Thirteenth Serogroup of *Legionella pneumophila* Isolated from Patients with Pneumonia

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A *Legionella*-like organism (strain 82A3105; ATCC 43736) was isolated from a lung aspirate taken from a patient with pneumonia. Results of physiologic, gas-liquid chromatographic, genetic, and serologic tests showed that strain 82A3105 and four additional clinical isolates belong to a new *Legionella pneumophila* serogroup 13.

Twelve serogroups of *Legionella pneumophila*, all of which can cause human pneumonia, have been described (2, 4–6). Here we describe *Legionella pneumophila* serogroup 13, based on five clinical isolates with cultural, biochemical, morphological, and Gram stain characteristics of *Legionella* species. None of the isolates reacted with fluorescent-antibody conjugates made to any of the species and serogroups of *Legionella* known and available at the time of isolation. A direct immunofluorescence assay conjugate prepared with the first isolate, which had been submitted to the Centers for Disease Control by the Washington State Department of Social and Health Services and designated Seattle 1, stained all five isolates 4+. No clinical information was available for strain Seattle 1. The other four isolates, either isolated by or submitted to the Department of Health Services, State of California, were from patients with pneumonia.

The first California patient, a 60-year-old man, was admitted to the hospital in late April 1982 with a fever (38.9°C), diarrhea, dry cough, and mental confusion. He developed renal failure. Two days after the onset of illness, he was treated with erythromycin. One day later, a lung aspirate was taken and submitted to the Department of Health Services, State of California, for culturing and direct fluorescent-antibody examination. Gram and Gimenez stains of the aspirate revealed no organisms. Direct fluorescent-antibody stains with available *Legionella* polyvalent conjugates were negative. By 4 days, a *Legionella*-like organism, strain 82A3105 (CDC 1425-CA-H), was growing on buffered charcoal-yeast extract (BCYE) agar. No other bacteria were isolated. Acute- and convalescent-phase sera drawn from the patient on 29 April and 24 June, respectively, were tested by the indirect fluorescent-antibody test. No rise in titer was demonstrated against the *L. pneumophila* serogroup 1 antigen. An eightfold rise in titer (512 to >4,096) was demonstrated when the isolate from the patient was used as the antigen.

During 1986, three additional isolates were submitted to the Department of Health Services, State of California, for identification. Strain 86A1687 (CDC 1411-CA-H) was isolated from a bronchial brush taken from a 70-year-old woman with pneumonia. Indirect fluorescent-antibody test results of her acute- and convalescent-phase sera were negative with the *L. pneumophila* serogroup 1 antigen. She had no apparent underlying conditions and recovered after treatment with erythromycin. Strain 86A3350 (CDC 1426-CA-H) was isolated from an endotracheal aspirate taken from a 44-year-old man. This patient, who was suffering from pneumonia and also had a history of lymphoma, recovered. Strain 86A3704 (CDC 1460-CA-H) was isolated from a bronchial wash taken from a 63-year-old man with pneumonia, diabetes, and lymphatic leukemia. He developed renal failure and died. No information was available on treatment or serologic testing for the last two patients. There was no apparent epidemiologic connection among the 3 patients.

The five *Legionella*-like strains were tested for catalase, oxidase, gelatinase, and urease activities as described previously (7), except that 1% *N,N*-dimethyl-phenylendiamine oxalate was substituted for tetramethyl-phenylenediamine dihydrochloride in the oxidase test. The ability to reduce nitrate was tested with BCYE broth supplemented with 0.1% KNO3. β-Lactamase testing was done with Cefinase disks (BBL Microbiology Systems). Hippurate hydrolysis was determined by the method of Hébert (1). Colonies were examined for autofluorescence with a Wood’s lamp (8). The isolates were tested for their ability to grow on BCYE agar, BCYE agar without cysteine, and sheep blood agar (8). Cellular fatty acids were analyzed by capillary-column gas-liquid chromatography (3). Previously described methods were used for the slide agglutination test (5) and DNA relatedness studies (9).

All five strains were gram-negative rods that grew on BCYE agar but not on BCYE agar without cysteine or on sheep blood agar. All were positive for catalase, gelatinase, hippurate hydrolysis, and β-lactamase and negative for urease, nitrate reduction, and autofluorescence. The oxidase reaction varied from negative to weakly positive among the five strains. Their cellular fatty acid profiles were consistent with the pattern described for *L. pneumophila* (3). DNAs from the five strains were 98% or more related to labeled DNA from *L. pneumophila*. The relatedness of *L. pneumophila* Philadelphia 1 (type strain) was 75% or higher at both optimal (60°C) and stringent (75°C) criteria for DNA reassociation, and related sequences contained only 1.5% unpaired bases (divergence). These data confirm the identity of the strains as *L. pneumophila*.

In the slide agglutination test, Seattle 1 antiserum at its use dilution (1:32) agglutinated all five strains 4+. Cross-reactions with strains Los Angeles 1 (serogroup 4) and Leiden 1...
(serogroup 10) and two Legionella-like organisms, 1335JD and 1347JD, were removed by absorbing Seattle 1 antiserum with strain Leiden 1 without affecting the homologous reaction. When Seattle 1 and the 4 Seattle 1-reactive California isolates were tested with unabsorbed antisera to all known Legionella species and serogroups, they agglutinated only with 1335JD and Leiden 1 antisera. The cross-reactions were removed by absorbing the antisera with strain Seattle 1.

These results show that the four California strains and Seattle 1 belong to a new serogroup of L. pneumophila, serogroup 13. The reference strain for this serogroup is 82A3105 (CDC 1425-CA-H; ATCC 43736).

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LITERATURE CITED