Legionnaires’ Disease Caused by *Legionella londiniensis*

Christina Stallworth,a Lisa Steed,a Mark A. Fisher,b, and Frederick S. Noltea

Department of Pathology and Laboratory Medicine, Medical University of South Carolina, Charleston, South Carolina, USA,a and Department of Pathology and ARUP Laboratories, University of Utah, Salt Lake City, Utah, USAb

*Legionella londiniensis* has been isolated from aqueous environments. However, to our knowledge, this organism has never been isolated from clinical specimens. A case of Legionnaires’ disease in a hematopoietic stem cell transplant recipient caused by this organism is described, which confirms that *L. londiniensis* can be an opportunistic pathogen.

**CASE REPORT**

A 57-year-old female presented to a primary care clinic in Charlotte, NC, with fever, chills, and a productive cough of thick green-brown sputum for 2 days’ duration, for which she was prescribed ciprofloxacin. She left for vacation in South Carolina, where she experienced worsening sputum production, increased shortness of breath, and a fever of 38.3°C. Her oxygen saturation was noted as 72% on 2 liters of oxygen/min. One day later, she was taken to a local emergency department for further evaluation.

Her medical history was significant for chronic lymphocytic leukemia (status, postmyeloablative), a sibling allogeneic bone marrow transplant 7 months prior, cytomegalovirus pneumonia, *Pneumocystis jiroveci* pneumonia, and parainfluenza respiratory viral infection 5 months prior, which left her oxygen dependent. Current medications included acyclovir, voriconazole, atovaquone, and long-term prednisone therapy.

On physical examination, the patient was febrile at 38.5°C, tachycardic at 123 beats per minute, and tachypneic at 30 breaths per minute. She was hypoxic, with oxygen saturation of 78% on room air. Auscultation of the lungs revealed coarse breath sounds. Pitting edema (grade 2 to 3+) was present in the lower extremities. Hematologic studies revealed a white blood cell count of 2,620/mm³, hemoglobin of 9.1 g/dl, hematocrit of 27.8%, and a platelet count of 43,000/mm³. Results of comprehensive metabolic panel tests were all within normal limits. Computed tomography of the chest revealed extensive consolidation within the left upper lobe and patchy consolidation in the left lower lobe. A small left pleural effusion and mediastinal lymphadenopathy were also present. The patient was intubated and admitted to the intensive care unit. She was empirically treated for pneumonia in an immunocompromised host with cefepime, linezolid, azithromycin, and trimethoprim/sulfamethoxazole.

The following tests were obtained: respiratory virus panel PCR (xTAG RVP; Luminex, Austin, TX), a procalcitonin test (Vidas BRAHMS; bioMérieux, Durham, NC), a serum cryptococcal antigen test, aerobic and anaerobic bacterial blood cultures, a *Legionella* urinary antigen test (Binax/Alere, Waltham, MA), a cytomegalovirus plasma viral load, a urine culture, and sputum cultures for bacteria, acid-fast bacilli, and fungi. The respiratory virus panel was positive for parainfluenza virus type 3. The procalcitonin test result was 6.34 ng/ml. The urine culture was positive for *Esherichia coli* at greater than 100,000 colonies/ml. All other tests were negative. Due to the severity of her symptoms, the patient underwent bronchoalveolar lavage (BAL), and the sample was sent for cytologic examination and microbiological studies.

Cytologic examination of the BAL fluid specimen revealed acute inflammation. Grocott’s methenamine silver stain for fungi and *Pneumocystis* and auramine rhodamine stain for acid-fast organisms were negative. Numerous white blood cells and no organisms were seen on Gram stain with safranin as the counterstain. The specimen was inoculated on the following media: Trypticase soy agar with 5% sheep blood, MacConkey II agar, chocolate GC II agar with hemoglobin and IsoVitaleX, and buffered charcoal yeast extract (BCYE) agar supplemented with α-ketoglutarate and containing vancomycin, polymyxin B, and anisomycin (BD BBL, Sparks, MD). All media were incubated at 37°C in 2 to 5% CO₂. No growth was seen on sheep blood, chocolate, and MacConkey agars. Tiny gray-blue, iridescent colonies were observed on BCYE agar on day 4 of incubation. A Gram stain of the colonies revealed thin, filamentous Gram-negative rods. The organism was subcultured to a second BCYE plate, and the pure culture was subsequently sent to ARUP Laboratories for further identification by partial ribosomal DNA sequencing using SmartGene and NCBI databases (8). The isolate was identified as most closely related to *Legionella londiniensis*.

The patient was treated for Legionnaires’ disease with 500 mg intravenous azithromycin daily for 21 days. The patient’s condition improved. She was subsequently extubated and was discharged comfortably on 3 liters of oxygen/min via nasal cannula 36 days after admission to the hospital.

