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11th Serogroup of *Legionella pneumophila* Isolated from a Patient with Fatal Pneumonia

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A *Legionella*-like organism (strain 797-PA-H; ATCC 43130) was isolated from a specimen taken from an endotracheal tube of a patient 4 days before death and from the left lung at autopsy. Growth characteristics were consistent with those for *Legionella* species. Strain 797-PA-H gave negative test results with available direct immunofluorescence assay conjugates and with slide agglutination test antisera prepared against the 22 *Legionella* species and 35 serogroups now recognized. Minimal reactivity (1 to 2+) was observed with both tests by using reagents prepared against the *Legionella*-like organism Lansing 3. Reciprocal absorption studies, however, showed that the cross-reactive antibodies could be removed easily. Physiologic, gas-liquid chromatographic, and DNA hybridization tests revealed that the strain belonged to the species *Legionella pneumophila*. Therefore, strain 797-PA-H was designated as the type strain of a new *L. pneumophila* serogroup, serogroup 11.

Legionella pneumophila comprises 10 serogroups (3, 4), all of which have been shown to cause pneumonia in humans. In this report, we describe the characterization of an 11th serogroup of *L. pneumophila* isolated from a patient with fatal pneumonia.

Case report. A 65-year-old man with multiple myeloma was admitted to Montefiore Hospital in October 1982 because of lower extremity weakness. A myelogram disclosed a complete spinal canal block at the level of the ninth thoracic vertebral body. The patient received radiation therapy and 128 mg of methylprednisolone per day. Although there was initial improvement, he became febrile (101°F [38.3°C]) on hospital day 15, and a chest roentgenogram disclosed a left lower lobe infiltrate. Blood, urine, and sputum were cultured, and therapy with intravenous nafcillin, tobramycin, and erythromycin was begun. On the following day, multiple blood cultures were positive for *Staphylococcus aureus*. The erythromycin and tobramycin were discontinued. Four days later, the patient developed hypotension and hypoxemia requiring endotracheal intubation. His course became complicated by disseminated intravascular coagulation and acute renal failure, and he died 4 days later. At autopsy, routine bacterial cultures of the lungs grew only rare *Torulopsis glabrata*, but the left lung grew a *Legionella*-like organism (LLO) on buffered charcoal-yeast extract (BCYE) agar which was negative by direct immunofluorescence assays (2) for *L. pneumophila* serogroups 1 to 6, *L. micdadei*, *L. dumoffii*, *L. bozemanii*, *L. gormanii*, and *L. longbeachae* serogroups 1 and 2. A culture obtained from the endotracheal tube 4 days before the death of the patient ultimately grew the same LLO.

Biochemical characteristics. The endotracheal tube isolate, strain 797-PA-H, was tested for the absence of growth on Trypticase soy agar (BBL Microbiology Systems) containing 5% sheep blood and BCYE agar without cysteine. Physiologic tests for catalase, gelatinase, oxidase, urease, beta-lactamase, hippurate hydrolysis, nitrate reduction, glucose fermentation, motility, autofluorescence, and browning of tyrosine-supplemented agar were done as described previously (1). Gas-liquid chromatography was performed by William R. Mayberry, East Tennessee State University, Johnson City.

Direct immunofluorescence assay. The strain was tested with working dilutions of the following direct immunofluorescence assay conjugates: *L. pneumophila* serogroups 1 to 9 and strains Seattle 1, Lansing 3, and San Francisco 9; *L. longbeachae* serogroups 1 and 2; *L. bozemanii* serogroup 1; *L. dumoffii*; *L. micdadei*; *L. gormanii*; *L. wadsworthii*; *L. oakridgensis*; *L. feeleii* serogroup 1; *L. sainthelensi*; *L. spiritensis*; *L. hackeliae* serogroup 1; *L. jordanis*; and *L. rubrilucens*.

Slide agglutination test. The slide agglutination test was performed with antisera to all previously published named *Legionella* species (n = 22) and serogroups (n = 35) (3–6). Antiserum to strain 797-PA-H was prepared as described previously (4), and cross-absorption tests were performed to prepare a serogroup-specific antiserum for strain 797-PA-H.

DNA hybridization. The strain was grown for 3 days at 35°C on 20 BCYE agar plates (100 by 15 mm). The preparation of unlabeled DNA and determination of DNA relatedness by the hydroxyapatite method at 60 and 75°C were performed as described previously (1).

