Quinolone-Resistant *Haemophilus influenzae* in a Long-Term Care Facility: Clinical and Molecular Epidemiology

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We describe a clonal outbreak of quinolone-resistant *Haemophilus influenzae* (QRHI) from an affiliated long-term care facility (LTCF-A); the outbreak was associated with the clinical use of levofloxacin, which was determined to be a risk factor for acquisition of QRHI. The minimum inhibitory concentration to which 90% of isolates were susceptible (MIC90), as determined by broth microdilution, was \( \frac{1}{4} \) mg/mL for levofloxacin, \( \frac{1}{2} \) mg/mL for moxifloxacin, \( \frac{1}{2} \) mg/mL for gatifloxacin, and 8 mg/mL for gemifloxacin. The MIC90, as determined by Etest (AB Biodisk), was \( \frac{1}{32} \) mg/mL for levofloxacin, ciprofloxacin, moxifloxacin, and gatifloxacin. Having been a resident at LTCF-A and having chronic obstructive pulmonary disease were significant risk factors for acquisition of QRHI at our 500-bed hospital (New York Hospital Queens). All QRHI isolates were found to be genetically related by pulsed-field gel electrophoresis, were nontypeable, were susceptible to ceftriaxone and azithromycin, and were negative for \( \beta \)-lactamase production. Emphasis on patient contact and respiratory isolation and placing colonized or infected patients in cohorts yielded a marked reduction in the prevalence of QRHI at LTCF-A.

*Haemophilus influenzae* is a common cause of both upper and lower respiratory tract infections. These organisms frequently colonize the lower respiratory tract of patients with chronic obstructive pulmonary disease (COPD) or cystic fibrosis and are closely associated with acute exacerbations of those diseases. They are also an important cause of pneumonia in elderly patients and in patients with HIV infection [1]. Quinolones are widely used for the treatment of respiratory tract infections, and their use has recently increased extensively in hospitals and long-term care facilities (LTCFs) in association with the evolution of penicillin resistance in *Streptococcus pneumoniae* [2]. Several reports of ciprofloxacin-resistant *H. influenzae* from Europe were published before 1997 [3–5], and a single case report from the United States was published in 1993 [6]. A more recent study from Spain [7] has described a gradual increase in low-level ciprofloxacin resistance in *H. influenzae*. Although national surveillance studies in the United States have documented *H. influenzae* isolates with reduced susceptibility to fluoroquinolones (MIC, \( \geq 0.12 \) µg/mL for \( \geq 2 \) fluoroquinolones), resistance to levofloxacin, as defined by standard criteria, has not been documented since its approval for clinical use in the United States in 1997 [8, 9].

This report describes an outbreak of genetically related levofloxacin-resistant strains of *H. influenzae* that were isolated by our clinical microbiology laboratory (New York Hospital Queens [NYHQ]; Flushing, New York).
York) from 1998 through 2002. All isolates of levofloxacin-resistant *Haemophilus influenzae* (LRHI) were recovered from patients at or recently transferred from an affiliated LTCF (LTCF-A). We report the clinical and molecular epidemiology and antibiotic use associated with this outbreak. We also conducted case-control studies to define risk factors for acquisition of LRHI at our hospital (NYHQ) and at LTCF-A.

**PATIENTS, MATERIALS, AND METHODS**

The clinical microbiology laboratory at NYHQ reports antibiotic susceptibility rates of pathogenic bacteria annually. This laboratory processes all clinical cultures from our acute care hospital and from 5 LTCFs affiliated with the hospital. In 1996, the clinical microbiology laboratory began routine susceptibility testing of quinolones against *H. influenzae* by the Kirby-Bauer disk diffusion method using ofloxacin; this was changed to levofloxacin in 1998. The infectious disease research laboratory subsequently quantified the level of resistance by both Etest methodology (AB Biodisk) and broth microdilution (STPI Sensititre plates; Trek Diagnostic Systems), according to manufacturer recommendations. The clinical microbiology laboratory uses *Haemophilus* test medium agar plates (Becton Dickinson) for Kirby-Bauer susceptibility testing. MIC determination using Etest was performed in the infectious disease research laboratory at NYHQ, although NCCLS does not recognize this method for susceptibility testing of *H. influenzae*.

