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Legionella lansingensis sp. nov. Isolated from a Patient with Pneumonia and Underlying Chronic Lymphocytic Leukemia

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A *Legionella*-like organism, strain 1677-MI-H, was isolated from the bronchoscopy washings of a patient with pneumonia who had a 2-year history of progressive, chronic lymphocytic leukemia. The growth characteristics, cellular fatty acids, and ubiquinone content of the isolate were consistent with those for *Legionella* spp. The isolate was serologically distinct in the slide agglutination test with absorbed antisera. DNA hybridization studies showed that strain 1677-MI-H (ATCC 49751) represents a new *Legionella* species which is named *Legionella lansingensis*.

Legionella pneumophila, the etiologic agent of Legionnaires' disease, was first recognized in 1977 following an epidemic of acute pneumonia in Philadelphia, Pa. (9). Since then, 33 *Legionella* species and 52 serogroups have been characterized (1, 10, 12–14). Isolates of 35 of the 52 serogroups (15 species) have been obtained from clinical material (1, 13). In this report, we describe a new *Legionella* species, *L. lansingensis*, that was isolated from a patient with pneumonia who had chronic lymphocytic leukemia.

CASE REPORT

The patient was a 54-year-old woman with a 2-year history of progressive, chronic lymphocytic leukemia. She was admitted to Ingham Medical Center, Lansing, Mich., in February 1987 with a 5-day history of fever, nonproductive cough, and left-sided pleuritic chest pain. Prior to admission, she had received a 5-day course of amoxicillin but had experienced a worsening of her pulmonary symptoms. Her past medical history was significant for diabetes mellitus, recurrent pulmonary emboli, splenectomy (because of thrombocytopenia), cholecystectomy, and hysterectomy with bilateral salpingo-oophorectomy. On admission, the patient's temperature was 105°F (ca. 41°C), her pulse was 118/min, and her respiration was 26/min. Physical findings were remarkable only for signs of lung consolidation in the upper lobe and right lower lobe. Notable laboratory results were a leukocyte count of 40,000/mm^3 with 87% lymphocytes and 2% neutrophils. Arterial blood gas analysis, performed when the patient was receiving 60% oxygen, showed a partial pressure of 75 mm Hg, a pH of 7.41, and a partial CO_2 pressure of 48 mm Hg. A chest roentgenogram revealed consolidation of the left upper and right basilar lobes. The patient initially was treated with cefazolin, tobramycin, and piperacillin, but she failed to respond. After 5 days of treatment, she continued to deteriorate and was transferred to Sparrow Hospital, Lansing, Mich. Trimethoprim-sulfamethoxazole and erythromycin were added to her therapeutic regimen. On hospital day 8, the patient was intubated, and a bronchoscopy was performed. Piperacillin and tobramycin were discontinued, and ceftazidime and amikacin were added. Stains and routine cultures of bronchoscopically obtained secretions were nondiagnostic. The bronchoscopy washings were also inoculated onto buffered charcoal-yeast extract (BCYE) agar (BBL Microbiology Systems, Cockeysville, Md.). The plates were incubated aerobically at 35°C and observed daily for suspected *Legionella* colonies. On hospital day 11, a biopsy was performed on the left upper lobe of the lung, and it showed acute interstitial pneumonia with diffuse alveolar damage. Stains for pneumocystis, fungi, acid-fast bacilli, and routine bacteria were all negative. Immunofluorescent stains for cytomegalovirus and herpes simplex virus were also found to be negative. On hospital day 12, all antimicrobial agents except erythromycin were discontinued when a *Legionella*-like organism was recovered on BCYE agar from bronchoscopy washings obtained on day 8. The patient stabilized and improved, and after 3 weeks of erythromycin treatment, antibiotics were discontinued and she was discharged.

