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Microbiological and Clinical Studies of Legionellosis in 33 Patients with Cancer

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***Legionella*, a large group of environmental Gram-negative bacteria, represents an occasional cause of pneumonia. We analyzed the microbiological and clinical features of 33 consecutive cases of *Legionella* infections that occurred at the University of Texas MD Anderson Cancer Center, Houston, TX, from 2002 to 2014. The *Legionella* strains were isolated from bronchoscopy specimens (32 strains) and a blood culture (1 strain) and were identified by sequencing analysis of the full-length 16S rRNA gene. The 33 strains involved 12 *Legionella* species or subspecies: 15 strains of *L. pneumophila* subsp. *pneumophila*, 3 strains of *L. pneumophila* subsp. *fraseri* or *L. pneumophila* subsp. *pascullei*, 4 strains of "*L. donaldsonii*," 3 strains of *L. micdadei*, and one each of *L. bozemanae*, *L. feeleii*, *L. gormanii*, *L. longbeachae*, *L. maceachernii*, *L. parisiensis*, *L. sainthelensi*, and *Legionella* sp. strain D5382. All patients except one asymptomatic carrier showed pneumonia, including one with concurrent bacteremia. Nine patients died, with this infection being the immediate cause of death in six. Twenty-seven patients had underlying hematologic malignancies. Twenty-three patients were leukopenic. Six patients were recipients of allogeneic hematopoietic stem cell transplant, with their infections caused by five *Legionella* species. Together, these results suggest that diverse *Legionella* species infect patients with cancer in the Houston area and its vicinity. The five cases of pneumonia due to *L. donaldsonii* and *Legionella* sp. D5382 are likely the first reports of human infection with these organisms.**

The genus *Legionella* was proposed in 1979 as the causative agent of Legionnaires' disease, a form of severe pneumonia (1, 2). Currently, this genus contains 58 species and three subspecies (www.bacterio.net). *Legionella* organisms are environmental Gram-negative bacteria that can be found in bodies of water and soil (3, 4). They are intracellular parasites of freshwater protozoa. They are aerobic, nonfermentative, asaccharolytic, and fastidious and require L-cysteine and iron salt for growth and isolation on solid culture medium. Of the 61 species and subspecies comprising >70 serogroups, ≥20 have been implicated as causative agents of pneumonia. *L. pneumophila* is the most common species of *Legionella* pneumonia; it includes ≥16 serogroups, of which serogroups 1, 4, and 6 are most common (5). Known human pathogens also include *Legionella longbeachae*, *Legionella micdadei*, *Legionella bozemanae*, *Legionella dumoffii*, and others.

The clinical spectrum of *Legionella* infection ranges from mild Pontiac fever to severe pneumonia. Rare extrapulmonary manifestations of infection have been reported, such as necrotizing cellulitis, abscesses, and endocarditis (6, 7). The principal known risk factor for *Legionella* pneumonia is the suppression of cellular immunity (5, 8). Thus, the patients at greatest risk include those being treated with corticosteroids, immunosuppressive medications after organ transplantation, and antineoplastic chemotherapy. The nonspecific presentation of *Legionella* pneumonia necessitates empirical therapy for patients diagnosed with community-acquired pneumonia, particularly those presenting with respiratory failure or shock (9).

The laboratory tests available to diagnose *Legionella* infection include culture of lower respiratory secretions, tissues, or body fluids, direct fluorescent staining of specimens with polyvalent antibodies and microscopy, immunoassays for *Legionella pneumophila* serogroup 1 antigen in the urine, and molecular amplification. The direct fluorescent antibody (DFA) staining of clinical specimens lacks sensitivity (10), and its sensitivity and specificity are not precisely known for species aside from *L. pneumophila* (7). Culture may be the only valid option for non-*pneumophila Legionella* species. Thus, most clinical investigations on *Legionella* have focused on the role of *L. pneumophila*. The fastidious growth and limited yet overlapping biochemical reactions also make the accurate identification of various *Legionella* species difficult in most hospital settings, where the majority of infection occurs.

Analysis of several genes, singly or in combination, has been shown to accurately differentiate *Legionella* species (11, 12). For instance, sequencing of the 16S rRNA gene, in particular the full-length gene, resolves most *Legionella* species with considerable confidence. The universal nature of the 16S gene also means there is a broad application for bacterial identification. In this study, we used the 16S gene sequencing method to identify 33 strains of *Legionella* species that were isolated consecutively from patients with cancer. We also paid attention to the clinical and pathogenic features of those non-*pneumophila Legionella* species.

