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Legionella tucsonensis sp. nov. Isolated from a Renal Transplant Recipient

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A *Legionella*-like organism (strain 1087-AZ-H) was isolated from a pleural-fluid specimen from a renal transplant patient undergoing immunosuppressive therapy. Growth characteristics and gas-liquid chromatography profiles of the isolate were consistent with those for *Legionella* spp. The isolate fluoresced blue-white under long-wave UV light. Strain 1087-AZ-H was serologically distinct in the slide agglutination test with absorbed antisera. DNA hybridization studies placed it in a new *Legionella* species, *Legionella tucsonensis* (ATCC 49180).

Twenty-eight species have thus far been characterized in the genus *Legionella*, with 45 serogroups identified by their agglutinating surface antigens (1, 11, 12). Isolates of 31 of the 45 serologically distinct groups have been obtained from clinical material (11). Additional *Legionella*-like organisms that do not belong to existing *Legionella* species or serogroups have been isolated from human and environmental sources. One of these, an isolate from a renal-transplant patient undergoing immunosuppressive therapy, is the subject of the present report.

CASE REPORT

The patient was a 63-year-old man who was admitted to the Tucson Veterans Administration Medical Center on 6 October 1984 for accidental overdose of insulin, which he began taking 2 days previously for worsening hyperglycemia. He was also taking continuous immunosuppressive doses of prednisone and azathioprine for maintenance of a renal transplant that he had received on 1 March 1983.

On admission to the hospital, the patient was somnolent but easily aroused. He had no pulmonary complaints, syncope, vomiting, or dizziness. His temperature was 100.9°F (ca. 38.2°C), his pulse was 112/min, and his respiration was 14/min. Physical findings were decreased breath sounds at the left lung base and ankle edema. Chest roentgenograph films showed a new, large left-pleura effusion and a small right-pleura effusion with diffuse bilateral lower-lobe infiltrates, consistent with pulmonary edema. The diagnosis was congestive heart failure, pulmonary edema, and possible sepsis. The patient was treated with furosemide, erythromycin, gentamicin, and trimethoprim-sulfamethoxazole. A thoracentesis was performed, and 40 ml of cloudy yellow fluid was removed. Gram stains of pleural fluid showed large numbers of neutrophils but no microorganisms. Direct immunofluorescence staining for *Legionella pneumophila* serogroups 1 through 6 was negative.

On hospital day 3, the pleural-fluid specimen was positive, with small grey colonies that grew only on charcoal yeast extract agar. The colonies contained poorly staining, small, gram-negative bacilli that were catalase positive and exhibited blue-white autofluorescence. Immunofluorescence staining for *L. pneumophila* serogroups 1 through 6 was negative. On the basis of these results, therapy was changed to erythromycin and rifampin. The patient rapidly improved, was extubated on hospital day 3, and was discharged 10 days later.

During the following year the patient was hospitalized on several occasions for pulmonary coccidioidomycosis infection, worsening congestive heart failure, and atrial fibrillation. He died on 17 December 1985 from cardiopulmonary arrest. A significant postmortem finding was the presence of left-pleura fibrosis. Retrospective Dieterle staining of a cell block prepared from the pleural fluid from which the *Legionella*-like organisms were isolated failed to show *Legionella*-like cells. Both the block and the *Legionella*-like isolate were submitted to the Centers for Disease Control for further identification.

MATERIALS AND METHODS

Growth and biochemical tests. Strain 1087-AZ-H was grown on buffered charcoal-yeast extract agar for all but two tests. The buffer was omitted for determining autofluorescence, and cysteine was omitted for determining cysteine requirement (11). Physiologic tests for catalase, gelatinase, oxidase, urease, β-lactamase, hippurate hydrolysis, nitrate reduction, glucose fermentation, flagella, autofluorescence, and browning of tyrosine-supplemented agar were done as described previously (3).

