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NOTES

Pleural Infection Caused by *Legionella anisa*

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The first case of infection caused by *Legionella anisa* with isolation of the organism is reported here. The presence of *L. anisa* in the water supply of the hospital and the isolation of this species in the pleural fluid of a patient suffering from nosocomial pleurisy confirm the potential pathogenicity of this *Legionella* species.

The observation of significant levels of antibodies to *Legionella anisa* in nosocomial cases of pneumonia in the same hospital and concomitant isolation of *L. anisa* in the hot water system of the hospital had suggested the potential pathogenicity of this species (1). However, this was not confirmed by isolation of the strain from patient samples.

As far as we know, we report here the first case of *L. anisa* isolated from a patient with a respiratory infection. The patient, a 32-year-old male, was admitted on 15 September 1987 for a left axillary lymph node carcinoma of unexplained origin. He received two successive chemotherapies (adriamycin, 135 mg; cyclophosphamide, 2.2 g; vincristine, 13 mg; bleomycin sulfate, 50 mg; cisplatin, 220 mg; and glucosteroids, 550 mg), as well as local and cerebral radiotherapy (from 15 October to 26 November). On 1 December he presented with a dyspnea and a left pleural effusion. He was given 5-fluorouracil (1.8 g) and cisplatinum (40 mg). Because of a leukocyte count of 10,800/mm^3 with 88% polymorphonuclear leukocytes and previous *Staphylococcus aureus* infection and after blood samplings, antibiotic treatment with cloxacillin and netilmicin was started. The patient became anuric and was transferred to the intensive care unit on 7 December. Physical examination revealed a temperature of 37°C, a respiratory rate of 20, a heart rate of 128, and systolic blood pressure of 105 mm Hg. There were signs of pleural effusion in the left lung. Laboratory data revealed a leukocyte count of 36,000/mm^3 with 89% granulocytes, blood urea nitrogen of 17.9 mmol/liter, serum creatinine of 289 μmol/liter, TGP of 176 IU/liter, TOG of 118 IU/liter, lactic dehydrogenase of 740, GGT of 67 IU/liter, bilirubin of 9 μmol/liter, and room arterial blood gases as follows: pO_2 = 42 mm Hg, pCO_2 = 41 mm Hg, pH = 7.44 (1 mm Hg = 133.3 Pa). Urinary sediment was highly characteristic of acute tubular necrosis. The X ray showed a full left thoracic pleural effusion; biological and cytologic examination of pleural fluid revealed a protein concentration of 23.4 g/liter, abundant erythrocytes and polymorphonuclear leukocytes, and a few malignant cells. Evidence of pneumonia was indicated by dosage of blood gases but could not be confirmed by radiologic examination because of the extent of pleurisy. Despite two pleural punctions, the patient developed respiratory failure requiring orotracheal intubation and mechanical ventilation; he died on 9 December. Autopsy was not performed. No anti-*Legionella* antibodies were detected in the blood sample collected on 8 December. The other serodiagnoses performed (virus, mycoplasma, chlamydia, coxiella, candida) were also negative. All the conventional tests for bacteria, mycobacteria, viruses, and fungi (blood culture, pleural culture) remained negative.

However, pleural fluid sampled on 8 December and shown to be negative by microscopic examination (Gram stain) demonstrated typical *Legionella* colonies after incubation for 5 days on buffered charcoal yeast extract agar (BCYE): growth on BCYE at 35°C in the presence of CO_2 (2.5%) but no growth on L-cysteine-free medium and cut-glass appearance of colonies observed under the microscope.

The isolate was positive for β-lactamase and negative for urease, nitrate reduction, and carbohydrate acidification. Like most of the species other than *L. pneumophila*, it failed to hydrolyze hippurate (1, 2). The colonies examined under Wood's light (366 nm) revealed the blue fluorescence shared by *L. anisa*, *L. bozemanii*, *L. cherrii*, *L. dumoffii*, *L. gormanii*, *L. parisiensis*, and *L. steigerwaltii*. Direct immunofluorescence assay using hyperimmune rabbit sera specific for the various *Legionella* species described showed a strongly positive reaction (+++) with the *L. anisa*-specific antiserum, as well as weaker reactions (+ to ++) with the antisera specific for *L. bozemanii* serogroup 1 and *L. longbeachae* serogroup 2.