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MATERIALS AND METHODS

Study setting and location. The *Legionella* strains were consecutive (sporadic) isolates collected from 2002 to December 2014 at the University of Texas MD Anderson Cancer Center, Houston, TX. The institution is a comprehensive cancer center that had 500 beds in 2002, which increased to 620 beds in 2014. The majority of patients were from the greater Houston area and its vicinity (other parts of Texas and nearby Louisiana). In view of the environmental origin of *Legionella*, the climate in Houston includes abundant rainfall (around 140 cm each year) and warm temperatures (monthly means, 12.4°C in the coldest January to 29.1°C in the warmest July and August). The greater Houston area is known for abundant bodies of water (lakes, rivers, bayous, and the Gulf of Mexico coast).

Microbiological tests and cultures. Lower respiratory specimens, mostly bronchoalveolar lavage (BAL) fluid and bronchial washings that were obtained through bronchoscopy, were stained by a direct fluorescent antibody assay for *Legionella* species (Remel, Lenexa, KS). The assay is designed to detect 22 *Legionella* species with 33 serogroups, including serogroups 1 to 10 of *L. pneumophila*, *L. bozemanae*, *L. dumoffii*, *L. feeleii*, *L. gormanii*, *L. longbeachae*, *L. maceachernii*, *L. micdadei*, *L. parisiensis*, *L. sainthelensi*, and others.

The respiratory specimens were also set up to culture *Legionella* (3). They were diluted approximately 5 times with low-tone acidic KCl solution (pH 2.2) and then plated. Four buffered charcoal yeast extract (BCYE) agar plates, two selective and two nonselective (BBL; BD Diagnostic Systems, Sparks, MD), were inoculated and incubated at 35°C for 7 days. Colonies of Gram-negative slender bacilli were subcultured onto sheep blood agar plates, and those with little or no growth on this agar represented potential *Legionella* species. Starting in 2011, BCYE plates with or without cysteine were also used for subcultures, which indicated *Legionella* growth with cysteine and no growth without cysteine. Potential *Legionella* isolates, particularly those with light blue- or pink-tinted colonies on BCYE agar, were further identified.

Blood cultures were performed using the Bactec 9240 automated culturing system (BD Diagnostic Systems) and manual Isolator tubes (Wampole Laboratories, Princeton, NJ). A blood culture set typically consisted of a 20-ml blood draw from a patient, which was divided equally and inoculated into a Bactec Plus Aerobic/F bottle and an Isolator tube. The Bactec bottles were incubated for 7 days at 35°C, with aeration. The Isolator tubes were processed by centrifugation, and the resulting blood sediments were spread on two chocolate agar plates and two sheep blood agar plates for incubation at 35°C with 5% carbon dioxide for 4 days. The Isolator plates allowed quantitation of bacterial colonies from the 10-ml blood draw. Approximately 30,000 sets of blood cultures were performed annually in the institution.

Definitive identification of *Legionella* species. Definite species identification was achieved by sequencing the 16S rRNA gene, as previously described (13). Briefly, for DNA extraction, the isolated colonies were suspended in 100 μl of extraction buffer (PrepMan Ultra; Applied Biosystems, Inc., Carlsbad, CA), boiled for 10 min at 105°C, and centrifuged (8,000 × *g* for 10 min). One microliter of the supernatant was used as a template in a 25-μl PCR, including activation of the enzyme (at 92°C for 2 min), 35 cycles of denaturation (at 95°C for 20 s), annealing (at 62°C for 20 s), and extension (at 72°C for 40 s), and a final extension for 5 min. At the time of isolation of each strain, a 594-bp PCR amplicon in the mid-region of the 16S gene using two universal bacterial primers (5′-TGCCA GCAGCCGCGGTAATAC [forward, UBFO] and 5′-CGCTCGTTGCGG GACTTAACC [reverse, UBRE]) was sequenced. During this study from 2009 to 2014, each sequence was extended to near full length (~1,460 bp) of the gene for more accurate species assignment. Two additional sets of primers were used for sequence extension, including 5′-GCGTGCTTAA CACATGCAAGTC (forward, AFBFO) and 5′-GCACAAGCGGTGGAG CATGTG (reverse, 16R3), and 5′-GGTGCAAGCGTTAATCGGA (forward, 16F4) and 5′-AGGAGGTGATCCAACCGCA (reverse, 16R). Sequence matches were made through the GenBank Basic Local Alignment Search Tool (BLAST), and the best match, usually at 99.4% to 100% with a type or reference strain, yielded species identification.

Clinical correlation and GenBank deposition. Clinical data were extracted from the medical records during isolation of a strain and/or later review and included demographics, underlying disease, presentation of symptoms and signs, physical and laboratory examination findings, radiographs, treatments, clinical course, and outcome. At least two physician authors assessed the clinical data for consistency. In view of the severity of these infections, nearly all patients also had chest computed tomography (CT) scans in addition to plain chest X rays. The review of the records was approved by the University of Texas MD Anderson Cancer Center institutional review board.

Nucleotide sequence accession numbers. The 16S rRNA gene sequences of an *L. donaldsonii* strain and an *L. donaldsonii*-like strain in this study were deposited in the GenBank under accession numbers KM504126 and KM504127.