The *Legionella*-like organism obtained was a weakly staining gram-negative bacillus which was negative by direct immunofluorescence assays for *L. pneumophila* serogroups 1 to 6, *L. micdadei*, *L. dumoffii*, *L. bozemanii* serogroup 1, *L. gormanii*, and *L. longbeachae* serogroups 1 and 2. However, the isolate reacted strongly with Gen-Probe *Legionella* reagents (Gen-Probe, San Diego, Calif.). The isolate was sent first to the Michigan Department of Health in Lansing and subsequently to the Centers for Disease Control for further studies.

MATERIALS AND METHODS

Direct immunofluorescence assay. The *Legionella*-like organism (strain 1677-MI-H) was tested with working dilutions of the following direct immunofluorescence assay conjugates: *L. pneumophila* serogroups 1 to 11 and Lansing 3; *L. micdadei*; *L. bozemanii* serogroups 1 and 2; *L. dumoffii*; *L. gormanii*; *L. longbeachae* serogroups 1 and 2; *L. jordanis*;

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L. hackeliae serogroups 1 and 2; *L. wadsworthii*; *L. feeleii* serogroups 1 and 2; *L. oakridgensis*; and *L. maceachernii*. No fluorescence was observed with any of the conjugates tested.

Growth and biochemical tests. A single-colony pick of strain 1677-MI-H was grown on BCYE agar for all but two tests. The buffer was omitted for the determination of autofluorescence, and cysteine was omitted for the determination of the cysteine requirement (13). Physiologic tests for catalase, gelatinase, oxidase, urease, β-lactamase, hippurate hydrolysis, nitrate reduction, glucose fermentation, flagella, autofluorescence, and browning of tyrosine-supplemented agar and initial analysis for whole-cell fatty acid content were done as described previously (3, 4).

Cellular fatty acids. Twenty batches of cells of strain 1677-MI-H, obtained from growth on BCYE agar, were analyzed for cellular nonhydroxy, monohydroxy, and dihydroxy fatty acids by gas-liquid chromatography after sequential alkaline saponification and acid hydrolysis to effect fatty acid liberation (1, 6, 7). Fatty acid profiles, adjusted for the relative molar responses of the individual components, were calculated on the basis of relative abundance, with the most abundant fatty acid in each class given a value of 100. The class moles percent of each of the individual components was also calculated.

Isoprenoid quinones. The isoprenoid quinone content of strain 1677-MI-H was determined as described previously (4).

Slide agglutination test. Strain 1677-MI-H was tested with antisera to all previously characterized *Legionella* species ($n = 33$) and serogroups ($n = 52$) (1, 10, 13). Antiserum to strain 1677-MI-H was prepared and tested as described previously (11).

DNA studies. The preparation and labeling of DNA and the hydroxyapatite method for DNA hybridization have been described previously (2, 15). DNA hybridization was done at 60°C. Unlabeled DNA was prepared from type strains of all *Legionella* species except *L. anisa*, *L. rubrilucens*, *L. cherrii*, *L. steigerwaltii*, and *L. santicrucis*. DNA from these five species is at least 50% related to DNA from the species that were tested. The G+C content of strain 1677-MI-H was determined spectrophotometrically by denaturation (5).

TABLE 1. Major fatty acids of *Legionella lansingensis*[a]

Fatty acid	Relative abundance in class[b]	Mol% in class
Nonhydroxy (approx 85 mol% of total)		
i-$C_{15:0}$	5	2
a-$C_{15:0}$	96	29
n-$C_{15:0}$	5	2
i-$C_{16:0}$	40	12
n-$C_{16:1}$	12	4
n-$C_{16:0}$	20	6
a-$C_{17:1}$	12	4
i-$C_{17:0}$	9	3
a-$C_{17:0}$	100	30
C_{17} cyc	6	2
3-Hydroxy (approx 10 mol% of total)		
i-C_{14}h	9	3
n-C_{14}h	6	2
a-C_{15}h	100	37
n-C_{20}h	21	8
a-C_{21}h	6	2
n-C_{21}h	33	12
n-C_{22}h	48	18
a-C_{23}h	15	6
n-C_{23}h	7	3
2,3-Dihydroxy (approx 5 mol% of total)		
n-C_{12}h2	2	2
n-C_{13}h2	2	2
i-C_{14}h2	10	8
n-C_{14}h2	9	7
a-C_{15}h2	100	81

