Pseudo-Outbreak of *Legionella pneumophila* Serogroup 8 Infection Associated With a Contaminated Ice Machine in a Bronchoscopy Suite

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**Objective.** To investigate the marked increase noted over an 8-month period in the number of *Legionella pneumophila* isolates recovered from bronchoalveolar lavage fluid specimens obtained during bronchoscopy in our healthcare system.

**Setting.** Bronchoscopy suite that serves a 580-bed tertiary care center and a large, multisite, faculty practice plan with approximately 2 million outpatient visits per year.

**Methods.** Cultures of environmental specimens from the bronchoscopy suite were performed, including samples from the air and water filters, bronchoscopes, and the ice machine, with the aim of identifying *Legionella* species. Specimens were filtered and acid-treated and then inoculated on buffered charcoal yeast extract agar. Serogrouping was performed on all isolates recovered from patient and environmental samples.

**Results.** All *L. pneumophila* isolates recovered from patients were serogroup 8, a serogroup that is not usually recovered in our facility. An epidemiologic investigation of the bronchoscopy suite revealed the ice machine to be contaminated with *L. pneumophila* serogroup 8. Patients were exposed to the organism as a result of a recently adopted practice in the bronchoscopy suite that involved directly immersing uncapped syringes of sterile saline in contaminated ice baths during the procedures. At least 1 patient was ill as a result of the pseudo-outbreak. Molecular typing of isolates recovered from patient and environmental samples revealed that the isolates were indistinguishable.

**Conclusions.** Extensive cleaning of the ice machine and replacement of the machine’s water filter ended the pseudo-outbreak. This episode emphasizes the importance of using aseptic technique when performing invasive procedures, such as bronchoscopies. It also demonstrates the importance of reviewing procedures in all patient areas to ensure compliance with facility policies for providing a safe patient environment.

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Legionellosis is caused by infection with gram-negative bacteria belonging to the family Legionellaceae. Infection with these bacteria can result in 2 distinct clinical entities, pneumonia due to Legionnaires disease or Pontiac fever, a milder, influenzalike illness. The Legionellaceae family comprises 50 species and at least 64 serogroups, with *Legionella pneumophila* serogroup 1 implicated in the majority (70%-90%) of cases of human *Legionella* infection.1 *Legionella* species are ubiquitous in freshwater environments. The most common mechanism of transmission for humans is through the inhalation of contaminated aerosols.2 Many environmental sources have been implicated in *L. pneumophila* infection, including contaminated water from condensers, showers, cooling towers, humidifiers, whirlpool spas, and a decorative fountain.3-7 Recently, residential water systems have been recognized as important sources of infection, particularly for immunocompromised individuals.8 In the spring of 2007, we became aware of a marked increase in the number of *L. pneumophila* isolates recovered from patients who underwent bronchoscopy in our healthcare system. This report details our investigation into the cause of this pseudo-outbreak and the use of environmental intervention to control it.

**Methods**

The pseudo-outbreak occurred in a bronchoscopy suite that serves a 580-bed tertiary care center and a large, multisite, faculty practice plan that has approximately 2 million outpatient visits per year. Over an 8-month period, cultures of bronchoalveolar lavage (BAL) fluid specimens from 13 pa-
tients grew sparse colonies of *L. pneumophila* serogroup 8. All 13 patients had undergone bronchoscopic procedures at 1 bronchoscopy suite in the hospital. None of the patients showed clinical evidence of infection with *Legionella* species at the time the organism was initially isolated. An investigation was initiated to locate the source of the organism.

Cultures of environmental samples from the bronchoscopy suite were performed with the aim of identifying *Legionella* species; samples were obtained from bronchoscope suction channels and biopsy ports, water filters, inlet and outlet water valves, air filters, and the ice machine in the unit. *Legionella* cultures were performed by the Clinical Microbiology Laboratory of Emory University Hospital, in accordance with the procedure suggested by the Centers for Disease Control and Prevention for recovery of *Legionella* species from the environment. In brief, 100 mL of water from various specimen sites was filtered through a 0.2-micron-pore filter (Nalgene). The filter was then mixed in a vortex mixer with 10 mL of the filtered water to obtain a 10-fold dilution of the sample. A total of 1.1 mL of acid solution (0.2 mol/L HCl [pH 2.2]) was added to the concentrated solution (1:10) and incubated at room temperature for 15 minutes to kill other bacteria present in the sample. Finally, 0.1 mL of the specimen was inoculated onto buffered charcoal yeast extract agar and evaluated at 2, 5, and 7 days for growth.

All patient specimens and environmental cultures positive for *Legionella* were sent to the Centers for Disease Control and Prevention for serogrouping, at which in-house slide agglutination with serogroup-specific antisera was used, followed by in-house direct fluorescent antibody confirmation. Molecular typing was performed by repetitive polymerase chain reaction using the DiversiLab system (bioMérieux) with *Legionella*-specific primers.

