The First Clinical Isolate of *Legionella parisiensis*, from a Liver Transplant Patient with Pneumonia

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A bluish white autofluorescent strain of *Legionella* was isolated from the tracheal aspirate of a female liver transplant patient who developed hospital-acquired pneumonia. This strain had biochemical characteristics compatible with those of *L. cherrii*, *L. anisa*, and *L. parisiensis* and could not be differentiated from *L. bozemanii* and *L. parisiensis* by the direct fluorescent-antibody assay. Phylogenetic analysis of partial 16S rRNA gene sequences of this strain (ATCC 700174) revealed the closest homology to the species *L. parisiensis* (99.5%). An *L. parisiensis* species-specific profile was also identified by a random amplified polymorphic DNA technique. This is the first report of *L. parisiensis* isolation from humans.

Since the first isolation of the bacterium responsible for Legionnaires' disease (21) and its description as *Legionella pneumophila* (5), numerous other species have been characterized. At present, the family *Legionellaceae* comprises 42 species (2, 20), among which 18 have been isolated from patients with pneumonia (12, 16, 28).

Following the description of *L. parisiensis* isolated from a cooling tower in Paris (4), no further isolation of this species has been reported, either from the environment or from humans. *L. parisiensis* has biochemical characteristics identical to those of *L. anisa* and *L. cherrii*, and antibodies directed against *L. parisiensis* cross-react with *L. bozemanii* (31). All these species belong to the bluish white autofluorescent group of *Legionella*. Within this group, identification at the species level is difficult but can be achieved by molecular methods such as 16S rRNA gene sequencing or ribotyping. However, ribotyping has not been sufficiently evaluated as a taxonomic tool for the autotrophic Legionella, since most of the ribotypes were determined with a very limited number of *Legionella* isolates, usually the type strain only (15).

Random amplification of polymorphic DNA (RAPD) techniques, which have been used for the identification of species of *Leptospira*, *Brucella*, and yeasts (11, 24, 26), have to date not been applied to *Legionella*.

Here, we report the first description of *L. parisiensis* associated with pneumonia, in a liver transplant patient. The strain was identified according to its biochemical characteristics, the direct fluorescent-antibody assay (DFA), and partial 16S rRNA gene sequencing. Moreover, when comparing the RAPD profiles of *L. pneumophila* to those of the eight species of bluish white autofluorescent *Legionella*, species-specific patterns were observed, facilitating the recognition of the patient isolate as *L. parisiensis*.

CASE REPORT

The patient was a 34-year-old female. She had a history of sarcoidosis that was recognized in 1990 and treated by corticotherapy, but she had a relapse in November 1993 and in May 1994 she developed cirrhosis of the liver. In December 1995, she received a liver transplant in Hôpital Paul Brousse (Villejuif, France), near Paris, and immunosuppressive treatment (corticotherapy and antilymphocyte sera). Postsurgical follow-up was complicated by renal failure requiring hemodialysis and graft rejection which was treated with immunosuppressants as a cure. Four weeks after admission to the hospital, she developed a right lower lobe pneumonia associated with severe dyspnea. She was febrile (39°C) and presented a neurological deterioration followed by a coma. The patient was first unsuccessfully treated with teicoplanin, imipenem, amikacin, and ganciclovir, followed by erythromycin for 3 weeks, allowing resolution of the pneumonia. She received a second liver transplant for hepatic necrosis and recovered. A tracheal aspirate was obtained before the treatment with erythromycin was initiated.

Sewty-two hours after inoculation of 0.1 ml of the tracheal aspirate onto buffered charcoal yeast extract agar supplemented with 0.1% α-ketoglutarate (BCYEa) (9), more than 10 bacterial colonies grew. The colonies had two different morphologies: one corresponding to a creamy yellow isolate (designated FLP1) and the other corresponding to a creamy white isolate (designated FLP2). The two colony morphologies had the ground glass appearance of the *Legionellaceae* and were Gram stain negative. No other microorganisms were recovered from the tracheal aspirate. A bronchoalveolar lavage was performed at the same time but the specimen was not cultured for *Legionella*; it did yield cytomegalovirus and rare colonies of *Staphylococcus aureus*. No other significant microorganisms were cultured from blood or urine during the course of pneumonia.

