

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

FRANÇOIS LO PRESTI,^{1*} SERGE RIFFARD,¹ FRANÇOIS VANDENESCH,¹ MONIQUE REYROLLE,¹
ESTHEL RONCO,² PHILIPPE ICHAI,³ AND JEROME ETIENNE¹

Centre National de Référence des *Legionella*, UPRES EA 1655, Faculté de Médecine R. Laënnec, 69372 Lyon Cedex 08,¹ Service de Microbiologie, Hôpital Raymond Poincaré, 92380 Garches,² and Centre Hépatobiliaire, Service de Chirurgie Pr H. Bismuth, Hôpital Paul Brousse, 94808 Villejuif,³ France

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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 belongs 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 autofluorescent *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.

Seventy-two hours after inoculation of 0.1 ml of the tracheal aspirate onto buffered charcoal yeast extract agar supplemented with 0.1% α -ketoglutarate (BCYE α) (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, HEH 15 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.*

* Corresponding author. Mailing address: Centre National de Référence des *Legionella* UPRES EA 1655, Faculté de Médecine R. Laënnec, Rue Guillaume Paradin, 69372 Lyon Cedex 08, France. Phone: (33) 04-78-77-86-57. Fax: (33) 04-78-77-86-58. E-mail: derba@cimac-res.univ-lyon1.fr.

cherrii ATCC 35525; *L. dumoffii* ATCC 33279; *L. dumoffii* ATCC 35850, Aix 80 B3, Toulouse 23 no. 3, and Strasbourg-92101226; *L. gormanii* ATCC 33297; *L. gormanii* Greoux 9 C3, Greoux 9 C5, and Toulouse 29 no. 16; *L. parisiensis* ATCC 35299; *L. pneumophila* ATCC 33152 serogroup 1; *L. pneumophila* ATCC 33155 serogroup 3; *L. pneumophila* ATCC 43283 serogroup 10; *L. steigerwaltii* ATCC 35302; *L. tucsonensis* ATCC 49180; and clinical *Legionella* isolates FLP1 and FLP2 of unknown species (see Case Report above). Cultures and controls were tested by using BCYE α agar, glycine-vancomycin-polymyxin B-colistin (GVPC) agar, blood agar, and BCYE α agar plates without L-cysteine (7). Biochemical reactions, DFA with unadsorbed and adsorbed sera corresponding to all existing serogroups, and serology were performed as described previously (17-19). Strain FLP2 is deposited in the American Type Culture Collection as strain ATCC 700174.

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'-CGGTGTGTACAAGGCC-3'), corresponding in the *Escherichia coli* numbering (6) to positions 8 to 27 and 1384 to 1400, respectively. Sequencing of the amplification product was done by using the dideoxy chain termination method (30) with the Sequenase, version 2.0, sequencing kit (Amersham International plc, Little Chalfont, England) after cloning onto the pCR2.1 vector (TA Cloning Kit; Invitrogen, Leek, The Netherlands) in *E. coli*. Sequences were compared with those in the DDBJ, EMBL, and GenBank databases with the BLAST program (1) obtained through the Internet (<http://www.ncbi.nlm.nih.gov>).

RAPD technique. Bacteria were lysed by the boiling water method (13), and DNA was extracted with phenol-chloroform-isoamyl alcohol (25:24:1), followed by ethanol precipitation. Primer SK2 (5'-GCGGCGGCGG-3') (23) or 910-05 (5'-CCGGCGGCGG-3') (22) was used for RAPD analysis. The reaction mixture consisted of 5 μ g 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) deoxynucleoside triphosphate (Pharmacia Biotech, Uppsala, Sweden) in 1 \times 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 (Polaroid, 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. U59698 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 α 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 (4+) 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 the *L. parisiensis* 16S rRNA gene 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*,