RESULTS

General clinical features. The clinical characteristics of the 33 patients are detailed in Table 1. These patients included 19 men and 14 women, with a mean age of 60 years (range, 28 to 74 years). Twenty-seven patients had underlying hematologic malignancies, while six patients had solid tumors. Nine patients were also hematopoietic stem cell transplant (HSCT) recipients; six HSCTs were allogeneic, and three were autologous. Twenty-three of the 33 patients were leukopenic, with total white blood cell (nonleukemic) counts ranging from 0 to 3.8×10^9 cells/liter; the absolute neutrophil count ranged from 0 to 1.66×10^9 cells/liter. Thirty-one patients acquired infection in the greater Houston area and its vicinity, and two patients likely acquired infection farther away, with case 4 acquired in Puerto Rico and case 16 acquired in Florida, due to their short arrival time on presentation.

All patients except one, who was found to be an asymptomatic carrier, had clinical signs, symptoms, and radiographic images that were consistent with pneumonia. Twenty-four of the 33 patients recovered with therapy, while nine patients died, with the *Legionella* pneumonia likely the immediate cause of death in six of them. Nine patients had evidence of respiratory failure that required mechanical ventilation on presentation or shortly after hospitalization. The antibiotics most commonly given were the broad class of fluoroquinolones (24 cases), consistent with current recommendations (9), with or without addition of azithromycin, doxycycline, cephalosporins, and/or carbapenems as part of empirical coverage for neutropenic pneumonia.

Culture and microbiological features. Thirty-two of the 33 *Legionella* strains were isolated from respiratory specimens obtained through bronchoscopy, while one was isolated from a blood culture. With near-full-length 16S gene sequencing (~1,460 bp), all strains except one showed 99.4% to 100% match with at least one reference sequence or species in GenBank to allow a confident species assignment. The match details are also shown in Table 1.

The most common species was *L. pneumophila* subsp. *pneumophila*, with 15 strains. These strains matched with many reference strains (genomes) that showed minor differences of one to two nucleotides in their 16S gene sequences. Fourteen of these 15 strains were serogrouped, with 10 being serogroup 1, 2 being serogroup 6, and one each from serogroups 3 and 5. Thus, serogroup 1 accounted for 10 of the total 32 typed strains in this study. The two patients who likely acquired their infection far from Houston had this subspecies (one serogroup 6 and one not typed).

TABLE 1 Microbiological and clinical features of 33 cancer patients with legionellosis