**RESULTS**

**Patients**

The first patient from whom *L. pneumophila* serogroup 8 was isolated was a 64-year-old man who had undergone a bronchoscopy to evaluate a mass lesion seen on a computed tomography scan of the chest. The patient had recently been going bronchoscopy for evaluation of possible sarcoidosis. Over the next 3 weeks, cultures of BAL fluid from 2 more patients grew sparse colonies of *L. pneumophila* serogroup 8. Both of these patients were immunosuppressed but did not appear to be acutely ill with *L. pneumophila* infection. The Centers for Disease Control and Prevention confirmed that the patients’ *L. pneumophila* isolates were serogroup 8, a rare serogroup among the patient population served by the healthcare center and its laboratory.

The laboratory had previously isolated *Legionella* species from patient specimens fewer than 2 times per year. Because of the rising number of specimens that contained *L. pneumophila* serogroup 8 isolates (5 specimens over a 5-month period) obtained from patients who received bronchoscopies in a particular suite, an epidemiologic investigation of the bronchoscopy unit was initiated. Eight more patients had culture results positive for *L. pneumophila* serogroup 8 over the next 4 months, during the time the epidemiologic investigation was taking place. Table 1 provides demographic and clinical characteristics for the 13 patients from whom *L. pneumophila* serogroup 8 was isolated.

Culture plates from all 13 patients showed sparse colonies of *L. pneumophila*, with 1–3 colonies per plate. Although 8 of the 13 patients were immunosuppressed, none showed clinical evidence of legionellosis. One of the 13 patients (patient 8) had chronic lung disease and was observed to have multiple bullae on initial bronchoscopy. No evidence of infection was found, although BAL fluid from this patient grew 3 colonies of *L. pneumophila* serogroup 8. Three weeks later, the patient underwent a bullectomy and partial pneumonectomy. Pathology results from that procedure revealed acute bacterial infection, and culture of the bullous resected lung tissue once again grew *L. pneumophila* serogroup 8.

Because the clinicians, epidemiologists, and microbiology laboratory staff were communicating closely during the pseudo-outbreak, patients were monitored for clinical signs of legionellosis. Since there was little clinical suspicion of legionellosis, serologic testing for *Legionella* was not performed for any of the patients, although 1 patient (patient 13) did have a urinary *Legionella* antigen test performed, the result of which was negative.

No other cases of *Legionella* infection were noted in the hospital or clinic during the time of this investigation. Over 1 year after the pseudo-outbreak, 1 patient with mixed connective tissue disease who was receiving long-term steroid therapy developed severe legionellosis with acute respiratory distress syndrome. *L. pneumophila* serogroup 8 was the only organism isolated from the patient’s BAL fluid. This case was determined to be community acquired. Regardless, repeated cultures of environmental samples were performed, including water, ice, and filters from the bronchoscopy suite, all of which were negative for *Legionella* species. Apart from this
<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age, years</th>
<th>Sex</th>
<th>Date of BAL</th>
<th>Patient history</th>
<th>Indication for BAL</th>
<th>Outpatient</th>
<th>Other organism recovered from BAL fluid culture</th>
<th>Other pathology results for BAL fluid</th>
<th>Action taken by clinician</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>64</td>
<td>M</td>
<td>11/24/06</td>
<td>Recently treated community-acquired pneumonia; mass lesion on chest CT scan</td>
<td>Mass lesion</td>
<td>No</td>
<td>Noardia nova</td>
<td>Cytology negative for malignancy</td>
<td>Nocardia treatment</td>
</tr>
<tr>
<td>2</td>
<td>74</td>
<td>F</td>
<td>2/20/07</td>
<td>History of sarcoidosis and increasing shortness of breath</td>
<td>Decreasing pulmonary function</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>3</td>
<td>46</td>
<td>F</td>
<td>3/23/07</td>
<td>Possible sarcoidosis with chronic adenopathy; no acute symptoms</td>
<td>Sarcoidosis</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>4</td>
<td>70</td>
<td>M</td>
<td>3/28/07</td>
<td>Chronic wheezing following lobectomy for lung cancer</td>
<td>Wheezing</td>
<td>Yes</td>
<td>Cryptococcus neoformans</td>
<td>None</td>
<td>Cryptococcus treatment</td>
</tr>
<tr>
<td>5</td>
<td>54</td>
<td>F</td>
<td>4/10/07</td>
<td>Stem cell transplant, with pneumonia</td>
<td>Pneumonia</td>
<td>No</td>
<td>None</td>
<td>Granulomas on biopsy</td>
<td>None</td>
</tr>
<tr>
<td>6</td>
<td>57</td>
<td>F</td>
<td>4/16/07</td>
<td>Lung transplant</td>
<td>Routine surveillance for rejection</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>7</td>
<td>69</td>
<td>F</td>
<td>4/19/07</td>
<td>Lung transplant, with acute shortness of breath</td>
<td>Pneumonia</td>
<td>No</td>
<td>Parainfluenza 3</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>8</td>
<td>58</td>
<td>M</td>
<td>5/11/07</td>
<td>Hemoptysis and weight loss, with bullae and mass lesion on chest CT scan</td>
<td>Mass lesion</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>Bullae excision¹</td>
</tr>
<tr>
<td>9</td>
<td>66</td>
<td>M</td>
<td>5/25/07</td>
<td>Hemoptysis and nodules on chest CT scan</td>
<td>Nodules</td>
<td>Yes</td>
<td>None</td>
<td>Chronic inflammation on biopsy</td>
<td>Diagnostic evaluation for hemoptysis</td>
</tr>
<tr>
<td>10</td>
<td>54</td>
<td>M</td>
<td>6/1/07</td>
<td>Leukemia on chemotherapy, with infiltrate on chest CT scan</td>
<td>Infiltrate</td>
<td>Yes</td>
<td>None</td>
<td>Organizing pneumonia on biopsy</td>
<td>None</td>
</tr>
<tr>
<td>11</td>
<td>27</td>
<td>M</td>
<td>6/7/07</td>
<td>Mediastinal adenopathy</td>
<td>Adenopathy</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>12</td>
<td>66</td>
<td>F</td>
<td>6/12/07</td>
<td>Pulmonary fibrosis, with 1-year history of lung nodule</td>
<td>Nodule</td>
<td>Yes</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>13</td>
<td>56</td>
<td>M</td>
<td>8/30/07</td>
<td>Lung transplant</td>
<td>Routine surveillance for rejection⁰</td>
<td>Yes</td>
<td>Few dematiaceous mold colonies</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>