MATERIALS AND METHODS

Strains and bacteriological methods. Twenty-nine strains representing nine *Legionella* species were used for this study: *L. anisa* ATCC 35292; *L. anisa* CH47-C1, CH47-C3, HEH15 D3, and Strasbourg 14 no. 14; *L. bozemanii* ATCC 33217 serogroup 1; *L. bozemanii* GAPH, Arizona 1, Portugal II no. 18, and Paris-96010250 serogroups 1 and 2; *L. bozemanii* ATCC 35545 serogroup 2; *L.
consisted of 5'CCGGCGGCG-3' (5' DDBJ, EMBL, and GenBank databases with the BLAST program (1)) obtained DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1), followed obtained with primer S15. and DDBJ Nucleotide Sequence Database under accession no. U59697 for the 16S rRNA gene of strain FLP2 have been submitted to the GenBank, EMBL, (Institut Pasteur, Paris, France) by use of the Dice coefficient by scoring positive (Polaroid, Cambridge, Mass.). The profiles were analyzed by Taxotron software ethidium bromide, and photographed under UV light with an MP-4 Camera extension step at 72°C for 3 min. PCR products were run on standard 1.5% Perkin-Elmer Cetus). PCR cycles were 4 min of denaturation at 94°C, followed den) in 197.27 and 98.74% homologies, respectively; and 16S rRNA amplification, cloning, and sequencing. DNA amplification of small-subunit rRNA genes was performed by using synthetic oligonucleotide primers A (5'-AGAGTTTGATCATGGCTCAG-3') and S15 (5'-CGGCGGCGGCGC-3') (22) was used for RAPD analysis. The reaction mixture contained of 5 μg of DNA template, 3 mM MgCl₂, 0.4 mM (each) primer, 2.5 U of Taq DNA polymerase (AmpliTaq; Perkin-Elmer Cetus, Branchburg, N.J.), and 0.1 mM (each) deoxynucleotide triphosphate (Pharmacia Biotech, Uppsala, Sweden) in 1× PCR buffer (10 mM Tris-HCl [pH 8.3], 50 mM KCl, 0.001% gelatin; Perkin-Elmer Cetus). PCR cycles were 4 min of denaturation at 94°C, followed by 42 cycles of 1 min at 94°C, 1 min at 30°C, and 2 min at 72°C and a final extension step at 72°C for 3 min. PCR products were run on standard 1.5% agarose gel (SeaKem GTG; FMC BioProducts, Rockland, Maine), stained with ethidium bromide, and photographed under UV light with an MP-4 Camera (Polariod, Cambridge, Mass.). The profiles were analyzed by Taxotron software (Institut Pasteur, Paris, France) by use of the Dice coefficient by scoring positive and negative matches for all bands (8). Clustering was based on the unweighted pair group method with averages (UPGMA) or on the single-linkage method. Nucleotide sequence accession number. The two nucleotide sequences of the 16S rRNA gene of strain FLP2 have been submitted to the GenBank, EMBL, and DDBJ Nucleotide Sequence Database under accession no. U59697 for the sequence obtained with primer A and accession no. U59609 for the sequence obtained with primer S15. RESULTS Isolates FLP1 and FLP2 demonstrated bluish white autofluorescence under long-wavelength UV light (365 nm). The isolates also grew on GVPC medium, but no growth occurred on BCYE agar without l-cysteine or on blood agar plates. They had the following biochemical characteristics: positive reaction for catalase, oxidase, β-lactamase, and gelatinase, and browning of tyrosine-supplemented agar. They were negative for hippurate hydrolysis, urease production, fermentation of carbohydrates, and nitrate reduction. All these characteristics were similar to those of L. anisa, L. cherrii, and L. parisiensis (27). FLP1 and FLP2 reacted strongly by DFA (+) with unadsorbed and adsorbed sera raised against L. parisiensis and L. bozemanii serogroups 1 and 2. The only other reactions noted were weak reactions with unadsorbed sera against L. tucsonensis and L. anisa.

The 16S rRNA sequence was partially determined for isolate FLP2 only. Two sequences of 404 and 406 bp were obtained with oligonucleotides A and S15, respectively, and were deposited in GenBank under accession nos. U59697 (primer A) and U59698 (primer S15). These sequences showed maximum homology with L. parisiensis 16S rRNA gene sequences deposited in GenBank (accession no. Z49731): 98.41 and 99.50% base homologies with U59697 and U59698, respectively. The nearest species were L. anisa with 97.51 and 98.49% U59697 and U59698 sequence homologies, respectively; L. cherrii, with 97.27 and 98.74% homologies, respectively; and L. dumoffii, with 97.5 and 97.9% homologies, respectively. Our two sequences had less than 97.5% homology with the sequences of the other 37 Legionella species for which sequence data were available for comparison.