Case no., age (yr)/sex; culture source[a]	Cancer type, HSCT status; baseline WBC, baseline ANC ($\times 10^9$ cells/liter)[b]	Presentation	Best match % (no./total no.) to species/strain, GenBank accession no.; serogroup	Treatment; outcome, note[c]
15 cases with *L. pneumophila* subsp. *pneumophila*				
24, 51/F; BAL fluid	Lung; 3.8, 2,4	Pneumonia, resp. failure[d]	100 (1,475/1,475) to strains Paris, Lorraine, and Phily-1; 1[e]	FEP, AZM, VAN; death @ 5 days
6, 64/F; BAL fluid	AML; 3.1, 0.26	Pneumonia, neutropenic fever	99.6 (1,401/1,406) to strains Paris, Lorraine, and Phily-1; 1	LVX, MXF, AZM; recovered
28, 75/M; BAL fluid	AML, allo-HSCT; 7.0, 6.5	Pneumonia, resp. failure	100 (1,478/1,478) to strains Paris, Lorraine, and Phily-1; 1	MEM, MXF, VAN; recovered, urine antigen (+)
32, 74/F; BAL fluid	Myeloma, auto-HSCT; 5.0, 3.0	Pneumonia	100 (1,443/1,443) to strains Paris, Lorraine, and Phily-1; 1	CIP, CRO, AZM; recovered, urine antigen (−)
15, 55/F; BAL fluid	AML; 0.1, 0	Pneumonia, neutropenic fever	100 (1,451/1,451) to strains Paris, Lorraine, and Phily-1; 6	TZB, LZD, CIP; recovered
16, 66/M; BAL fluid	Thyroid; 7.3, 4.42	Pneumonia, resp. failure	99.8 (1,449/1,451) to strains Paris, Lorraine, and Phily-1; 6	AZM, CIP; recovered, infected in Florida
4, 60/F; BAL fluid	Breast; 0.8, 0.4	Pneumonia, resp. failure, neutropenic fever	100 (1,444/1,444) to strains Paris, Lorraine, and Phily-1; not serogrouped	VAN, MEM, AZM, SXT; death @ 18 days, infected in Puerto Rico
21, 61/M; BAL fluid	Large-cell lymphoma; 0.1, 0	Pneumonia, resp. failure, shock, neutropenic fever	100 (1,450/1,450) to strains Paris, Lorraine, and Phily-1; 3	MEM, VAN, LVX; recovered, direct DFA of BAL fluid (+)
14, 56/M; BAL fluid	Acute promyelocytic leukemia; 34.7-leukemic, 0	Pneumonia, resp. failure, neutropenic fever	99.9 (1,452/1,454) to strain Corby, CP000675; 1	CIP, MEM, VAN; death @ 16 days
29, 58/M, BAL fluid	Multiple myeloma; 4.1, 2.4	Pneumonia	100 (1,477/1,477) to strain Corby, CP000675; 1	FEP, LZD, MXF; recovered
22, 60/M; BAL fluid	CLL; 2.7, 1.56	Pneumonia	100 (1,434/1,434) to strain Corby, CP000675; 5	VAN, FEP, LVX; recovered
17, 56/F; BAL fluid	Myeloma, auto-HSCT; 3.1, 1.66	Pneumonia, neutropenic fever	100 (1,451/1,451) to strain Lens, CR628337; 1	TZB, AZM, LVX; recovered
19, 69/M; BAL fluid	AML; 51.4-leukemic, 0	Pneumonia	100 (1,443/1,443) to strain Lens, CR628337; 1	FEP, TGC, MXF; recovered
30, 68/M; BAL fluid	AML; 0, 0	Pneumonia, resp. failure, neutropenic fever	99.9 (1,458/1,459) to strain Lens, CR628337; 1	FEP, LZD, IPM, CIP; death @ 30 days
10, 55/F; BAL fluid	AML, allo-HSCT; 0.4, 0.28	Pneumonia, neutropenic fever	99.6 (1,403/1,408) to strain Alcoy, CP001828; 1	FEP, LZD; recovered
3 cases with *L. pneumophila* subsp. *fraseri* or subsp. *pascullei*				
18, 56/M; bronchial washings	Esophageal; 3.6, 2.63	No symptoms	99.9 (1,443/1,445) to HQ287902; 6	LVX after culture; no infection
5, 74/M; blood	Renal cell; 8.3, 4.92	Pneumonia-bacteremia, resp. failure, coagulopathy	100 (1,441/1,441) to HQ287902; not serogrouped	FEP, CIP, VAN; recovered
7, 54/F; BAL fluid	AML; 1.8, 1.10	Pneumonia, neutropenic fever	99.9 (1,436/1,438) to HQ287902; not serogrouped	LVX; recovered
4 cases with *L. donaldsonii*				
1, 35/M; BAL fluid	CML, allo-HSCT; 2.5, 1.13	Pneumonia, neutropenic fever	99.6 (1,403/1,409) to Z49724	FEP, AZM, MEM; recovered
12, 43/F; BAL fluid	ALL; 0, 0	Pneumonia, neutropenic fever	99.6 (1,403/1,409) to Z49724	CIP, AZM, SXT; recovered
33, 72/F; BAL fluid	CLL; 21.6-leukemic, 0.65	Pneumonia, neutropenic fever	99.6 (1,403/1,409) to Z49724	MEM, LZD, CIP; recovered
26, 66/M; BAL fluid	CLL; 15.8-leukemic, 0.4	Pneumonia, neutropenic fever	98.6 (1,389/1,409) to Z49724	FEP, CIP, LZD; recovered
3 cases with *L. micdadei*				
9, 54/M; BAL fluid	ALL, allo-HSCT; 8.8, 6.59	Pneumonia	100 (1,405/1,405) to AF227162	CIP, LZD; recovered
23, 57/M; BAL fluid	Large-cell lymphoma; 2.9, 0.7	Pneumonia, neutropenic fever	100 (1,443/1,443) to AF227162	FEP, MEM, VAN; recovered
25, 71/F; BAL fluid	CLL; 11.8-leukemic, 0.8	Pneumonia-severe, neutropenic fever, coagulopathy	100 (1,442/1,442) to AF227162	FEP, MEM, VAN; recovered
8 cases with other *Legionella* species				
3, 65/F; BAL fluid	Myeloma, auto-HSCT; 2.2, 1.15	Pneumonia, resp. failure, neutropenic fever	99.4 (1,387/1,396) to *L. bozemanae*, Z49718	SXT, DOX; death @ 23 days
11, 59/M; BAL fluid	Lung; 10.5, 8.67	Pneumonia (postobstructive, severe)	99.7 (1,404/1,408) to *L. feeleii*, X73406	LVX; hospice, death

(Continued on following page)

TABLE 1 (Continued)

