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Pulsed Field Electrophoresis of Genomic Restriction Fragments for the Detection of Nosocomial *Legionella pneumophila* in Hospital Water Supplies

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Ten *Legionella pneumophila* strains isolated from different sources were analyzed according to their restriction fragment patterns obtained by cleavage of genomic DNA with *Not*I and *Sfi*I and separation by pulsed field electrophoresis. Three *L. pneumophila* isolates from a nosocomial outbreak in Lübeck (Germany) and three other *L. pneumophila* strains independently isolated from a water tap located in the care unit where the patients were hospitalized exhibited identical restriction fragment profiles. Therefore, we concluded that these environmental specimens were the source of the Legionnaires disease. Another two isolates from patients and two strains from the environment, all unrelated to the outbreak described, showed different cleavage patterns.

Nosocomial infections account for a high percentage of cases of legionellosis in different countries (16). The causative agent of Legionnaires disease is mostly *Legionella pneumophila* (15). *L. pneumophila* strains can be subtyped by serological methods (3, 7, 9), isoenzyme analysis (12), plasmid profiles (1, 8), and DNA-DNA hybridizations with DNA probes which are often derived from rRNA genes (9, 12, 13). Recent studies have favored the assumption that strains from environmental sources, often domestic water systems, cooling towers, and air-conditioning systems, were the origin of Legionnaires disease (10, 12, 14).

In this study we investigated *L. pneumophila* isolates from a nosocomial outbreak in a hospital in Lübeck, Germany. Strains S-594, S-597, and S-686 came from an intensive care unit outbreak, whereas strain S-734A was isolated from a patient with community-acquired pneumonia. Strain S-594 was cultured from an autopsy lung specimen of a 74-year-old male patient who developed pneumonia 8 days after a plastic patch of the posterior cerebral artery was performed. Strain S-597 was also isolated from an autopsy lung specimen of a 55-year-old male patient who was operated on for an aneurysm of the abdominal aorta and who died on the fifth postoperative day of pneumonia. The third strain (S-686) was isolated from the tracheal secretions of a 41-year-old male patient who had had a car accident and who developed a pneumonia on day 10 after admission to the intensive care unit. Strain S-734A was cultivated from the bronchial washings of a 42-year-old male patient who presented with pneumonia on admission to the hospital.

Environmental isolates S-621, S-695, and S-696 were grown from tap water specimens from the intensive care unit during the time of the outbreak. Strains *L. pneumophila* Philadelphia I, the causative agent of the outbreak during a meeting of the American Legion Convention in Philadelphia (15), and the environmental isolates U21S6 and MSP19S1 (2) were used as controls. All strains belonged to serogroup 1, with the exception of isolate U21S6, which was serogroup 6. Determination of serogroups was carried out by using monoclonal antibodies, as described previously (4, 7). Bacteria were grown on buffered charcoal yeast extract agar (Oxoid, Wesel, Germany) at 37°C in a 5% CO_2 atmosphere, as described previously (5).

For cleavage of genomic DNA and subsequent pulsed field electrophoresis, bacterial cells were harvested into SE buffer (75 mM NaCl, 25 mM EDTA [pH 7.4]), and the turbidity, measured at 600 nm, was adjusted to the equivalent of an optical density of 2.0. DNA isolation and cleavage with restriction enzymes was performed essentially as described recently (6). Pulsed field electrophoresis was carried out in 0.5× TBE–1% agarose gels (11) at 8°C by using the CHEF DR II system (Bio-Rad, Richmond, Calif.). Physical parameters of electrophoresis are given in the legend to Fig. 1. Lambda concatemers (Pharmacia, Germany), yeast chromosomes (*Saccharomyces cerevisiae* WAY 5-4A; Biometra, Germany), and *Hin*dIII-cleaved lambda DNA were used as DNA size markers. Restriction enzymes *Not*I and *Sfi*I were purchased from Gibco, Germany.

In order to evaluate the origin of the nosocomial *L. pneumophila* strains, genomic DNA was extracted from these specimens and from environmental isolates (see above). After cleavage with either *Not*I or *Sfi*I, the length of DNA fragments was determined by pulsed field electrophoresis. Furthermore, DNA preparations of the reference strain *L. pneumophila* Philadelphia I, *L. pneumophila* S-734A originating from a preclinically acquired case of legionellosis (see above), and two unrelated *L. pneumophila* isolates (serogroups 1 and 6) from a water tap were analyzed.