Cellular fatty acids. Three separate batches of strain 1087-AZ-H were analyzed for cellular nonhydroxy, monohydroxy, and dihydroxy fatty acids by gas-liquid chromatography with two separate fatty acid liberation methods (7, 8, 13). Fatty acid profiles were adjusted for the relative molar response of each component, and relative abundance was calculated with the most abundant fatty acid in each class considered equal to 100.

Slide agglutination test. Strain 1087-AZ-H was tested with antisera to all previously characterized *Legionella* species (n = 28) and serogroups (n = 45). Antiserum to strain 1087-AZ-H was prepared, tested, and absorbed as described previously (10).

DNA studies. The preparation and labeling of DNA and the hydroxyapatite method for DNA hybridization have been

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described elsewhere (2, 13). DNA hybridization was done at 60°C and, for some strains, at 75°C. The guanine-plus-cytosine (G+C) content of strain 1087-AZ-H was determined in a spectrophotometer by thermal denaturation (6). Unlabeled DNAs were prepared from type strains of 23 *Legionella* species and from five additional *L. bozemanii* strains.

Examination of pleural fluid block by indirect immunofluorescence. Sections were cut from the pleural fluid block and affixed to slides by heating for 15 min at 60°C. The slides were deparaffinized and hydrated by sequentially dipping them in two changes each of xylene, absolute ethanol, 95% ethanol, and distilled water. After air drying, the slides were stained with rabbit antiserum prepared against the *Legionella*-like organism isolated from the patient pleural fluid for 30 min at 37°C, washed in phosphate-buffered saline (pH 7.2) for 15 min, and air dried. Next, an antirabbit conjugate was added to the slides, which were again incubated and washed with phosphate-buffered saline as described above. The slides were then rinsed with distilled water, allowed to dry at 25°C, mounted with a cover slip and buffered glycerol consisting of 1 part of 0.5 M carbonate-bicarbonate buffer (pH 9.0) in 9 parts glycerol, and examined with a fluorescence microscope.

RESULTS

Growth characteristics and biochemical tests. Strain 1087-AZ-H was typical of *Legionella* species in that it required cysteine for growth. Blue-white autofluorescence was observed when the organism was exposed to long-wave (365-nm) UV light. Microscopic examination showed a filamentous, gram-negative bacillus with a single polar flagellum. Physiologic test results for strain 1087-AZ-H were negative for nitrate reduction, urease, oxidase, glucose fermentation, browning of tyrosine-supplemented agar, and hippurate hydrolysis and positive for catalase, gelatinase, and β-lactamase.

Cellular fatty acid analysis. Gas-liquid chromatography profiles of strain 1087-AZ-H showed the presence of nonhydroxy and 3-hydroxy fatty acids in a molar ratio of approximately 9:1. No 2,3-dihydroxy fatty acids were found. The fatty acids detected, in order of relative abundance, and their mole percent composition in each class are shown in Table 1.

Slide agglutination test. When tested with antisera to the 28 species and 45 serotypes previously described, strain 1087-AZ-H showed a 4+ reaction with *L. longbeachae* serogroup 1 antiserum and a 1+ reaction with *L. bozemanii* serogroup 2 and *L. cincinnatiensis* antisera. Cross-reactivity with *L. longbeachae* serogroup 1 antiserum was removed by absorption with strain 1087-AZ-H cells. Since *L. bozemanii* serogroup 2 and *L. cincinnatiensis* antisera cross-reacted only at a 1+ level, absorptions were not carried out to remove these reactions. Strain 1087-AZ-H antiserum at the optimal working dilution of 1:8 gave a 4+ agglutination reaction with the 1087-AZ-H antigen and the *L. santicrucis* antigen and a 3+ agglutination reaction with the *L. anisa* antigen. All cross-reactions were removed from the 1087-AZ-H antiserum by absorbing the antiserum with *L. anisa* and *L. santicrucis* cells.