Case no., age (yr)/sex; culture source[a]	Cancer type, HSCT status; baseline WBC, baseline ANC ($\times 10^9$ cells/liter)[b]	Presentation	Best match % (no./total no.) to species/strain, GenBank accession no.; serogroup	Treatment; outcome, note[c]
8, 65/M; BAL fluid	ALL; 0.4, 0.22	Pneumonia (severe), neutropenic fever	99.4 (1,415/1,424) to *L. gormanii*, Z32639	AMK, MEM, VAN, AZM; death @ 14 days
31, 59/M; BAL fluid	AML; leukemic, 3.0	Pneumonia	100 (1,455/1,455) to *L. longbeachae*, FN650140	FEP, TGC, CIP; hospice, death
27, 81/F; BAL fluid	Myelodysplastic syndrome; 2.0, 0.6	Pneumonia (low grade)	99.9 (1,442/1,443) to *L. maceachernii*, NR_041790	FEP, LZD; death @ 4 days, unrelated
20, 50/M; BAL fluid	CML, allo-HSCT, GVHD; 4.9, 4.12	Pneumonia	99.5 (1,396/1,403) to *L. parisiensis*, Z49731	LVX, DOX; recovered
2, 28/M; BAL fluid	Acute leukemia, allo-HSCT, GVHD; 5.5, 4.4	Pneumonia	99.5 (1,445/1,452) to *L. sainthelensi*, X73399	CRO, AZM, GAT; recovered
13, 38/F; BAL fluid	CLL; 3.1, 0.42	Pneumonia, neutropenic fever	99.9 (1,360/1,361) to *Legionella* sp. strain D5382, JN380990	LVX, DOX; recovered

[a] F, female; BAL, bronchoalveolar lavage; M, male.
[b] HSCT, hematopoietic stem cell transplant; WBC, white blood cell count; ANC, absolute neutrophil count; AML, acute myelogenous leukemia; allo-HSCT, allogeneic HSCT; auto-HSCT, autologous HSCT; CML, chronic myelogenous leukemia; ALL, acute lymphoblastic leukemia; CLL, chronic lymphocytic leukemia; GVHD, graft-versus-host disease.
[c] FEP, cefepime; AZM, azithromycin; VAN, vancomycin; LVX, levofloxacin; MXF, moxifloxacin; MEM, meropenem; (+), positive; CIP, ciprofloxacin; CRO, ceftriaxone; (−), negative; TZP, piperacillin-tazobactam; LZD, linezolid; SXT, trimethoprim-sulfamethoxazole; DFA, direct fluorescent assay; TGC, tigecycline; DOX, doxycycline; GAT, gatifloxacin.
[d] resp., respiratory.
[e] Phily-1, strain Philadelphia-1.

There were 3 strains of *L. pneumophila* subsp. *fraseri* or subsp. *pascullei*. These two subspecies had identical 16S gene sequences and were not further differentiated. However, they differed from *L. pneumophila* subsp. *pneumophila* by 12 nucleotides in the 1,500-bp 16S gene to allow confident separation. One of these three strains was typed as being serogroup 6.

The other 15 strains of *Legionella* species included 4 strains of *L. donaldsonii*, 3 strains of *L. micdadei*, and one each of *L. bozemanae*, *L. feeleii*, *L. gormanii*, *L. longbeachae*, *L. maceachernii*, *L. parisiensis*, *L. sainthelensi*, and *Legionella* sp. D5382. It is notable that the strains isolated from six patients with allogeneic HSCT included two *L. pneumophila* subsp. *pneumophila* serogroup 1 and one each of *L. donaldsonii*, *L. micdadei*, *L. parisiensis*, and *L. sainthelensi*.

During the study years, direct fluorescent antibody staining with microscopy was also applied to all bronchoscopy-derived specimens; positive specimens rarely matched the culture results (data not shown), with only one specimen being positive with both methods. This strain, *L. pneumophila* subsp. *pneumophila* serogroup 3, was isolated from a 61-year-old man with lymphoma who had no countable blood neutrophils (Table 1, case 21). Thus, despite polyvalent antibodies of the fluorescent assay that covered all the *Legionella* species and serogroups in this study except *L. donaldsonii*, this direct assay failed to detect 28 strains in the specimens. The urine *Legionella* antigen test was not used consistently; in two patients with infection due to *L. pneumophila* subsp. *pneumophila* serogroup 1, one was positive (case 28) and one negative (case 32).

The above results suggest that diverse *Legionella* species may cause pneumonia in patients with cancer in the Houston area, and culture recovery from bronchoscopy-derived respiratory specimens likely provides the most reliable, sensitive, and specific diagnosis. Selected cases are presented below to illustrate unusual features or the first report of an organism in human infection, along with brief discussions.

Four cases of *L. donaldsonii* infection. *L. donaldsonii* is a tentative name used in the GenBank accession for a clinical isolate (Glasgow 86/35785, without clinical details) (11). The isolation of this organism from the environment was reported recently in Taiwan (14). The four cases in this series (cases 1, 12, 26, and 33) all involved patients with refractory leukemia. They occurred in 2002, 2010, 2014, and 2014, respectively, with acquisition of infection likely occurring in the greater Houston area.

