-day course of therapy. He improved and was discharged receiving oral levofloxacin (500 mg q.d.) to complete a 14-day course of therapy. Because he was not clinically improving, a specimen was obtained via bronchoalveolar lavage on 20 November 2000. 

Table 1. Microbiological characteristics of *Streptococcus pneumoniae* isolated from sputum samples obtained from patients during different episodes of community-acquired pneumonia.

<table>
<thead>
<tr>
<th>Patient (isolate)</th>
<th>Source</th>
<th>Episode</th>
<th>Date of isolation</th>
<th>Serotype/ PFGE pattern</th>
<th>MIC in μg/mL, by agent</th>
<th>Amino acid substitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>A (9807)a</td>
<td>Sputum</td>
<td>1</td>
<td>30 Jun 2000</td>
<td>6A/AA</td>
<td>(&lt;0.06 &lt;0.12 1 0.25 0.12 0.03)</td>
<td>None</td>
</tr>
<tr>
<td>A (9808)b</td>
<td>Sputum</td>
<td>2</td>
<td>10 Nov 2000</td>
<td>6A/AA</td>
<td>(&lt;0.06 &lt;0.12 16 4 4 0.5)</td>
<td>None</td>
</tr>
<tr>
<td>A (9843)c</td>
<td>Blood</td>
<td>3</td>
<td>25 May 2001</td>
<td>9N/A</td>
<td>(&lt;0.06 &lt;0.12 1 0.25 0.12 0.03)</td>
<td>None</td>
</tr>
<tr>
<td>A (9844)b</td>
<td>Blood</td>
<td>4</td>
<td>23 June 2001</td>
<td>9N/A</td>
<td>(&lt;0.06 &lt;0.12 0.5 0.25 0.12 0.03)</td>
<td>None</td>
</tr>
<tr>
<td>A (9845)</td>
<td>Sputum</td>
<td>5</td>
<td>14 Sep 2001</td>
<td>18C/AA</td>
<td>(&lt;0.06 &lt;0.12 1 0.25 0.12 0.03)</td>
<td>None</td>
</tr>
<tr>
<td>A (9847a)</td>
<td>Blood</td>
<td>6</td>
<td>22 Dec 2001</td>
<td>7F/A</td>
<td>(&lt;0.06 &lt;0.12 1 0.25 0.12 0.03)</td>
<td>None</td>
</tr>
<tr>
<td>A (9847b)</td>
<td>Blood</td>
<td>7</td>
<td>22 Dec 2001</td>
<td>18C/AA</td>
<td>(&lt;0.06 &lt;0.12 16 4 2 0.5)</td>
<td>S-79(-F S-81(+F)</td>
</tr>
<tr>
<td>A (9848)b,c</td>
<td>Blood</td>
<td>8</td>
<td>4 Jan 2002</td>
<td>18C/AA</td>
<td>(&lt;0.06 &lt;0.12 16 4 2 0.5)</td>
<td>S-79(-Y S-81(+F)</td>
</tr>
<tr>
<td>B (9849)a</td>
<td>Sputum</td>
<td>1</td>
<td>30 May 2000</td>
<td>11A/ND</td>
<td>(&lt;0.06 &lt;0.12 1 0.25 0.12 0.03)</td>
<td>None</td>
</tr>
<tr>
<td>B (9850)c</td>
<td>Sputum</td>
<td>2</td>
<td>8 Oct 2001</td>
<td>18C/AA</td>
<td>(&lt;0.06 &lt;0.12 1 0.25 0.12 0.03)</td>
<td>None</td>
</tr>
<tr>
<td>B (9851)b</td>
<td>Sputum</td>
<td>3</td>
<td>12 Jan 2002</td>
<td>18C/AA</td>
<td>(&lt;0.06 &lt;0.12 8 2 2 0.25)</td>
<td>D-83(-Y S-81(+Y)</td>
</tr>
<tr>
<td>C (9809)</td>
<td>Sputum</td>
<td>1</td>
<td>5 Oct 2000</td>
<td>23F/ND</td>
<td>(&lt;0.06 &lt;0.12 1 0.25 0.06 0.015)</td>
<td>None</td>
</tr>
<tr>
<td>C (9810)</td>
<td>Sputum</td>
<td>2</td>
<td>20 Nov 2000</td>
<td>11A/ND</td>
<td>(&lt;0.06 &lt;0.12 4 2 0.5)</td>
<td>S-79(-F S-81(+F)</td>
</tr>
<tr>
<td>D (9841)a</td>
<td>Sputum</td>
<td>1</td>
<td>12 Oct 2001</td>
<td>19F/A</td>
<td>2 8 4 0.25 &lt;0.03 0.015</td>
<td>S-79(-F None</td>
</tr>
<tr>
<td>D (9842)b</td>
<td>Sputum</td>
<td>2</td>
<td>27 Nov 2001</td>
<td>19F/A</td>
<td>2 8 16 4 2 0.25</td>
<td>S-79(-F S-81(+F)</td>
</tr>
</tbody>
</table>