DNA relatedness studies. $^{32}PO_4$-labeled DNA from strain 1087-AZ-H was most highly related to strains of *L. bozemanii*, *L. anisa*, and *L. parisiensis*, species that exhibit blue-white autofluorescence (Table 2). Reciprocal relatedness values obtained using labeled DNA from *L. bozemanii* WIGA confirmed that strain 1087-AZ-H represented a new species (Table 2). Relatedness of 1087-AZ-H to labeled DNAs from the type strains of *L. birminghamensis*, *L. cincinnatiensis*, *L. brunensis*, *L. moravica*, and *L. quinlivanii* was 1 to 11% (data not shown).

TABLE 1. Major fatty acids of *L. tucsonensis*[a]

Fatty acid	Relative abundance	Mol%
Nonhydroxy		
a-$C_{15:0}$	100	25
n-$C_{16:1}$	78	20
i-$C_{16:0}$	77	20
a-$C_{17:0}$	49	12
n-$C_{16:0}$	41	10
cyc17	26	7
i-$C_{14:0}$	8	2
n-$C_{18:0}$	4	1
cyc16	3	1
n-$C_{17:0}$	3	1
3-Hydroxy		
n-C_{14}h	100	21
n-C_{16}h	72	15
i-C_{16}h	63	13
n-C_{18}h	60	12
a-C_{15}h	38	8
a-C_{17}h	33	7
n-C_{20}h	28	6
n-C_{15}h	23	5
a-C_{19}h	12	3
n-C_{19}h	10	2
i-C_{14}h	9	2
n-C_{17}h	9	2
i-C_{15}h	6	1
i-C_{17}h	6	1
n-C_{13}h	4	1
i-C_{18}h	4	1

[a] Minor components (relative abundance, <3) were n-$C_{15:0}$ and n-$C_{20:0}$ nonhydroxy fatty acids. The letter preceding C indicates the configuration of the chain. Abbreviations: i, iso-branched; a, anteiso-branched; n, normal (straight chain); cyc, cyclopropane; h, monohydroxy fatty acid.

Indirect immunofluorescence examination of pleural fluid. Indirect fluorescent-antibody staining of the cell block prepared from the culture-positive pleural fluid revealed several bacilli consistent with *Legionella* morphology that fluoresced at a level of 4+.

DISCUSSION

The patient from whose pleural fluid strain 1087-AZ-H was isolated and subsequently demonstrated by indirect fluorescent-antibody staining had pulmonary edema but no substantial inflammatory infiltrate. The presence of pleural effusions and the paucity of pulmonary infiltrates are uncommon in published cases of *Legionella* infection but may represent a very early manifestation of infection that was attenuated by antibiotic therapy (5, 9). This case most probably represents a community-acquired, opportunistic infection in an immunocompromised host with deficient cell-mediated immunity. The patient had a history of pulmonary infections, and the year after his episode of *Legionella* infection, he developed coccidioidomycosis. As with other diseases caused by opportunistic pathogens, *Legionella* infections are not uncommon among renal- or cardiac-transplant recipients (4, 13).

Strain 1087-AZ-H was presumptively identified as a *Legionella* species by its tinctorial, morphologic, and growth characteristics. This identification was confirmed by the presence of predominantly branched-chain cellular fatty acids in the isolate. DNA hybridization studies placed strain

TABLE 2. DNA relatedness of strain 1087-AZ-H to *Legionella* species[a]