Case 1 (Table 1) was a 35-year-old man who received an allogeneic HSCT for chronic myelogenous leukemia. The patient had suffered from graft-versus-host disease that required methylprednisolone and tacrolimus therapy. Approximately 2 months following HSCT, he was admitted to the hospital, complaining of subjective fevers and shortness of breath. On chest examination, there were inspiratory crackles and dullness to percussion. His labs showed pancytopenia. A chest radiograph showed new bilateral pulmonary opacities with pleural effusions (Fig. 1), and a CT also revealed peripheral lung nodules. A bronchoscopy was performed, and BAL fluid cultures grew *L. donaldsonii* without other pathogens. The patient was treated with cefepime, azithromycin, and meropenem until recovery during the 46-day hospitalization. Intubation with mechanical ventilation was also required.

Case 12 was a 43-year-old woman being treated for leukemia, with zero blood neutrophils per microliter. She presented to the emergency department with fever (39°C) and tachycardia (150 beats/min). The admission chest radiograph showed pulmonary opacities most consistent with pneumonia. A chest CT performed later also showed worsening nodular opacities in the left upper lobe compared to an earlier CT. The BAL fluid cultures grew *L. donaldsonii* without other pathogens. The patient was treated empirically and then specifically during the 45-day hospitalization. She recovered.

Case 33 was a 72-year-old woman with a history of chronic lymphocytic leukemia (CLL) for 7 years. While being treated for relapsed leukemia with neutropenia, she presented to the emergency room with fever (39.1°C), mild nonproductive cough, and rhinorrhea. Chest radiograph and CT scan revealed the new development of left lower lobe opacities. A BAL fluid sample was obtained 2 days later, which grew *L. donaldsonii*. A rhinovirus was also detected in the nasal wash sample. The patient was hospital-

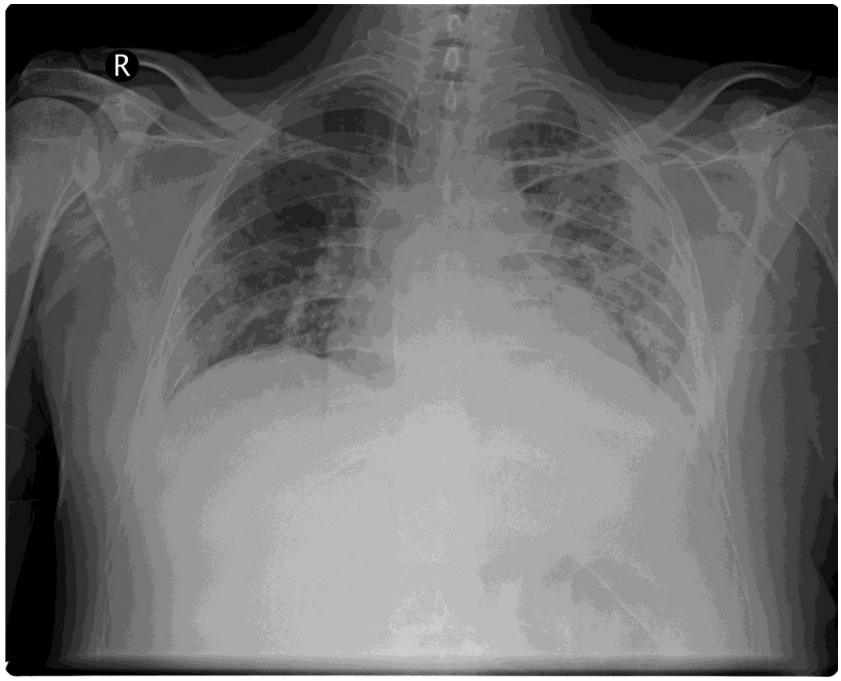

FIG 1 Chest radiograph of *L. donaldsonii* pneumonia in a 35-year-old man with a hematopoietic stem cell transplant.

ized for 6 days and treated with meropenem, ciprofloxacin, and linezolid. She also recovered.

Case 26 involved a 66-year-old man with CLL for 3 years. Within the 2-month period after the initiation of chemotherapy, the patient experienced two episodes of fever and possible pneumonia with admission to a community hospital, but the etiology was not ascertained. This admission began with the presentation of fever (39°C), dry cough, and dyspnea in the emergency room. Chest radiography and CT scan showed left lower lobe consolidation and bilateral multifocal opacities. A bronchoscopy was performed the next day, and the culture grew an *L. donaldsonii*-like organism without other pathogens. The patient was hospitalized for 7 days and treated with cefepime, ciprofloxacin, and linezolid. He recovered.