The *Not*I restriction fragment length pattern of nosocomial isolates S-594, S-597, and S-686 (Fig. 1A, lanes 4 to 6, respectively) was identical to the pattern obtained from the water isolates S-621, S-695, and S-696 (Fig. 1A, lanes 7 to 9, respectively). In contrast, the *Not*I patterns of *L. pneumophila* Philadelphia I (Fig. 1A, lane 1) and *L. pneumophila* S-734A (Fig. 1A, lane 10) did not exhibit any similarities to each other or to the patterns of the isolates mentioned above. Two other isolates from a water tap (U21S6 and MSP19S1; Fig. 1A, lanes 2 and 3) also showed totally different *Not*I patterns that were not similar to those of any of the other strains. In order to characterize the strains further, we used the restriction enzyme *Sfi*I. Figure 1B shows that *Sfi*I cleavage led to a larger number of fragments

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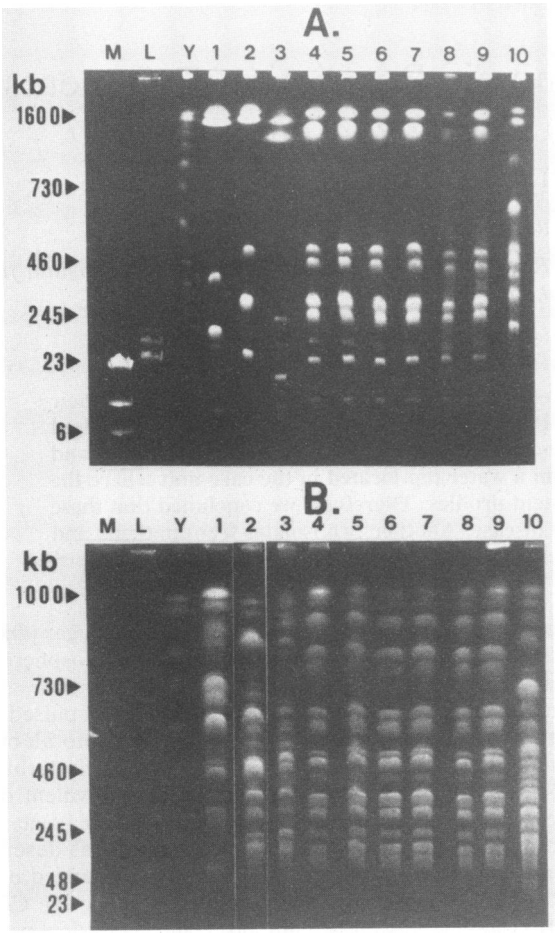

FIG. 1. Pulsed field gel electrophoresis of *Not*I (A)- and *Sfi*I (B)-cleaved genomic DNA of *L. pneumophila* strains. Lanes: 1, Philadelphia I; 2, U21S6; 3, MSP19S1; 4, S-594; 5, S-597; 6, S-686; 7, S-621; 8, S-695; 9, S-696; 10, S-734A. Lambda concatemers (lanes L), yeast chromosomes (lanes Y), and *Hin*dIII-cleaved lambda DNA (lanes M) were used as size markers. DNA sizes are indicated. Electrophoresis was run at 200 V with an increasing pulse time from 60 to 90 s for 24 h, followed by a constant pulse time of 90 s for 6 h in the case of the *Not*I digestions only (A).

compared with those obtained by *Not*I restriction pattern analysis. Those strains, however, which exhibited identical *Not*I fragments also displayed highly related *Sfi*I restriction fragment patterns (cf. Fig. 1A, lanes 4 to 9), and the strains which had different *Not*I patterns showed differences in their *Sfi*I profiles (Fig. 1A, lanes 1 to 3 and 10).

In a previous report, we demonstrated the usefulness of the genomic *Not*I restriction fragment pattern elaborated by orthogonal field alternation gel electrophoresis for analyzing *Legionella* strains (2). The low number of fragments (5 to 10, depending on the strain) obtained after cleavage of genomic DNA with *Not*I facilitates the interpretation of results. In this study we used the pulsed field technique to investigate *L. pneumophila* strains originating from a nosocomial outbreak in Lübeck. We evaluated the environmental source of the infection by comparing *L. pneumophila* strains isolated from a water tap in the care unit where the patients were hospitalized with those isolated from patients. According to the *Not*I pattern, the three isolates from patients were identical to three strains independently isolated from a water tap. These data favor the assumption that the patients were infected by the environmental isolates.