**Note.** CT, computed tomography.

¹ Lung tissue from excised bullae obtained from patient 8 grew *L. pneumophila* serogroup 8 on culture.

⁰ Patient 13 also underwent bronchoscopies on April 3 and May 31, 2007.
case, no other Legionella serogroup 8 isolates have been recovered since the investigation of the pseudo-outbreak began.

**Epidemiologic Investigation**

A targeted investigation was initiated in the bronchoscopy suite. The bronchoscopy procedures during which specimens were obtained that yielded Legionella species on culture were performed by 3 physicians who used 3 different bronchoscopes. Cultures of water samples from the suction channels and biopsy ports of the bronchoscopes as well as the water filters of the intake water lines of the bronchoscopy suite were performed. Figure 1 shows the time line of the epidemiologic investigation.

After culture results for samples from the 3 bronchoscopes and the water system were repeatedly negative, a nurse from the bronchoscopy suite pointed out a relatively new practice that was being used to keep sterile saline chilled. Several months previously, the staff had begun to immerse uncapped syringes of sterile saline directly into ice baths. This saline was used to flush the bronchoscope when there was excessive bleeding during the procedure. The ice in the ice bath was taken from an ice machine in the unit. Subsequent cultures of ice from the machine grew L. pneumophila serogroup 8, as did samples from the machine’s water filters. The practice of immersing syringes of saline directly into ice was discontinued and replaced by a practice of immersing the entire bottle of saline in ice and drawing aliquots of saline directly from the bottle using sterile technique, to avoid contact with the ice. It was further noted that the other bronchoscopy suite served by the hospital laboratory, which had not observed an increase in the number of cultures yielding Legionella species, did not immerse their saline-filled syringes directly into ice baths. Although the samples from the ice machine were not cultured at the other facility, a retrospective review of patient Legionella culture results from that facility showed no increase over baseline during the past 7 years. In addition, that bronchoscopy suite was in a facility served by a different water supply company.

The ice machine was removed and disinfected, and the inlet water filter was replaced. Reports that the ice machine filter had not been changed for several years could not be confirmed; however, hospital facilities did not have a record of filter changes for the contaminated ice machine from the time it had been installed, which was several years previously. Although the facility had a policy of routinely servicing all ice machines and changing their water filters, the ice machine in this suite had been installed by an outside contractor during renovation of the suite. The hospital facilities department had never been informed about the ice machine and had not added it to their maintenance log. Follow-up cultures of samples from the ice machine obtained several months later were negative for Legionella species. Fifteen months later, in September 2008, repeated cultures of ice and samples from the machine’s filters remained negative for Legionella.

Molecular typing was performed on all patient isolates (excluding the isolate recovered from patient 1, which had not been saved), as well as the isolate recovered from the ice machine. Repetitive polymerase reaction typing showed that

**Figure 1.** Time line of patient cultures positive for Legionella pneumophila serogroup 8 and the corresponding time line for the epidemiologic investigation initiated in the bronchoscopy suite where these 13 patients underwent bronchoscopy.
11 of 12 isolates recovered from patients were indistinguishable from one another and from the isolate recovered from the ice machine (greater than 99% correlation). The single patient isolate that typing showed to be similar but distinguishable from the other isolates differed by only 1 band.