Three strains from cases 1 (in 2002), 12 (strain MDA4726 in 2010), and 33 (in 2014) showed almost identical 16S gene sequences (zero to one mismatch of 1,444 bp). They all matched 99.6% with *L. donaldsonii* (GenBank accession no. Z49724, 1,403 of 1,409 bp) (Table 1). They matched second with *L. feeleii* (GenBank accession no. X73406, 99.3% [1,439/1,449]). The fourth strain (MDA6655 from case 26 in 2014) matched best with both *L. donaldsonii* and *Legionella tunisiensis* at 98.6% (1,389/1,409 and 1,449/1,470, respectively). This strain was thus considered *L. donaldsonii*-like and potentially novel. Notably, *L. tunisiensis* is a newly described species in 2012 that was isolated from environmental water with hitherto unknown clinical significance (15). The 16S sequences of strains MDA4726 and MDA6655 showed considerable difference, matching at 98.7% (1,460/1,479). These two sequences were deposited to the GenBank in view of the clinical significance of these organisms, sequence quality, and/or novelty.

Case of *Legionella* sp. D5382 infection. Strain D5382 is a *Legionella* organism without species status, and its 16S gene sequence was directly deposited to GenBank from Australia (GenBank accession no. JN380990, 1,363 bp, R. M. Ratcliff, 2011). No infection by this strain was known prior to this study. The strain recovered in this study matched best (99.9% [1,360/1,361]) with D5382, and the next-level matches showed a difference of nearly 20 nucleotides with several *Legionella anisa* strains in GenBank (~98.7%). The patient was a 38-year-old female with CLL (case 13) who presented to the emergency department with fever (39.3°C) and hypotension (87/51 mm Hg). Her admission chest radiograph showed a large left upper lobe consolidation; a CT confirmed the air space opacities in the left upper lobe, with additional bilateral ground glass nodular opacities. On review of prior radiographs, it was noted that subtle evidence of a developing pneumonia likely started 2 months earlier. A BAL fluid culture on the day of admission grew *Legionella* sp. strain D5382. The patient was treated empirically with ciprofloxacin, piperacillin-tazobactam, and linezolid. She was discharged on levofloxacin, and her condition improved gradually over 7 weeks.

Case of *L. parisiensis* infection. *L. parisiensis*, initially isolated in Paris from a cooling tower in 1985 (16), has been reported to cause pneumonia in two cases, in a 34-year-old French woman who had undergone liver transplant and immunosuppressive therapy (17), and in a German patient with immunosuppression (lack of clinical details) (18). The present case thus represented the third confirmed infection and likely the first in the United States. The patient was a 50-year-old male with chronic myelogenous leukemia post-allogeneic HSCT for 5 months (case 20). He had experienced severe graft-versus-host disease, for which he received tacrolimus and high-dose prednisone. He presented to the emergency department with a 2-day history of fever, productive cough with yellow sputum, left-sided pleuritic chest pain, and dyspnea. On admission, his temperature was 39.4°C, and his blood was pancytopenic. The admission chest imaging showed the

new development of multifocal pneumonia, along with nodules involving the left lower lobe and left parahilar region. A BAL fluid sample obtained on hospital day 2 grew *L. parisiensis*. He was treated with levofloxacin, piperacillin-tazobactam, and vancomycin. His condition improved further after discharge with additional antimicrobial therapy.

Bacteremia with *L. pneumophila* subsp. *fraseri* or subsp. *pascullei*. Cases of *L. pneumophila* bacteremia were reported a few times decades ago (19–21), but the bacterial strains were rarely further investigated for additional features due to technical limits and their uncertain significance. Similarly, since the initial descriptions of *L. pneumophila* subsp. *fraseri* and subsp. *pascullei* (22), there have been limited studies of these organisms and clinical reports. Thus, the case of bacteremia caused by *L. pneumophila* subsp. *fraseri* or subsp. *pascullei* in this series may be instructive.

The patient was a 74-year-old male nonsmoker post-chemotherapy for metastatic renal cell carcinoma (case 5). He presented in May 2007 with fever, cough, dyspnea, profound generalized weakness, and episodes of confusion. Bilateral lung crackles were noted on physical examination, along with pulmonary opacities on chest radiography, acute respiratory acidosis on arterial blood gas analysis, and coagulopathy in view of positive tests for D-dimers and prolonged thrombin time. His white blood cell count rose from $8{,}300 \times 10^6$/liter at baseline to $12{,}900 \times 10^6$/liter, with 97% neutrophils. In the emergency room, blood cultures in a Bactec bottle and an Isolator tube were drawn, and he was treated empirically with intravenous cefepime and levofloxacin and admitted for intensive care.

While the Bactec bottle remained negative for 7 days, the Isolator tube culture, upon spread of the blood sediments on agar plates and incubation for ~90 h, grew 75 colonies of a Gram-negative rod on the two chocolate agar plates but not on the two sheep blood agar plates. The fastidiousness of this organism, along with a slender Gram-negative microscopic morphology, prompted the use of 16S gene sequencing, which led to the identification of *L. pneumophila*. The pneumonia was refractory to antimicrobial therapy, and hemoptysis also developed. At 11 days after admission, a bronchoscopy was performed, which revealed a diffuse alveolar hemorrhage, but cultures of the BAL fluid specimen were negative. The condition of the patient eventually improved, and he was discharged after 50 days of hospitalization. Further 16S sequencing of the strain later led to the subspecies assignment. The strain did require cysteine to grow.