By comparing the isolates from the hospital in Lübeck with four unrelated *L. pneumophila* strains of either human or environmental origin, it is evident that marked differences in the sizes of the *Not*I restriction fragments exist. This demonstrates the discriminating power of the *Not*I profile. The results obtained by *Sfi*I cleavage were in total agreement with those of the *Not*I restriction fragment analysis. Precise typing, however, was restricted to the *Not*I profile, since the high number of *Sfi*I fragments led to a crowded arrangement of bands which also could not be resolved by improving the physical parameters of pulsed field electrophoresis (data not shown).

Typing of *Legionella* strains for epidemiologic tracking has been carried out by rDNA restriction fragment length polymorphism (9, 12–14) and plasmid (1, 8, 12) analyses, as well as by isoenzyme analysis (12). The long-range DNA mapping used in this study has not previously been described for epidemiologic tracking. Our data show that pulsed field studies are useful in determining the origins of *L. pneumophila* isolates that cause disease. This is important in controlling and preventing the dissemination of environmental *Legionella* isolates to humans. It is interesting that the World Health Organization recommends the use of the DNA fingerprinting technique (16) to assess epidemiological relationships of Legionnaires disease bacteria.

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REFERENCES

1. **Aye, T., K. Wachsmuth, J. C. Feeley, R. J. Gibson, and S. R. Johnson.** 1981. Plasmid profiles of *Legionella* species. Curr. Microbiol. **6**:389–394.
2. **Bender, L., M. Ott, R. Marre, and J. Hacker.** 1990. Genome analysis of *Legionella ssp.* by orthogonal field alternation gel electrophoresis (OFAGE). FEMS Microbiol. Lett. **72**:253–258.
3. **Benson, R. F., W. L. Thacker, H. W. Wilkinson, R. J. Fallon, and D. J. Brenner.** 1988. *Legionella pneumophila* serogroup 14 isolated from patients with fatal pneumonia. J. Clin. Microbiol. **26**:382–387.
4. **Conlan, J. W., and L. W. Ashworth.** 1986. The relationship between serogroup antigen and lipopolysaccharide of *Legionella pneumophila*. J. Hyg. **96**:39–48.
5. **Edelstein, P. H.** 1981. Improved semiselective medium for isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. J. Clin. Microbiol. **14**:298–303.
6. **Grothues, D., and B. Tümmler.** 1987. Genome analysis of *Pseudomonas aeruginosa* by field inversion gel electrophoresis. FEMS Microbiol. Lett. **48**:419–422.
7. **Joly, J. R., Y. Y. Chen, and D. Ramsay.** 1983. Serogrouping and subtyping of *Legionella pneumophila* with monoclonal antibodies. J. Clin. Microbiol. **18**:1040–1046.
8. **Maher, W. E., J. F. Plouffe, and M. F. Para.** 1983. Plasmid profiles of clinical and environmental isolates of *Legionella pneumophila* serogroup 1. J. Clin. Microbiol. **18**:1422–1423.
9. **Pfaller, M., R. Hollis, W. Johnson, R. M. Massanari, C. Helms, R. Wenzel, N. Hall, N. Moyer, and J. Joly.** 1989. The application of molecular and immunologic techniques to study the epidemiology of *Legionella pneumophila* serogroup 1. Diagn. Microbiol. Infect. Dis. **12**:295–302.
10. **Ribeiro, C. D., S. H. Burge, S. R. Palmer, J. O. Tobin, and I. D. Watkins.** 1987. *Legionella pneumophila* in a hospital water system following a nosocomial outbreak. Epidemiol. Infect. **98**: 253–262.

11. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.
12. **Tompkins, L. S., N. J. Troup, T. Woods, W. F. Bibb, and R. M. McKinney.** 1987. Molecular epidemiology of *Legionella* species by restriction endonuclease and alleloenzyme analysis. J. Clin. Microbiol. **25:**1875–1880.
13. **van Ketel, R. J.** 1988. Similar DNA restriction endonuclease profiles in strains of *Legionella pneumophila* from different serogroups. J. Clin. Microbiol. **26:**1838–1841.
14. **van Ketel, R. J., and B. de Wever.** 1989. Genetic typing in a cluster of *Legionella pneumophila* infections. J. Clin. Microbiol. **27:**1105–1107.
15. **Winn, W. C., Jr.** 1988. Legionnaires disease: historical perspective. Clin. Microbiol. Rev. **1:**60–81.
16. **World Health Organization.** 1990. Epidemiology, prevention and control of legionellosis. Memorandum from a WHO meeting. Bull. W.H.O. **68:**155–164.