RAPD profiles were determined for three L. pneumophila type strains of serogroups 1, 3, and 10, respectively, and the 24 Legionella strains of the bluish white autofluorescent group (including the 9 type strains and 16 environmental or clinical strains) and were compared to the profiles for isolates FLP1 and FLP2. The patterns were identical after three independent determinations in which the reproducibility of the technique was assessed (Fig. 1). Twenty-five distinctive RAPD patterns were observed for the 29 Legionella strains tested. When more than one isolate of a given species was tested, a group of three to eight species-specific bands could be identified within the profiles, defining a species-specific pattern (Fig. 1 and 2). The patterns of strains FLP1 and FLP2 differed from each other by a single band. Analysis of these patterns by the Taxotron software revealed that they were closely related to that of L. parisiensis (90% similarity with SK2 primer and 81% with 910-05 primer) (Fig. 2 and data not shown). Similar results were found by both the UPGMA and the single-linkage aggregation methods.

Water samples from the hospital where the patient was admitted could be obtained only 4 months after the isolation of isolates FLP1 and FLP2. No legionellae were found in this water sample. A serum sample obtained from the patient 2 days after appearance of fever was negative (titers, less than 16) for all Legionella species except L. parisiensis, which gave the highest titer of 64. The same titers were obtained with two additional serum samples taken 21 days and 4 months after the onset of illness, respectively.

DISCUSSION Legionella account for approximately 5% of cases of pneumonia. Seventy to 90% of Legionella infections are caused by L. pneumophila, particularly serogroups 1 and 6, followed by L. micdadei. Other species cause 5 to 30% of the cases of infection (10, 27). Unusual species are not readily identified by all laboratories because biochemical tests are of little use in the differentiation of Legionella species. The usefulness of the hippurate hydrolysis test for confirmation of identification of L.
pneumophila has been hampered by the frequent positivity of more recently isolated Legionella species, such as L. feeleii, L. geestiana, L. londiniensis, L. spiritensis, and L. waltersii (2, 27). Autofluorescence of colonies under UV light also differentiates certain Legionella species, but fluorescent species are rarely isolated from humans with disease, which compromises the clinical usefulness of the test. In our study, the biochemical reactions of our isolates were similar to those of other bluish white autofluorescent species such as L. anisa, L. cherrii, and L. parisiensis (27). Legionella isolates are identified at the species level by DFA with various polyclonal antibodies, but the utility of DFA analysis is limited by cross-reactions among Legionella species and serogroups (31). By DFA isolates FLP1 and FLP2 were not differentiated from L. bozemanii and L. parisiensis.

The use of genotypic methods is often needed for differentiating between the serologically cross-reacting Legionella species (3), and definitive identification of unusual Legionella species isolated from humans most often requires 16S rRNA gene sequencing. In the present study, this method confirmed the identification of strain FLP2 (ATCC 700174) as L. parisiensis. During this study, the convenience of RAPD analysis for the identification of bluish white autofluorescent Legionella species was assessed. Species-specific bands defining species-specific patterns were observed when multiple isolates of the same species were tested (Fig. 2). Hence, the RAPD profiles of the clinical isolates of L. parisiensis were highly similar to that of the type strain, indicating the usefulness of the method in laboratory practice. The high degree of similarity between the two isolates also revealed that the patient could have been infected by two different isolates of L. parisiensis (Fig. 1). However, the dendrogram derived from the RAPD profiles does not match the phylogenetic tree derived by rRNA gene analysis or DNA-DNA hybridization (data not shown). This suggests that the RAPD profile can be of use for taxonomic but not for phylogenetic purposes. This approach by arbitrary amplification must be extended to all other Legionella species (with multiple isolates of each species) to determine if RAPD analysis could be an efficient and rapid method for the identification of clinical isolates of Legionella. In fact, this technique has mainly been used for the typing of L. pneumophila serogroup 1 isolates in epidemiological surveys and has never previously been applied to taxonomic analysis (13, 14, 25, 29, 32).

This is therefore the first description of a clinical case of human infection due to L. parisiensis. The clinical diagnosis was made on the basis of clinical and radiological signs of pneumopathy in a liver transplant patient and was confirmed by microbiological data. The possibility that the strain could be a contaminant or a colonizing organism is rather unlikely since the strain was isolated from the tracheal aspirate and the patient became apyretic only after the introduction of erythromycin. The low constant titer of 64 against L. parisiensis found in patient sera with no increase during a 4-month period...
can be explained by the immunosuppressive treatment. If this titer is not theoretically significant for a presumptive diagnosis of legionellosis, it should be noted that it is the highest titer found in comparison to those obtained against the other species of *Legionella*.

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**REFERENCES**