The blood source, sole growth on chocolate agar, and large number of colonies made this culture unusual for *Legionella* species. This case led us to include a BCYE agar plate in exchange for a sheep blood agar plate in our Isolator blood cultures starting in September 2009; so far, no *Legionella* organism has been isolated this way, but this medium has improved the recovery of *Methylobacterium radiotolerans*, another environmental Gram-negative bacillus (23).

Case of asymptomatic carriage. Airway colonization or carriage of *Legionella* species has been rarely reported (24–27). Thus, the present case of an asymptomatic carrier is noteworthy. The patient (case 18) was a 56-year-old male ex-smoker with metastatic esophageal cancer who had undergone chemoradiation therapy. A restaging CT showed patchy pulmonary opacities in the right upper lobe that correlated with the radiation treatment history to suggest radiation effects. Prior to surgery, a bronchoscopy was performed for staging, and the bronchial washings (without BAL in view of the absence of significant lung pathology) grew *L. pneumophila* subsp. *fraseri* or subsp. *pascullei* serogroup 6. The patient had no complaints to suggest a respiratory tract infection, nor did repeat chest imaging reveal significant abnormalities. As a precaution, he was empirically treated with levofloxacin as an outpatient.

DISCUSSION

By using 16S gene sequencing in this study, we determined the *Legionella* species with confidence. We noted 12 *Legionella* species or subspecies in 33 cases. These results suggest that diverse *Legionella* species may cause infection in patients with cancer in the Houston area. We attribute the recovery of diverse species to our inclusive cultures, the timely lavage of the infection site, and the increased vulnerability of our cancer patient population to opportunistic pathogens, particularly those with hematologic malignancies (27 of the 33 patients).

In the general population, most *Legionella* infections are caused by *L. pneumophila*; for instance, a multinational study of community-acquired Legionnaires' disease identified 508 culture-confirmed cases, in which *L. pneumophila* was responsible for 91.5% of the cases, and among these, serogroup 1 accounted for 84.2% (28). In the environment, however, *Legionella* species are diverse, as revealed in a French study (29). Another large-scale French study compared clinical and environmental *Legionella* isolates and noted that *L. pneumophila* serogroup 1 accounted for 28% of the environmental isolates, compared to 95% of the clinical isolates (30). Therefore, the clinical dominance of *L. pneumophila*, among the large number of *Legionella* species described so far, is a reflection of the higher pathogenicity of this species.

In contrast, our data showed that the 31 infections that were likely acquired in the greater Houston area and its vicinity involved 13 strains of *L. pneumophila* subsp. *pneumophila* and 18 strains of other species or subspecies. In particular, serogroup 1 of *L. pneumophila*, with or without a subspecies designation, accounted for 10 of the 31 strains, or 32.3%, a frequency similar to the 28% in the French environmental strains (30), as noted above. This dominance of non-*pneumophila Legionella* species should reflect the presence of these diverse *Legionella* species in the Houston area environment and the vulnerability of our cancer patient population to these organisms. Despite the lack of data on the environmental distribution of *Legionella* species, the Houston area is known for warm temperatures, ample rain, many water bodies, and frequent standing water. During the 13 study years, we did notice yearly fluctuation of the isolation of these *Legionella* strains, and the reason behind this is under investigation. Rainfall is a reported risk factor for legionellosis (31, 32). We previously noted that the clinical recovery of rapidly growing mycobacteria, also of an environmental origin, followed the seasonal rise and fall of rainfall in Houston (33). Thus, future studies of *Legionella* species in the Houston area environment may be revealing.

The four cases of *L. donaldsonii* pneumonia and the one due to *Legionella* sp. D5382 are likely the first reports of human infection with these organisms. All five patients had underlying leukemia with leukopenia and/or neutropenia. Upon antimicrobial treatment, they all recovered, although two patients required long hospitalizations. These infections also hint that *L. donaldsonii* is likely not rare in the Houston area environment, similar to *L. micdadei*, a known species that infected three of our patients. *L. donaldsonii*

clusters with *L. feeleii* (11), and they are relatively distant from other species. Our findings suggest that the species designation *L. donaldsonii* may be warranted, pending additional phenotypic studies of our strains and other strains.

It has been reported that among recipients of HSCT or solid organ transplant, *L. pneumophila*, *L. micdadei*, and *L. bozemanae*, in descending order, are the most commonly isolated *Legionella* species (8, 34–36). Our six patients with allogeneic HSCT were infected by five different *Legionella* species, including *L. pneumophila* subsp. *pneumophila* serogroup 1, *L. micdadei*, *L. donaldsonii*, *L. parisiensis*, and *L. sainthelensi*. This finding thus adds *L. donaldsonii*, *L. parisiensis*, and *L. sainthelensi* to the list of opportunistic pathogens.

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