Identification was established by determination of the cell wall fatty acid profiles using gas-liquid chromatography and by determination of ubiquinone content by high-pressure liquid chromatography (4, 5). The predominance and proportions of acids a-$C_{15:0}$ (27%), i-$C_{16:0}$ (26%), and $C_{16:1}$ (12%) were characteristic of the species *L. anisa*, *L. gormanii*, and *L. hackeliae* according to our previous data (4). The association of direct immunofluorescence assay results and fatty acid patterns suggested that the isolate could belong to the *L. anisa* species, which was confirmed by the ubiquinone content (Q9:1.3, Q10:3.8, Q11:2.9, Q12:4).

Identification was completed by examination of membrane proteins by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and compared with those of four other *L. anisa* strains (two reference and two environmental isolates) and two species with similar characteristics (*L. bozemanii* serogroup 1 and *L. longbeachae* serogroup 2). Membrane proteins were extracted by the modified method of Lema and Brown (3). Electrophoresis was performed with the Phast

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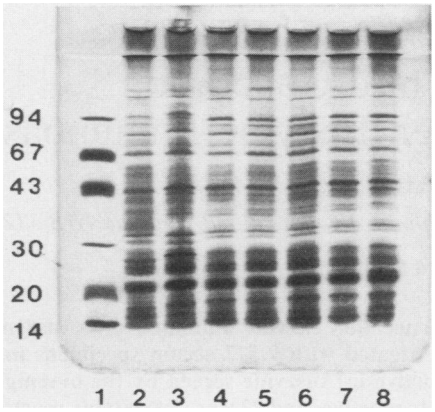

FIG. 1. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis of extracts of strains of *L. anisa*, *L. bozemanii* serogroup 1, and *L. longbeachae* serogroup 2. The gel (12.5% acrylamide) was stained with silver nitrate. Lanes: 1, standards of proteins (LMW; Pharmacia) with molecular sizes in kilodaltons; 2, *L. bozemanii* serogroup 1 (ATCC 33217); 3, *L. longbeachae* serogroup 2 (ATCC 33484); 4, *L. anisa* of environmental origin (822011); 5, *L. anisa* of environmental origin (86201313); 6, *L. anisa* isolated from the patient (pleural fluid) (87101758); 7, *L. anisa* CH47C3; and 8, *L. anisa* ATCC 35292.

system (Pharmacia, Uppsala, Sweden) (6). After silver staining, the five *L. anisa* strains showed identical protein profiles (Fig. 1) with some bands (66, 42, 32, 24, and 21 kilodaltons) common to *L. bozemanii* and *L. longbeachae* and, conversely, one clear band at 92 kilodaltons and two close bands at 41 to 40 kilodaltons characteristic of *L. anisa* only.

Lastly, the genetic identification of *L. anisa* was confirmed by hybridization studies of the bacterial genome done by P. A. D. Grimont (Institut Pasteur, Paris, France) using the S1 nuclease method at 60°C.

Our isolate was 100% related to the type strain of *L. anisa*; 50 to 52% related to *L. parisiensis* and *L. bozemanii*; 24 to 26% related to *L. gormanii*, *L. dumoffii*, and *L. cherrii*; 10 to 16% related to *L. longbeachae*, *L. sainthelensi*, *L. wadsworthii*, and *L. jordanis*; and 1 to 5% related to *L. pneumophila*, *L. rubrilucens*, *L. spiritensis*, *L. micdadei*, *L. oakridgensis*, *L. feeleii*, and *L. jamestowniensis*.

Isolation of an *L. anisa* strain from the pleural fluid of an immunosuppressed patient with a severe respiratory infection definitively confirmed the pathogenic role of the species. The lack of a serum reaction was probably the result of sampling early in the course of the disease. The origin of the disease seemed to be nosocomial. In this hospital previous cases of legionellosis caused by *L. pneumophila* serogroups 1, 5, and 8 had been observed and bacteriologically confirmed; these isolates, as well as *L. erythra* and *L. anisa*, were present in the hot water system at concentrations of 10^2 to 10^4 CFU/liter, depending on the sites sampled.

Chlorine pumps providing free chlorine concentrations of 2 to 5 ppm (μg/ml) at tap outlets had been installed in the units concerned by the outbreak (intensive care and hematology); the pumps had been functioning for 6 months. The case described above occurred while the chlorine pump in the hematology unit was temporarily disconnected. The pump is now working again, and no further case has been observed. This underlines the potential danger of the presence of legionellae in the environment, especially for immunosuppressed patients, and the need for epidemiological, clinical, and environmental surveillance.

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