Source of unlabeled DNA	Labeled DNA from:					
	Strain 1087-AZ-H			*L. bozemanii* WIGA^T		
	RBR, 60°C[b]	D[c]	RBR, 75°C	RBR, 60°C	D	RBR, 75°C
Legionella strain 1087-AZ-H	100	0.0	100	58	7.0	35
L. bozemanii 956-MA-H	66	6.5	19	86	1.5	79
L. bozemanii 352-CT-H	64	6.5	13	84	1.5	73
L. bozemanii 62-MA-H	62	7.0	15	86	1.5	70
L. bozemanii 918-ISR-U	60	7.0	32	81	2.0	70
L. bozemanii 1127-AUS-H	60	6.0	19	85	1.0	79
L. bozemanii WIGA^T	55	6.5	33	100	0.0	100
L. anisa WA-316-C3^T	54	6.5	44			
L. parisiensis PF-209C-C2^T	54	7.5	25	45	5.5	20
L. gormanii LS-13^T	39	9.5	11			
L. wadsworthii 81-716^T	36					
L. longbeachae Long Beach 4^T	35					
L. micdadei TATLOCK^T	34					
L. dumoffii NY-23^T	33	9.5	11			
L. sainthelensi Mount Saint Helens 4^T	27					
L. steigerwaltii SC-18-C9^T	25	10.0	9			
L. pneumophila Philadelphia 1^T	25					
L. cherrii ORW^T	23	10.0	9			
L. jordanis BL-540^T	10					
L. maceachernii PX-1-G2-E2^T	10					
L. jamestowniensis JA-26-G1-E2^T	10					
L. spiritensis Mount Saint Helens 9^T	10					
L. santicrucis SC-63-C7^T	9	11.5	3			
L. feeleii WO-44C-C3^T	9					
L. erythra SE-32A-C8^T	8					
L. hackeliae Lansing 2^T	7					
L. israelensis Bercovier 4^T	7					
L. oakridgensis Oak Ridge 10^T	4					
L. rubrilucens WA-270A-C2^T	2					

[a] $^{32}PO_4$-labeled DNAs were reacted with unlabeled DNAs from the same strain (homologous reaction) and from a series of other legionellae. Each reaction was done at least twice. Reassociation values in homologous reactions ranged from 30 to 85% before normalization. Control reactions, in which labeled DNA was incubated in the absence of unlabeled DNA, showed 0.3 to 3.1% binding to hydroxyapatite. These control values were subtracted before normalization.

[b] The relative binding ratio (RBR) was calculated as follows: RBR = (percentage of DNA bound to hydroxyapatite in heterologous reaction/percentage of DNA bound to hydroxyapatite in homologous reaction) × 100.

[c] Percent divergence (D) was calculated on the assumption that a 1% decrease in the thermal stability of a heterologous DNA duplex compared with the thermal stability of the homologous DNA duplex was caused by 1% of the bases within the duplex being unpaired; it was calculated to the nearest 0.5%.

1087-AZ-H in a new *Legionella* species, *L. tucsonensis*. Fatty acid profiles distinguished *L. tucsonensis* from the 28 *Legionella* species previously characterized. Although the pattern of a-$C_{15:0}$ and 3-OH, n-$C_{14:0}$ as the most abundant nonhydroxy and monohydroxy fatty acids, respectively, is shared by several of the blue-white fluorescent species, *L. tucsonensis* differs from these species in several respects. The nonhydroxy and monohydroxy fatty acid profiles of *L. tucsonensis* are readily distinguished from those of the other blue-white fluorescent species (unpublished data). *L. tucsonensis* also differs serologically from the other 28 *Legionella* species. Cross-reactions of *L. tucsonensis* antiserum with *L. santicrucis* and *L. anisa* strains were removed by absorption. *L. tucsonensis* is the 29th species and the 46th serogroup described in the genus and the 15th *Legionella* species shown by culture to cause human illness.

Description of *L. tucsonensis* sp. nov. *L. tucsonensis* (tuc.so.nen'sis N. L. fem. adj. *tucsonensis*, coming from Tucson, Ariz.) is a gram-negative rod with a single polar flagellum. Its cellular fatty acids are predominantly branch chained. It is positive in reactions for catalase, gelatinase, and β-lactamase and requires cysteine for growth. It is negative in reactions for urease, reduction of nitrate to nitrite, oxidase, hydrolysis of hippurate, and acid production from D-glucose. It can be identified serologically in the slide agglutination test with absorbed antiserum. The type strain of *L. tucsonensis* is 1087-AZ-H (ATCC 49180), which has a G+C content of 44 mol%. It was isolated from human pleural fluid.

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