[a] Abbreviations: i, isobranched; a, anteisobranched; n, normal (straight chain); cyc, cyclopropane; h, monohydroxy fatty acid; h2, dihydroxy fatty acid. The number following the colon indicates the number of double bonds. The monohydroxy acids are 3-hydroxylated and saturated; the dihydroxy acids are 2,3-hydroxylated and saturated. Trace components (mol%, <2) include the i-$C_{14:0}$, n-$C_{15:1}$, i-$C_{16:1}$, i-$C_{17:1}$, n-$C_{17:0}$, n-$C_{20:0}$, a-$C_{21:0}$, and n-$C_{21:0}$ nonhydroxy acids; the i-C_{15}h, i-C_{16}h, n-C_{16}h, a-C_{17}h, n-C_{17}h, n-C_{19}h, i-C_{21}h, and i-C_{22}h monohydroxy acids; and the n-C_{15}h2 dihydroxy acid.

[b] The most abundant fatty acid in each class was given a value of 100.

RESULTS

Growth characteristics and biochemical tests. Microscopic examination of strain 1677-MI-H showed a filamentous, gram-negative bacillus with a single polar flagellum. Strain 1677-MI-H required cysteine for growth, as shown by its failure to grow on BCYE without cysteine. No autofluorescence was observed when the organism was exposed to long-wave (365-nm) UV light. Strain 1677-MI-H gave positive physiologic test results for catalase and oxidase. Negative results were obtained for nitrate reduction, urease, glucose fermentation, browning of tyrosine-supplemented agar, gelatinase, hippurate hydrolysis, and β-lactamase.

Cellular fatty acid analysis. The nonhydroxy, monohydroxy, and dihydroxy fatty acid profiles of strain 1677-MI-H are shown in Table 1. Branched- and straight-chain saturated and unsaturated nonhydroxy fatty acids represented some 85 mol% of the total fatty acids, while saturated 3-hydroxy fatty acids and 2,3-dihydroxy fatty acids, in a ratio of approximately 2:1, made up the remaining 15 mol%.

Isoprenoid quinones. Strain 1677-MI-H contained ubiquinones with 11, 12, 13, and 14 isoprene units (Q-11, Q-12, Q-13, and Q-14) in the polyprenoid side chain. Q-13 was the major ubiquinone and was present at a concentration approximately four times that of Q-11, Q-12, or Q-14.

Slide agglutination test. Antigen prepared from strain 1677-MI-H reacted 2+ with unabsorbed *L. pneumophila* Bloomington 2 serogroup 3 antiserum. However, no reaction was observed with *L. pneumophila* serogroup 3 antiserum that had been absorbed with *L. pneumophila* serogroup 6 cells in order to make it serogroup specific. No agglutination was observed with antisera to any of the other previously described *Legionella* species or serogroups. Strain 1677-MI-H antiserum at the optional working dilution of 1:16 gave a 4+ agglutination with 1677-MI-H antigen. No agglutination was observed with any other *Legionella* antigen.

DNA relatedness studies. DNA from strain 1677-MI-H had a G+C content of 42.6 ± 0.2 mol%. Labeled DNA from strain 1677-MI-H was hybridized at 60°C with homologous unlabeled DNA and with unlabeled DNAs from type strains of 26 of the 33 previously described *Legionella* species, and unlabeled 1677-MI-H DNA was hybridized with labeled DNAs from the type strains of *L. fairfieldensis* and *L. adelaidensis* (Table 2). The relatedness of strain 1677-MI-H to other legionellae was 1 to 12%. This low level of relatedness precluded relatedness to the five species not tested.