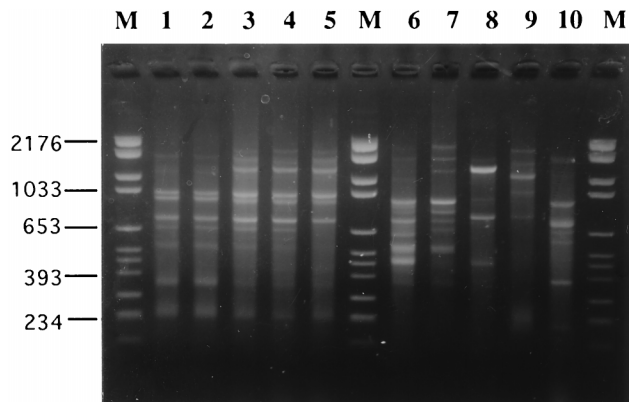


FIG. 1. RAPD amplification patterns of *Legionella* strains with primer SK2. Lanes M, molecular weight marker VI (Boehringer Mannheim); lanes 1 and 2, isolate FLP1; lanes 3 and 4, isolate FLP2; lane 5, *L. parisiensis* ATCC 35299; lane 6, *L. anisa* ATCC 35292; lane 7, *L. bozemanii* ATCC 33217 serogroup 1; lane 8, *L. dumoffii* ATCC 33279; lane 9, *L. gormanii* ATCC 33297; lane 10, *L. tucsonensis* ATCC 49180.

with 97.5 and 97.9% homologies, respectively. Our two sequences had less than 97.3% 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 (titer, 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.*

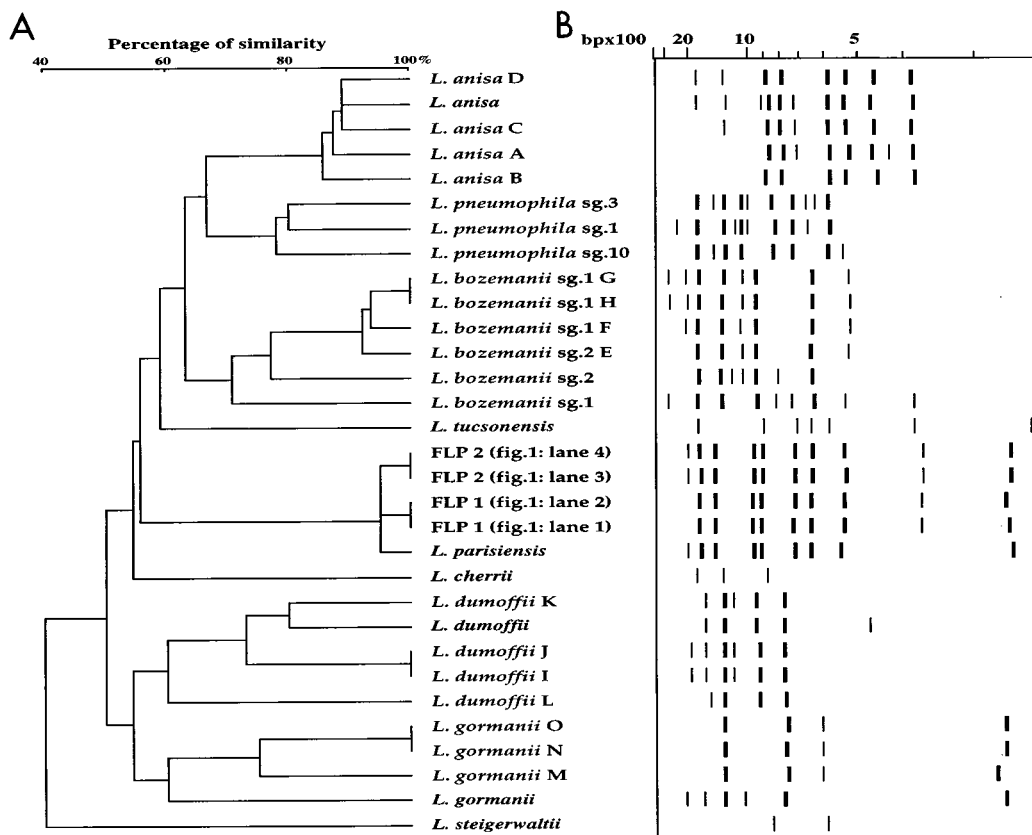


FIG. 2. Identification of clinical isolates of *L. parisiensis* by analysis of RAPD patterns. (B) Schematic of RAPD patterns. Each species except for the following is represented by the type strain: A, strain CH47-C1 (ATCC 35290); B, strain CH47-C3 (ATCC 35291); C, strain HEH 15 D3; D, strain Strasbourg 14 no. 14; E, strain Paris-96010250; F, strain Arizona 1; G, strain GAPH; H, strain Portugal II no. 18; I, strain Aix 80 B3; J, strain Toulouse 23 no. 3; K, strain Wadsworth 81-782A (ATCC 35850); L, strain Strasbourg-92101226; M, strain Greoux 9 C3; N, strain Greoux 9 C5; O, strain Toulouse 29 no. 16. Heavier bands correspond to those always present in a given species. (A) Dendrogram corresponding to panel B based on the single-linkage aggregation method (error, 2 to 3.5%) on a matrix by use of the Dice coefficient.