Data from 2001 indicated that 35% of clinical *H. influenzae* isolates were resistant to levofloxacin. We then reviewed data from 1998 and 1999 retrospectively. Data from 2000 were not available. Subsequently, all LRHI isolates retrieved from 1 January 2002 through 30 September 2002 were saved; in addition, a single LRHI isolate that was obtained in 2001 and that remained viable was also saved.

The clinical records of all patients from whom LRHI isolates were recovered in 2001 were reviewed. All of the case patients from whom LRHI was isolated were identified as being from a distinct unit in LTCF-A in which patients receiving mechanical ventilatory support are treated. Four control subjects who were present in the same ventilatory unit in 2001 but from whom LRHI was not recovered were selected and matched for every case patient. Separate case-control studies were conducted to identify risk factors associated with acquisition of LRHI at NYHQ and at LTCF-A. Case patients were defined as any patient from NYHQ or LTCF-A from whom an LRHI isolate had been recovered in 2001. Potential risk factors studied that could predispose a patient to infection with *H. influenzae* included age, sex, COPD, ventilator use, tracheostomy, ciprofloxacin use, levofloxacin use, HIV infection, and unresponsive mental status. Because all patients came from the same unit, mechanical ventilation was examined as a risk factor in the case-control studies at both NYHQ and LTCF-A. Prior residence at LTCF-A was included among possible risk factors in the NYHQ study. Unconditional logistic regression was used to analyze the data [10]. In this model, the natural logarithm of the OR is a linear function of a set of predictor variables (covariates) whose regression coefficients, when exponentiated, yield the adjusted OR for each covariate. Each OR was accompanied by an approximate 95% CI. Adjusted OR estimates were considered statistically significant if the value of 1.0 was excluded. All statistical calculations were performed using the Stata software package, version 8 (Stata) [11].

The genetic relationship of all LRHI isolates recovered in 2002 and of a single LRHI isolate from 2001 was determined by PFGE using previously published methods [12]. All isolates were serotyped according to manufacturers specifications (Becton Dickinson).

Quinolone use at LTCF-A was determined by the number of prescriptions for levofloxacin and ciprofloxacin, derived from computerized pharmacy profiles. The approximate number of annual days of therapy was derived by multiplying the number of prescriptions by 7, because each prescription that was written was dispensed as a 7-day supply.

After discovery of the cluster of LRHI at LTCF-A, the determination of its clonality by PFGE, and the recognition of its prevalence among the ventilator-dependent population, immediate steps were taken to enhance infection-control efforts within the unit in which patients receiving ventilatory support received care. Environmental specimens were obtained for culture from common surfaces and from ventilators (both internal and external specimens were obtained from ventilators). Extensive environmental cleaning with a diluted bleach solution (1 part bleach per 10 parts water) was undertaken. Staff reeducation and frequent observation of staff practices were performed. These practices included suctioning technique (using a closed suctioning system), changing of tracheostomy tubes, use of ventilators, and hand hygiene (including the use of alcohol-based products). Infected or colonized patients were placed in cohorts, and staff members were placed in cohorts to the extent that this was possible. Weekly sputum samples were obtained for culture from all patients in the ventilator

**Table 1. Total Haemophilus influenzae isolates and levofloxacin-resistant Haemophilus influenzae (LRHI) isolates, by institution.**

<table>
<thead>
<tr>
<th>Strains</th>
<th>NYHQ</th>
<th>All 5 affiliated LTCFs</th>
<th>LTCF-A only</th>
</tr>
</thead>
<tbody>
<tr>
<td>All <em>H. influenzae</em></td>
<td>66 (100)</td>
<td>35 (100)</td>
<td>30 (100)</td>
</tr>
<tr>
<td>LRHI</td>
<td>8 (12.1)</td>
<td>28 (80.0)</td>
<td>28 (93.3)</td>
</tr>
</tbody>
</table>

**NOTE.** LTCF, long term care facility; NYHQ, New York Hospital Queens.

* All patients at NYHQ from whom LRHI was recovered had been transferred from LTCF-A.

b All LRHI isolates were recovered from patients at LTCF-A.
Table 2. Antibiotic susceptibility of 27 levofloxacin-resistant *Haemophilus influenzae* isolates, by broth microdilution and Etest.