TABLE 2. DNA relatedness of strain 1677-MI-H to legionellae

Source of unlabeled DNA	% Relatedness at 60°C to labeled DNA from strain 1677-MI-H
Strain 1677-MI-H	100
L. fairfieldensis 1725-AUS-E[a]	12
L. brunensis 441-1	5
L. feeleii WO-44C-C3	5
L. hackeliae Lansing 2	5
L. adelaidensis 1762-AUS-E[a]	4
L. jamestownensis JA-26-G1-E2	4
L. jordanis BL-540	4
L. micdadei TATLOCK	4
L. bozemanii WIGA	3
L. gormanii LS-13	3
L. maceachernii PX-1-G2-E2	3
L. pneumophila Philadelphia 1	3
L. dumoffii NY-23	2
L. erythra SE-32A-C8	2
L. gratiana Lyon 8420412	2
L. longbeachae Long Beach 4	2
L. moravica 316-36	2
L. sainthelensi Mount Saint Helens 4	2
L. shakespearei 214	2
L. spiritensis Mount Saint Helens 9	2
L. wadsworthii 81-716 A	2
L. birminghamensis 1407-AL-H	1
L. cincinnatiensis 72-OH-H	1
L. israelensis Bercovier 4	1
L. oakridgensis Oak Ridge 10	1
L. parisiensis PF-209C-C2	1
L. quinlivanii 1442-AUS-E	1
L. tucsonensis 1087-AZ-H	1

[a] In these reactions, labeled DNA was from the species indicated and unlabeled DNA was from strain 1677-MI-H.

DISCUSSION

Strain 1677-MI-H was presumptively identified as a *Legionella* species by its tinctorial, morphologic, and growth characteristics. This identification was confirmed by the presence of predominately branched-chain cellular fatty acids and long-chain ubiquinones (4). As seen in Table 1, the fatty acid profiles of strain 1677-MI-H contain straight-chain and branched-chain components in all three fatty acid classes, which is consistent with inclusion in the genus *Legionella*. Although the nonhydroxy profile is reminiscent of other species with major contributions from a-$C_{17:0}$ and a-$C_{15:0}$, the domination of the monohydroxy and dihydroxy profiles by a-C_{15}h and a-C_{15}h2, respectively, is a distinguishing characteristic of this new species. This pattern differs from those of the other dihydroxy fatty acid-containing *Legionella* species: *L. israelensis*, *L. maceachernii*, *L. micdadei*, and the three subspecies of *L. pneumophila*, in which i-C_{14}h and i-C_{14}h2 are the dominant components; and *L. fairfieldensis*, in which n-C_{14}h and n-C_{14}h2 are the dominant components of the respective hydroxylated classes (6–8, 10).

We originally suspected that the isolate was an *L. pneumophila* strain, since it reacted with the unabsorbed *L. pneumophila* Bloomington 2 serogroup 3 antiserum. However, DNA hybridization studies in which strain 1677-MI-H was 12% or less related to other known *Legionella* species placed it in a new species, *L. lansingensis*. *L. lansingensis* is the 34th species and 53rd serogroup described in the genus and the 16th *Legionella* species shown by culture to cause human illness.

With the recognition of more strains of *L. lansingensis*, it is possible that divergence in the fatty acid patterns and phenotype markers will emerge. As is true for most *Legionella* species, *L. lansingensis* cannot be identified biochemically. It must be identified phenotypically on the basis of its specific reaction in the slide agglutination test.

Description of *L. lansingensis* sp. nov. *L. lansingensis* sp. nov. (lan. sing. en' sis N.L. fem. adj. *lansingensis* coming from Lansing, Mich.) is a gram-negative rod with a single polar flagellum. It is positive in reactions for catalase and oxidase and requires cysteine for growth. It is negative in reactions for the reduction of nitrate to nitrite, urease, acid production from D-glucose, browning of tyrosine-supplemented agar, gelatinase, hippurate hydrolysis, and β-lactamase. Its cellular fatty acids are predominately branch chained, and it contains ubiquinones with more than 10 isoprene units in the side chain. It is serologically distinct from other described legionellae and can be identified by using the slide agglutination test. The type strain of *L. lansingensis* is 1677-MI-H (ATCC 49751), which has a G+C content of 42.6 ± 0.2 mol%. It was isolated from human bronchoscopy washings.

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