pneumophila has been hampered by the frequent positivity of more recently isolated *Legionella* species, such as *L. feeleyi*, *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

- Altschul, S. F., W. Gish, W. Miller, E. W. Myers, and D. J. Lipman. 1990. Basic local alignment search tool. *J. Mol. Biol.* **215**:403–410.
- Benson, R. F., W. L. Thacker, M. I. Daneshvar, and D. J. Brenner. 1996. *Legionella waltersii* sp. nov. and an unnamed *Legionella* genomospecies isolated from water in Australia. *Int. J. Syst. Bacteriol.* **46**:631–634.
- Brenner, D. J. 1986. Classification of *Legionellaceae*. Current status and remaining questions. *Isr. J. Med. Sci.* **22**:620–632.
- Brenner, D. J., A. G. Steigerwalt, G. W. Gorman, H. W. Wilkinson, W. F. Bibb, M. Hackel, R. L. Tyndall, J. Campbell, J. C. Feeley, W. L. Thacker, P. Skaliy, W. T. Martin, B. J. Brake, B. S. Fields, H. V. McEachern, and L. K. Corcoran. 1985. Ten new species of *Legionella*. *Int. J. Syst. Bacteriol.* **35**:50–59.
- Brenner, D. J., A. G. Steigerwalt, and J. E. McDade. 1979. Classification of the Legionnaires' disease bacterium: *Legionella pneumophila*, genus novum, species nova, of the family *Legionellaceae*, family nova. *Ann. Intern. Med.* **90**:656–658.
- Brosius, J., M. L. Palmer, P. J. Kennedy, and H. F. Noller. 1978. Complete nucleotide sequence of a 16S ribosomal RNA gene from *Escherichia coli*. *Proc. Natl. Acad. Sci. USA* **75**:4801–4805.
- Dennis, P. J. L. 1988. Isolation of legionellae from environmental specimens, p. 31–44. *In* T. G. Harrison and A. G. Taylor (ed.), *A laboratory manual for Legionella*. John Wiley & Sons Ltd., Chichester, United Kingdom.
- Dice, L. R. 1945. Measures of the amount of ecological association between species. *Ecology* **26**:297–302.
- Edelstein, P. H. 1981. Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. *J. Clin. Microbiol.* **14**:298–303.
- Edelstein, P. H., and R. D. Meyer. 1994. *Legionella* pneumonias, p. 455–484. *In* J. E. Pennington (ed.), *Respiratory infections: diagnosis and management*. Raven Press, Ltd., New York, N.Y.
- Fekete, A., J. A. Bantle, S. M. Halling, and R. W. Stich. 1992. Amplification fragment length polymorphism in *Brucella* strains by use of polymerase chain reaction with arbitrary primers. *J. Bacteriol.* **174**:7778–7783.
- Fry, N. K., T. J. Rowbotham, N. A. Saunders, and T. M. Embley. 1991. Direct amplification and sequencing of the 16S ribosomal DNA of an intracellular *Legionella* species recovered by amoebal enrichment from the sputum of a patient with pneumonia. *FEMS Microbiol. Lett.* **83**:165–168.
- Gomez-Lus, P., B. S. Fields, R. F. Benson, W. T. Martin, S. P. O'Connor, and C. M. Black. 1993. Comparison of arbitrarily primed polymerase chain reaction, ribotyping, and monoclonal antibody analysis for subtyping *Legionella pneumophila* serogroup 1. *J. Clin. Microbiol.* **31**:1940–1942.
- Grattard, F., P. Berthelot, M. Reyrolle, A. Ros, J. Etienne, and B. Pozzeto. 1996. Molecular typing of nosocomial strains of *Legionella pneumophila* by arbitrarily primed PCR. *J. Clin. Microbiol.* **34**:1595–1598.
- Grimont, F., M. Lefevre, E. Ageron, and P. A. D. Grimont. 1989. rRNA gene restriction patterns of *Legionella* species: a molecular identification system. *Res. Microbiol.* **140**:615–626.
- Harrison, T. G., and N. A. Saunders. 1994. Taxonomy and typing of legionellae. *Rev. Med. Microbiol.* **5**:79–90.
- Harrison, T. G., and A. G. Taylor. 1988. The diagnosis of Legionnaires' disease by estimation of antibody levels, p. 113–134. *In* T. G. Harrison and A. G. Taylor (ed.), *A laboratory manual for Legionella*. John Wiley & Sons Ltd., Chichester, United Kingdom.