Results and discussion. Growth characteristics of strain 797-PA-H were consistent with those of *Legionella* species. Growth was obtained on BCYE agar but not on BCYE agar without cysteine or on blood agar. By direct immunofluorescence assay, strain 797-PA-H was stained minimally (1 to 2+) only with the conjugate for LLO strain Lansing 3. In addition, the only agglutination observed was with antiserum to LLO strain Lansing 3. However, the reaction strength (2+) was below the minimal positive level. Reciprocal absorption studies with the Lansing 3 and 797-PA-H antisera and strains showed that cross-reactive antibodies could be removed by absorption with the heterologous strain (Table 1).

Physiologic test results were identical to those for Philadelphia 1, the type strain of the species *L. pneumophila* (1).

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TABLE 1. Slide agglutinating antibody titers of unabsorbed and absored antisera against *L. pneumophila* 797-PA-H and LLO strain Lansing 3

Antiserum		SAT[a] titer against antigen	
Immunizing strain	Absorbed with	Lansing 3	797-PA-H
797-PA-H		16[b]	64
	Lansing 3	<2	32
Lansing 3		64	16
	797-PA-H	16	<2

[a] SAT, Slide agglutination test.
[b] Reciprocal of dilution giving 2+ or greater agglutination.

Both strains gave positive test results for catalase, gelatinase, beta-lactamase, oxidase, hippurate hydrolysis, motility, and browning of tyrosine-supplemented agar. Negative results were obtained for urease, nitrate reduction, glucose fermentation, and autofluorescence. Gas-liquid chromatography profiles were consistent with those for strain Philadelphia 1 (William R. Mayberry, personal communication).

DNA studies showed that strain 797-PA-H belonged to the species *L. pneumophila*. Labeled DNA from strain Philadelphia 1 was 79% related to unlabeled DNA from 797-PA-H, and divergence in the related sequences was only 1.5%. These results support the designation of 797-PA-H (ATCC 43130) as a new *L. pneumophila* serogroup, serogroup 11.

This report further supports the need for isolation attempts in suspected cases of legionellosis. The number of *Legionella* species, 22, and serogroups, 35, continues to increase (3–6), and serologically negative test results may also continue for a small percentage of specimens. LLOs that give negative results or that react minimally or show multiple cross-reactions with existing *Legionella* reagents should be characterized further.

LITERATURE CITED

1. **Brenner, D. J., A. G. Steigerwalt, G. W. Gorman, H. W. Wilkinson, W. F. Bibb, M. Hackel, R. L. Tyndall, J. Campbell, J. C. Feeley, W. L. Thacker, P. Skaliy, W. T. Martin, B. J. Brake, B. S. Fields, H. V. McEachern, and L. K. Corcoran.** 1985. Ten new species of *Legionella*. Int. J. Syst. Bacteriol. **35**:50–59.
2. **McKinney, R. M., L. Thacker, P. P. Harris, K. R. Lewallen, G. A. Hébert, P. H. Edelstein, and B. M. Thomason.** 1979. Four serogroups of Legionnaires' disease bacteria defined by direct immunofluorescence. Ann. Intern. Med. **90**:621–624.
3. **Meenhorst, P. L., A. L. Reingold, D. G. Groothuis, G. W. Gorman, H. W. Wilkinson, R. M. McKinney, J. C. Feeley, D. J. Brenner, and R. van Furth.** 1985. Water-related nosocomial pneumonia caused by *Legionella pneumophila* serogroups 1 and 10. J. Infect. Dis. **152**:356–364.
4. **Thacker, W. L., B. B. Plikaytis, and H. W. Wilkinson.** 1985. Identification of 22 *Legionella* species and 33 serogroups with the slide agglutination test. J. Clin. Microbiol. **21**:779–782.
5. **Thacker, W. L., H. W. Wilkinson, B. B. Plikaytis, A. G. Steigerwalt, W. R. Mayberry, C. W. Moss, and D. J. Brenner.** 1985. Second serogroup of *Legionella feeleii* strains isolated from humans. J. Clin. Microbiol. **22**:1–4.
6. **Wilkinson, H. W., W. L. Thacker, A. G. Steigerwalt, D. J. Brenner, N. M. Ampel, and E. J. Wing.** 1985. Second serogroup of *Legionella hackeliae* isolated from a patient with pneumonia. J. Clin. Microbiol. **22**:488–489.