**NOTE.** D, aspartic acid; Em, erythromycin; F, phenylalanine; Gare, garenoxacin; Gati, gatifloxacin; Levo, levofloxacin; Moxi, moxifloxacin; ND, not done; Pen, penicillin; S, serine; Y, tyrosine.

- a Initial occurrence.
- b Relapse (2 strains with the same PFGE type and serotype).
- c Reinfection (2 strains of different serotypes and/or PFGE types).
- d Episode of clinical failure of infection to respond to levofloxacin therapy.

Three months later, on 12 January 2002, patient B was again admitted to the hospital with signs and symptoms compatible with CAP. After obtaining a sputum sample for culture, the patient was treated with IVIG and intravenous levofloxacin (500 mg q.d.) for an additional 13 days. *S. pneumoniae* isolated from the sputum sample was serotype 11A and was susceptible to levofloxacin.

On 8 October 2001, the patient was admitted to the hospital with signs and symptoms compatible with CAP. After obtaining a sputum sample for culture, the patient was treated with IVIG and intravenous levofloxacin (500 mg q.d.) for 2 days before discharge. He improved and was discharged receiving oral levofloxacin (500 mg q.d.) to complete a 14-day course of therapy. The pneumococcal isolate was the same serotype (18C) and had the same PFGE pattern as the pneumococci isolated from his relative’s sputum sample during episode 2, but it was now resistant to levofloxacin. Although this isolate was the same serotype, had the same PFGE pattern, and had the same antimicrobial drug susceptibilities as the pneumococci isolated from his relative’s blood culture<2 weeks before, it was found to have different amino acid substitutions in the QRDR of parC and gyrA (table 1).

**Patient C.** A patient with a history of chronic lymphoid leukemia and aplastic anemia, which was in remission, was admitted to the hospital on 5 October 2000 and received a diagnosis of CAP. Empirical treatment with intravenous levofloxacin (500 mg q.d. for 7 days) was initiated. Gram stain of a sputum specimen obtained at admission revealed grammegative diplococci, and culture grew *S. pneumoniae* susceptible to penicillin, erythromycin, and levofloxacin (table 1). The patient improved and was discharged receiving oral levofloxacin (500 mg q.d.) to complete 14 days of therapy.

Approximately 1 month after completing levofloxacin therapy, the patient was admitted to the hospital again on 14 November 2000 and received a diagnosis of CAP. He was treated with intravenous levofloxacin (500 mg q.d.). Because he was not clinically improving, a specimen was obtained via bronchoalveolar lavage on 20 November 2000. *S. pneumoniae* was isolated and was found to be resistant to levofloxacin (MIC, 16 μg/mL; table 1). The results of the bronchoalveolar lavage...
were not known at the time of discharge, and because the patient showed clinical signs of improvement, he was discharged receiving daily doses of oral levofloxacin to complete a 14-day course.

The *S. pneumoniae* isolated from this patient at the first hospital admission was found to be serotype 23F, and the isolate from the second admission was serotype 11A. PFGE confirmed that the isolates were not clonally related (table 1).