- Harrison, T. G., and A. G. Taylor. 1988. Identification of Legionellae by serological methods, p. 57–68. *In* T. G. Harrison and A. G. Taylor (ed.), *A laboratory manual for Legionella*. John Wiley & Sons Ltd., Chichester, United Kingdom.
- Harrison, T. G., and A. G. Taylor. 1988. Phenotypic characteristics of Legionellae, p. 45–56. *In* T. G. Harrison and A. G. Taylor (ed.), *A laboratory manual for Legionella*. John Wiley & Sons Ltd., Chichester, United Kingdom.
- Hooley, J. V., N. A. Saunders, N. K. Fry, R. J. Birtles, and T. G. Harrison. 1996. Phylogeny of *Legionellaceae* based on small-subunit ribosomal DNA sequences and proposal of *Legionella lytica* comb. nov. for *Legionella*-like amoebal pathogens. *Int. J. Syst. Bacteriol.* **46**:526–531.
- McDade, J. E., C. C. Shepard, D. W. Fraser, T. R. Tsai, M. A. Redus, W. R. Dowdle, and Laboratory Investigation Team. 1977. Legionnaires' disease. Isolation of a bacterium and demonstration of its role in other respiratory diseases. *N. Engl. J. Med.* **297**:1197–1203.
- Menard, C., and C. Mouton. 1993. Randomly amplified polymorphic DNA analysis confirms the biotyping scheme of *Porphyromonas gingivalis*. *Res. Microbiol.* **144**:445–455.
- Meunier, J.-R., and P. A. D. Grimont. 1993. Factors affecting reproducibility of random amplified polymorphic DNA fingerprinting. *Res. Microbiol.* **144**:373–379.
- Paffetti, D., C. Barberio, E. Casalone, D. Cavalieri, R. Fani, G. Fia, E. Mori, and M. Polsinelli. 1995. DNA fingerprinting by random amplified polymorphic DNA and restriction fragment length polymorphism is useful for yeast typing. *Res. Microbiol.* **146**:587–594.
- Pruckler, J. M., L. A. Mermel, R. F. Benson, C. Giorgio, P. K. Cassidy, R. F. Breiman, C. G. Whitney, and B. S. Fields. 1995. Comparison of *Legionella pneumophila* isolates by arbitrarily primed PCR and pulsed-field gel electrophoresis: analysis from seven epidemic investigations. *J. Clin. Microbiol.* **33**:2872–2875.
- Ralph, D., M. McClelland, J. Welsh, G. Baranton, and P. Perolat. 1993. *Leptospira* species categorized by arbitrarily primed polymerase chain reaction (PCR) and by mapped restriction polymorphisms in PCR-amplified rRNA genes. *J. Bacteriol.* **175**:973–981.
- Rodgers, F. G., and A. W. Pasculle. 1991. *Legionella*, p. 442–453. *In* A. Balows, W. J. Hausler, Jr., K. L. Hermann, H. D. Isenberg, and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 5th ed. American Society for Microbiology, Washington, D.C.
- Rowbotham, T. J. 1993. *Legionella*-like amoebal pathogens, p. 137–140. *In* J. M. Barbaree, R. F. Breiman, and A. P. Dufour (ed.), *Legionella: current status and emerging perspectives*. American Society for Microbiology, Washington D.C.
- Sandery, M., J. Coble, and S. McKersie-Donnolley. 1994. Random amplified polymorphic DNA (RAPD) profiling of *Legionella pneumophila*. *Lett. Appl. Microbiol.* **19**:184–187.
- Sanger, F., S. Nicklen, and A. R. Coulson. 1977. DNA sequencing with chain-terminating inhibitors. *Proc. Natl. Acad. Sci. USA* **74**:5463–5467.
- Thacker, W. L., B. B. Plikaytis, and H. W. Wilkinson. 1985. Identification of 22 *Legionella* species and 33 serogroups with the slide agglutination test. *J. Clin. Microbiol.* **21**:779–782.
- Van Belkum, A., M. Struelens, and W. Quint. 1993. Typing of *Legionella pneumophila* strains by polymerase chain reaction-mediated DNA fingerprinting. *J. Clin. Microbiol.* **31**:2198–2200.