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>Broth microdilution, µg/mL</th>
<th>Etest, µg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC&lt;sub&gt;50&lt;/sub&gt;</td>
<td>MIC&lt;sub&gt;90&lt;/sub&gt;</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>&gt;4</td>
<td>&gt;4</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Moxifloxacin</td>
<td>&gt;2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Trovafoxacin</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Gatifloxacin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>&gt;2</td>
<td>&gt;2</td>
</tr>
<tr>
<td>Gemifloxacin&lt;sup&gt;b&lt;/sup&gt;</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>≤0.25</td>
<td>≤0.25</td>
</tr>
<tr>
<td>Azithromycin</td>
<td>0.25</td>
<td>&gt;1</td>
</tr>
<tr>
<td>Penicillin</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>...</td>
<td>...</td>
</tr>
<tr>
<td>Amoxicillin/clavulanic acid</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

**NOTE.** NA, not available; ND, not done.

<sup>a</sup> All isolates were >2.

<sup>b</sup> Microdilution performed separately.

<sup>c</sup> All isolates were ≤0.25.

unit, and duplicate isolates of LRHI from the same patient were excluded.

**RESULTS**

**Sources of resistant isolates.** Antibiotic susceptibility data from the clinical microbiological laboratory at NYHQ revealed that the proportion of *H. influenzae* isolates resistant to levofloxacin increased from 5% in 1998, to 13% in 1999, to 35% in 2001, and to 44% in 2002. In 2001, *H. influenzae* isolates (excluding duplicates) were recovered from 66 patients at NYHQ, 8 of whom harbored LRHI (table 1). LRHI was found in the sputum samples of 7 patients and in the blood samples of 1 patient. All 8 LRHI strains were obtained from patients who had been transferred to NYHQ from LTCF-A. *H. influenzae* was recovered from 35 patients at 5 LTCFs. Of these pa-

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**Figure 1.** PFGE patterns of 27 levofloxacin-resistant *Haemophilus influenzae* isolates obtained during 2002 and 1 viable isolate obtained during 2001 (lane 1). Lanes 6, 13, and 26, λ ladder DNA standards.
Table 3. Multivariate analysis of a case-control study of patients with levofloxacin-resistant *Haemophilus influenzae* isolates treated at New York Hospital Queens.

<table>
<thead>
<tr>
<th>Variable</th>
<th>OR (95% CI)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Quinolone use</td>
<td>0.12 (0.01–1.69)</td>
<td>.116</td>
</tr>
<tr>
<td>Age</td>
<td>0.96 (0.91–1.02)</td>
<td>.168</td>
</tr>
<tr>
<td>Sex</td>
<td>12.67 (0.52–306.80)</td>
<td>.118</td>
</tr>
<tr>
<td>LTCF-A</td>
<td>19.04 (1.26–296.55)</td>
<td>.033</td>
</tr>
<tr>
<td>COPD</td>
<td>66.95 (1.60–2797.25)</td>
<td>.027</td>
</tr>
<tr>
<td>Ventilator dependence</td>
<td>24.49 (0.10–6089.69)</td>
<td>.256</td>
</tr>
<tr>
<td>Tracheostomy</td>
<td>0.06 (0.0003–11.7118)</td>
<td>.296</td>
</tr>
<tr>
<td>UNMS</td>
<td>1.19 (0.08–17.98)</td>
<td>.901</td>
</tr>
</tbody>
</table>

**NOTE.** COPD, chronic obstructive pulmonary disease; LTCF-A, residence at affiliated long-term care facility A; UNMS, unresponsive mental status.