**Patient D.** A patient with Kartagener syndrome and Bruton agammaglobulinemia presented to the emergency department on 15 October 2001 and was admitted to the hospital with complaints of productive cough, fever, and shortness of breath. Two days before hospitalization, on 13 October 2001, he initiated a course of oral levofloxacin (500 mg q.d.). Gram stain of the sputum revealed gram-positive diplococci, and culture grew *S. pneumoniae* that had intermediate resistance to penicillin G, was resistant to erythromycin, and was susceptible to levofloxacin (table 1). He was provided IVIG and treated empirically with intravenous levofloxacin (500 mg q.d.) for 2 days. He improved and was discharged receiving 500 mg of oral levofloxacin daily to complete a 14-day course of therapy.

Approximately 1 month after completing therapy, the patient was admitted to the hospital on 27 November 2001 and received a diagnosis of CAP. He had not received a fluoroquinolone since his last admission. He was provided IVIG and initiated therapy with intravenous levofloxacin (500 mg q.d.) plus piperacillin-tazobactam (3.375 g q6h). Cultures of sputum samples obtained in the emergency department before therapy grew *S. pneumoniae* that had intermediate resistance to penicillin G, was resistant to erythromycin, and was susceptible to levofloxacin (table 1). He was provided IVIG and treated empirically with intravenous levofloxacin (500 mg q.d.) for 2 days. He improved and was discharged receiving 500 mg of oral levofloxacin daily to complete a 14-day course of therapy.

The isolates of *S. pneumoniae* from both admissions were serotype 19F and had identical PFGE patterns (table 1). The *S. pneumoniae* isolated from a sputum sample on 12 October 2001 was found to have an amino acid substitution in the QRDR of parC, only, but the isolate from the second admission was serotype 11A. PFGE confirmed that the isolates were not clonally related (table 1).

**Discussion**

Several studies have shown that the most important risk factor for nasopharyngeal colonization or invasive infection with antibiotic-resistant pneumococci is the recent use of antibiotics [27–29], although the processes resulting in the development of resistance remain unclear. However, it appears that the development of fluoroquinolone resistance is due to the ability of the pneumococcus to give rise to in vivo mutants that are resistant. Previous reports have described the development of pneumococcal resistance to levofloxacin during or after therapy with levofloxacin in either previously healthy patients or patients with underlying lung disease, such as chronic bronchitis [14–18]. Immunocompromised patients may be at even greater risk for the development of resistance to the fluoroquinolones because they lack the immune response necessary to reduce frequency of colonization and length of carriage of *S. pneumoniae* and the density of organisms being carried [30]. Each of these is a risk factor for the development of resistance during therapy. Although there have been only isolated reports of individual strains of *Haemophilus influenzae* and *Moraxella catarrhalis* showing decreased susceptibility to the fluoroquinolones, when it has occurred, it has typically appeared in immunocompromised patients or patients with underlying chronic lung problems who have repeatedly been treated with a fluoroquinolone [31–34].

Two important questions should be considered. After clinical cure of pneumococcal infection, should the patient be screened for carriage state? Does the persistence of pneumococci in the posttreatment nasopharyngeal culture require further treatment to eradicate carrier state, especially among immunocompromised patients? We believe that it may be worthwhile to screen the immunocompromised patients for persistence of pneumococci. If persistence is present, these isolates should be checked for susceptibility and typed if possible. These patients should be observed closely without treatment if they are asymptomatic.

If fluoroquinolone therapy is a risk factor for subsequent fluoroquinolone-resistant pneumococcal infections, how long can a patient carry such a strain and be at the risk of relapse? In our patients, the interval between infection and relapse with a levofloxacin-resistant strain was 1–4 months. However, little is known regarding the length of carriage of pneumococci in the adult. Carriage for >2 years has been reported elsewhere [35, 36]. Immunocompromised patients may be at particular risk for prolonged carriage because immune defects contribute to the length of carriage, as well as increased rates of invasive fluoroquinolone. The reinfections occurred ~1–6 months after the initial episode, and the relapses occurred 1–4 months after the initial infection.
pneumococcal disease [37]. McEllistrem et al. [38] studied the recurrence rate of pneumococcal disease among persons with HIV infection to determine whether it was caused by either a relapse with the same strain or a reinfection with a different strain. Among 41 patients, there were 48 recurrences comprising 6 relapses and 42 reinfections. The median time interval between infections for relapses was 13.1 weeks (range, 2.7–43.3 weeks).