*Legionella pneumophila* serogroup 1 causes 95 to 98% of community-acquired Legionnaires’ disease, which is a severe and often life-threatening pneumonia in the immunocompromised host. The Pontiac monoclonal antibody (MAb) 3-1 subgroup accounts for 80 to 90% of clinical isolates of this serogroup. Infection by *L. pneumophila* serogroup 1 is less common in immunocompromised patients, and up to 60% of nosocomial Legionnaires’ disease cases may be caused by other *L. pneumophila* serogroups and *Legionella* spp. Of the 52 validly named species,
only 20 have been isolated from both humans and the environment (3).

The patient described in this case report had major risk factors for developing Legionnaires’ disease, which included immunosuppression with corticosteroids and chronic lung disease. L. londiniensis has been isolated from cooling tower pond water from an office building in London, United Kingdom, and from hot spring water samples in Miyazaki and Shizuoka Prefectures in Japan (2, 4), but to our knowledge this is the first isolate from a clinical specimen and the first reported isolate in the United States. The environmental factors and source of L. londiniensis causing the pulmonary disease in our patient are unknown. Our patient also had parainfluenza virus type 3 detected by PCR in the same BAL fluid sample from which we isolated L. londiniensis. It is interesting to note that she had a parainfluenza virus infection that left her dependent on supplemental oxygen 5 months prior to the episode of pneumonia described here. Parainfluenza viruses are associated with symptomatic upper and lower respiratory tract infections as well as prolonged, asymptomatic respiratory tract shedding in hematopoietic stem cell transplant recipients (8). Although her clinical presentation and response to azithromycin strongly implicate L. londiniensis as the cause of her pneumonia, prolonged shedding of parainfluenza virus in the respiratory tract may contribute to sustained inflammation or activation of an inflammatory process that leads to irreversible airway damage (8). This may have predisposed her to infection with L. londiniensis. Further support for a bacterial etiology of her pneumonia is provided by a procalcitonin level of 6.34 ng/ml. A level of ≥0.5 ng/ml is indicative of a bacterial infection (1).

Our case also highlights the importance of cultures in the evaluation of patients with suspected Legionnaires’ disease. Although the Legionella urinary antigen test is the primary diagnostic test for Legionnaires’ disease used throughout the world, it detects only disease caused by L. pneumophila serogroup 1, which explains the negative result of our patient’s test. Even among patients with L. pneumophila serogroup 1 infections, the sensitivities of the urine antigen tests vary with disease severity, with estimates ranging from 60 to 70% in those patients with mild to moderate disease to up to 90% in those with more severe disease (3). Also, more than half of nosocomial cases and infections in immunocompromised individuals are caused by other L. pneumophila serogroups and Legionella spp. (5). Culture in addition to a urinary antigen test should be performed in all patients with suspected Legionnaires’ disease to maximize the diagnostic yield.

Molecular methods, especially those targeting specific 16S rRNA or mip gene sequences, are very useful in identifying Legionella spp. Partial sequencing of the 16S rDNA gene is able to identify all Legionella spp. to the genus level and about 90% of species other than L. pneumophila to the species level (11). Correct species-level identification can be problematic for some less commonly encountered species because of the small numbers and integrity of the sequences available in GenBank (NCBI) and other databases. However, L. londiniensis is well separated from the other Legionella spp. by both full-length and partial 16S sequencing. In our case, partial 16S rRNA gene sequencing (10) was able to identify the isolate as L. londiniensis, with a sequence that was identical over the entire 491-bp length to an L. londiniensis isolate (ATCC 700510, GenBank accession no. AF129525) described by Lo Presti et al. (6) and also had 99% identity to the type strain of the species (ATCC 49505, GenBank accession no. Z49730). In addition, sequencing of the mip gene as described for the mip gene sequence database (http://www.hpa.org.uk/web/HPAweb &HPAwebStandard/HPAweb_C/1195733805138) confirmed the identification as L. londiniensis, with 100% identity to the type strain sequence (GenBank no. U92229) (9). Both fluoroquinolone and macrolide antibiotics are effective for treatment of infections with L. pneumophila, Legionella micdadei, Legionella longbeachae, Legionella bozemaniae, and Legionella dumoffii (7). It is not known whether the clinical efficacy of these antimicrobial agents can be extrapolated to treatment of infections with all other species of Legionella. Although our patient initially failed to respond to treatment with oral ciprofloxacin, she did respond to treatment with intravenous azithromycin.

The pathogenic potential of an environmental L. londiniensis isolate was examined by its cytotoxicity for J774.1 cells, a macrophage-derived canine histiocytic sarcoma cell line, and its ability to grow intracellularly in Acanthamoeba and in U937 cells, a human monocyte cell line (4). This isolate was cytotoxic for J774.1 cells, as indicated by a reduction of viable cells. It was able to grow within Acanthamoeba but unable to replicate within U937 cells. Based on these findings, the authors concluded that it had limited pathogenic potential for humans compared to L. pneumophila. To our knowledge, this is the first report of Legionnaires’ disease caused by L. londiniensis and confirms that in immunosuppressed patients, this bacterium is capable of causing human disease.

REFERENCES