**DISCUSSION**

We report a pseudo-outbreak of *L. pneumophila* serogroup 8 infection associated with a contaminated ice machine in a bronchoscopy suite. Cultures of BAL fluid from 13 patients grew sparse colonies of *L. pneumophila* serogroup 8, and 1 of the patients developed infection due to *L. pneumophila*, most likely as a result of bacteria introduced during bronchoscopy.

Bronchoscopes have been associated with multiple outbreaks and pseudo-outbreaks of lower respiratory tract infection in the past.10,11 Mitchell and colleagues12 reported a pseudo-outbreak of *L. pneumophila* serogroup 6 infection that was associated with the rinsing of bronchoscopes in contaminated tap water. However, to our knowledge, this represents the first reported pseudo-outbreak of *L. pneumophila* serogroup 8 infection that has been linked to contamination during bronchoscopy and the first reported case of nosocomial infection with *L. pneumophila* serogroup 8 secondary to bronchoscopic contamination.

*L. pneumophila* serogroup 8 was first isolated in 1981 from a postmortem lung culture.13 A severe case of Legionnaires disease due to serogroup 8 was reported in 1987 in Germany.14 Two more recent case reports implicate serogroup 8 as a coinfecting organism in pulmonary infections, accompanying *L. pneumophila* serogroup 1 and *Aspergillus fumigatus*.15,16 *L. pneumophila* serogroup 8 remains a rare cause of pneumonia, as evidenced by a European study of culture-proven legionellosis, which implicated serogroup 8 in less than 1% of cases.17 In France, serogroup 8 was identified in only 3 of 1367 cultures of samples from patients with Legionnaires disease from 1998 through 2005.16 In the United States, the most common *L. pneumophila* serogroups isolated—after serogroup 1—are serogroups 3 and 6; taken together, serogroups 7–14 account for less than 1% of the isolates that cause Legionnaires disease.18

Molecular typing confirmed that 11 of the 12 isolates recovered from patients in the present study were indistinguishable from one another and from the strain recovered from the ice machine, evidence that supports the conclusion that this was a pseudo-outbreak involving this unusual serotype. The rarity of serogroup 8 in our laboratory and in the United States further supports the conclusion that these isolates were related, as does the clustering of cases over a short time period. A review of all *Legionella* species isolated over the past 6 years at our microbiology laboratory revealed that the species identity, serogroup, and distribution of the 11 previously recovered *Legionella* isolates was as follows: *L. pneumophila* serogroup 1 (6 [54%]), *L. pneumophila* serogroup 6 (2 [18%]), *L. pneumophila* serogroup 13 (1 [9%]), *L. micdadei* (1 [9%]), and untypeable *L. pneumophila*, (1 [9%]).

We were able to identify this pseudo-outbreak before more clinical infections developed in patients because of the laboratory’s policy of automatically culturing lower respiratory tract, bronchoscopy, and lung biopsy specimens onto specialized *Legionella* culture media. *Legionella* species will not grow well on routine bacteriology media, and we inoculate buffered charcoal yeast extract agar plates for the isolation of *Legionella*. It is important to note, however, that not all clinical microbiology laboratories in the United States automatically culture lower respiratory tract specimens for isolation of *Legionella* species and may miss infection due to this fastidious bacterium.

The relatively new practice in the bronchoscopy suite of immersing the syringes containing saline directly into a beaker of ice water may have led to introduction of bacteria into the patients’ lungs. Only 1 patient became infected with *L. pneumophila*. This infection most likely represented introduction of the organism at the time of the patient’s initial bronchoscopy and subsequent development of nosocomial infection. Patient 13 might have been harboring *Legionella* for several months, as his cultures grew *L. pneumophila* serogroup 8 two months after the ice machine had been decontaminated. The patient had undergone 2 bronchoscopies during the time of the pseudo-outbreak, and we suspect that he was initially colonized as a result of unrecognized contamination. It is remarkable that, although 8 of the 13 patients in this series were immunosuppressed, there was only 1 clinically apparent infection due to *L. pneumophila*, which emphasizes the fact that the inoculum of bacteria introduced from the syringe during this procedure was likely to have been very small.

We report a pseudo-outbreak of infection due to *L. pneumophila* serogroup 8 that was ultimately traced to a contaminated ice machine in a bronchoscopy suite. One patient developed lower respiratory tract infection due to this organism. This episode emphasizes the importance of following aseptic technique when performing invasive procedures. It also demonstrates the importance of reviewing protocols in all areas where such procedures are performed to ensure compliance with facility policies for providing a safe patient environment.

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