Although patients A, B, and D were known to have low immunoglobulin levels and had been receiving immunoglobulin replacement regularly, we believe that patients with known hypogammaglobulinemia should receive IgG replacement regularly, we believe that patients with known immunoglobulin levels and had been receiving immunoglobulin replacement when infection is suspected. Patient C was treated with levofloxacin for a fluoroquinolone-resistant isolate (MIC, 16 µg/mL). He had a prolonged course of therapy with eventual resolution. This patient is an example that, even though pharmacodynamics would predict failure, even an immunocompromised patient may recover. Patient D’s second episode appeared to have initially responded to piperacillin-tazobactam therapy. This may be because of intermediate susceptibility to penicillin (the isolate had an MIC of penicillin of 2 µg/mL). We did not test the isolate against piperacillin-tazobactam. We presumed that the MIC of piperacillin will be similar to that of penicillin, as with other isolates.

Concern has been expressed that a patient being treated for pneumococcal infection with a fluoroquinolone may be at greater risk for clinical failure as a result of the development of resistance when the infecting strain harbors a mutation that has reduced its susceptibility to fluoroquinolones. Gillespie and Dickens [39] investigated the rate of mutation of clinical isolates of pneumococci to ciprofloxacin and gemifloxacin and found that once a mutation had arisen, a second round of mutations occurred at 12–450-fold higher rates. Conventional susceptibility testing is able to detect clinically relevant resistance but not necessarily low-level resistance.

Previous studies have found that current NCCLS criteria that define the susceptibility category for levofloxacin and moxifloxacin do not always identify those isolates with mutations [26, 40]. Bast et al. [26] characterized 61 pneumococcal isolates with amino acid substitutions at Ser-79 or Asp-83 in parC and/or Ser-81 or Glu-85 in gyrA QRDRs. Thirty-five of 36 isolates with only parC amino acid substitutions were susceptible to levofloxacin (MIC, ≤2 µg/mL). Three isolates had amino acid substitutions in both parC and gyrA, yet were susceptible to moxifloxacin (MIC, ≤1 µg/mL). The percentage of isolates with such amino acid substitution will increase as more fluoroquinolone-resistant pneumococci are isolated. In addition, this occurrence—isolates with first- and second-step mutations that are classified as susceptible—will increase as fluoroquinolones with even greater pneumococcal activity are developed and approved for the treatment of pneumococcal infections. Garenoxacin (BMS 284756) is a des-fluoro(6) quinolone antimicrobial drug currently under clinical development that displays a high degree of in vitro activity against a broad range of bacterial pathogens, including pneumococci [41]. Its in vitro activity and pharmacokinetic parameters suggest that susceptibility MIC breakpoints of at least ≤0.5 µg/mL would be appropriate [42, 43]. However, all 6 isolates from our patients with amino acid substitutions in the QRDR of gyrA and parC had garenoxacin MICs of ≤0.5 µg/mL.

Possible modification of current CAP guidelines may provide guidance for the physician treating similar groups of patients with CAP as reported here. Such patients are currently considered in guidelines from the American Thoracic Society and the Infectious Diseases Society of America as being at high risk for drug-resistant S. pneumoniae infection and, therefore, candidates for empirical therapy with levofloxacin, gatifloxacin, or moxifloxacin [1, 2]. In addition, in immunocompromised patients with suspected or proven pneumococcal infection, it may be prudent to not empirically prescribe fluoroquinolone as a single agent until efficacy data for higher doses of fluoroquinolones are available. At present, therapy with a combination of fluoroquinolone plus another antipneumococcal agent may be a better choice when the patient has previously been treated with a fluoroquinolone in at least the past 4 months.

References