Pertinent patients, 28 harbored LRHI, and all 28 were from LTCF-A. Among 30 patients at LTCF-A from whom *H. influenzae* was recovered, 28 (93.3%) were colonized or infected with LRHI. The majority of these patients had evidence of acute pulmonary infections, manifested by fever (in 19 of 28 patients), increased quantity of purulent tracheal secretions (in 16 of 28), and infiltrates noted on chest radiographs (in 7 of 28). All *H. influenzae* isolates that were obtained from the other 4 LTCFs during 2001 were susceptible to levofloxacin.

**Susceptibility studies.** Microdilution and Etest susceptibility studies revealed high-level resistance to levofloxacin, as well as to ciprofloxacin, moxifloxacin, trovafloxacin, gemifloxacin, and gatifloxacin (table 2). Most isolates were uniformly susceptible to several other antibiotics, including azithromycin, amoxicillin-clavulanic acid, ampicillin, ceftriaxone, and penicillin (table 2).

**PFGE and serotyping.** PFGE analysis revealed that all the LRHI isolates retrieved in 2002 from NYHQ and LTCF-A were highly related, as was the only viable isolate recovered in 2001 from LTCF-A (figure 1). One isolate was obtained from the sputum samples of a physician who was a patient at NYHQ and who had cared for patients at LTCF-A. All 27 LRHI isolates were nontypeable using standard serological methods.

**Case-control studies.** A multivariate logistic regression model that included quinolone use, age, sex, residence at LTCF-A, COPD, ventilator dependence, tracheostomy, and unresponsive mental status was used to analyze case-control data from patients at NYHQ. Residence at LTCF-A (OR, 19.0; 95% CI, 1.3–286.6) and COPD (OR, 67.0; 95% CI, 1.6–2797.3) were significant independent predictors of LRHI (table 3). A second multivariate logistic model that included levofloxacin or ciprofloxacin use, age, sex, COPD, ventilator dependence, and tracheostomy was used to analyze case-control data from LTCF-A. Table 4 shows that levofloxacin use was the only significant predictor of LRHI (OR, 3.0; 95% CI, 1.2–8.0).

**Quinolone use at LTCF-A.** The data in table 5 show a decrease in ciprofloxacin use and an increase in levofloxacin use from 2000 to 2001 at LTCF-A.

**Infection-control intervention at LTCF-A.** Infection-control interventions and sputum surveillance cultures (performed at admission and on a weekly basis thereafter) were initiated in the ventilator unit during the first week of November 2002. Surveillance and weekly cultures of sputum samples continued through the third week of December 2002. LRHI was cultured from sputum samples obtained from 12 of 47 patients in the unit during November 2002 (22 of 129 cultures grew LRHI). As enhanced infection-control procedures continued, 5 of 47 patients had cultures of sputum samples that yielded LRHI in December 2002 (6 of 60 cultures grew LRHI). Four of these 5 patients had cultures positive for LRHI during the previous month. From January through March 2003, LRHI was recovered from 1 patient during each month. Cultures of samples obtained from these patients had previously yielded LRHI. During the period from April through June 2003, LRHI was recovered from 2 new patients.

**DISCUSSION**

Respiratory tract infections are common among individuals in LTCFs and are associated with a high mortality rate [13, 14]. Studies have suggested that infections in LTCFs are not adequately evaluated and that antibiotic therapy is usually initiated empirically, enhancing selection of antibiotic-resistant bacteria [13–15]. As a result, quinolones are commonly used as first line agents because of their broad antimicrobial coverage, excellent gastrointestinal absorption, limited side effects, and once- or twice-daily dosing.

Quinolone resistance in *S. pneumoniae* has been increasing and is associated with increasing use of fluoroquinolones [16–18]. In contrast with prior surveillance studies in the United States, we identified clinical isolates of *H. influenzae* that had high-level resistance to levofloxacin and other quinolones. Such
resistance emerged exclusively in patients located in or previously residing in LTCF-A.

In this study, all isolates collected and analyzed by PFGE in 2002 and 1 viable isolate from 2001 showed highly related patterns. Our case-control study at NYHQ, which involved all patients from whom LRHI was recovered in 2001, identified residence in LTCF-A and presence of COPD as significant risk factors for the acquisition of LRHI. The results of a case-control study at LTCF-A showed that only levofloxacin use was a statistically significant risk factor by multivariate analysis. Although levofloxacin use, from the day of admission to LTCF-A to the date on which the first LRHI isolate was recovered, was examined as a risk factor in all patients from whom an LRHI was isolated, the duration and frequency of levofloxacin use varied from case to case. Ho et al. [17] reported that the presence of COPD, residence in a nursing home, and quinolone therapy were significant risk factors for the acquisition of levofloxacin-resistant S. pneumoniae. Our findings demonstrate similar risk factors and suggest that the emergence of quinolone resistance in H. influenzae may have been due to an increase in empirical use of levofloxacin at LTCF-A. A recent report from Spain by Bastida et al. [19] documented an LRHI isolate and the clinical failure of levofloxacin therapy in a patient who received moxifloxacin 2 months before the isolation of LRHI. Analysis of their strain revealed 4 mutations in the quinolone-resistance determining regions (QRDRs) of DNA gyrase and topoisomerase IV and was similar to mutational analysis of the QRDRs in our strains (X. Li, C. Urban, K. Drlica, unpublished data). The epidemiological study performed on all LRHI isolates obtained during the first 9 months of 2002 and on 1 isolate from 2001 revealed highly related PFGE patterns, suggesting clonal spread. The reasons for the successful persistence of this non-typeable clone are unknown at the present time, but its persistence may have been due to high expression of IgA protease, colonization factors, or other molecular determinants [20–22]. Our results also suggest that breaches in infection-control practices may have resulted in the outbreak and spread of this clone, as in a recently described outbreak of fluoroquinolone-resistant S. pneumoniae [18].

Because of an increase in quinolone use in the treatment of respiratory tract infections in hospitals and LTCFs, susceptibility to quinolones in H. influenzae and other respiratory pathogens should be monitored routinely. Because initial treatment of respiratory tract infections is usually initiated empirically, routine susceptibility surveillance is the only mechanism by which emerging resistance can be identified. Thus, the recent suggestion that quinolones may be acceptable agents for empirical treatment of respiratory tract infection in nursing home patients should apply only if susceptibility information is available to the treating physicians [13]. Our review of antibiograms from 19 neighboring hospitals in the New York City metropolitan area showed that only 4 hospitals routinely tested H. influenzae for susceptibility to any quinolone. Because of the frequent lack of infection-control personnel, on-site microbiology laboratories, and infectious disease physicians, control of emerging resistant pathogens may be less than adequate in LTCFs [22]. These factors, coupled with the debilitated patient population, suggest that LTCFs may be an underestimated reservoir for QRHI. Because patients are frequently transferred from LTCFs to neighboring hospitals, LTCFs may also provide a source for introduction and spread of antibiotic-resistant common respiratory pathogens.

This report highlights several important issues. First, it is crucial to maintain appropriate infection-control techniques in the long-term care environment. This is particularly important because it is increasingly common for LTCFs to care for patients who are dependent on ventilator assistance and who are placed in a common geographic unit, as in LTCF-A. Second, the close administrative, clinical, and laboratory relationship between NYHQ and LTCF-A provided an opportunity for oversight of clinical antibiotic susceptibility data, including that from LTCF-A, by a university-affiliated infectious disease section. And, finally, it demonstrates the potential benefits of interinstitutional collaborative research involving clinical and molecular epidemiology, infection-control, and antimicrobial resistance mechanisms in controlling antibiotic resistance in both tertiary and long-term care settings.

References

