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 NotI and SfiI and separation by pulsed field electrophoresis. Three L. pneumophila isolates from a nosocomial outbreak in Lübeck (Germany) and three other causative agents of the outbreak were 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 U2156 and MSP1951 (2) were used as controls. All strains belonged to serogroup 1, with the exception of isolate U2156, 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% CO2 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 concatamers (Pharmacia, Germany), yeast chromosomes (Saccharomyces cerevisiae WAY 5-4A; Biometra, Germany), and HindIII-cleaved lambda DNA were used as DNA size markers. Restriction enzymes NotI and SfiI 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 NotI or SfiI, the length of DNA fragments was determined by pulsed field electrophoresis. Furthermore, DNA preparations of the reference strain L. pneumophila Philadelphia 1, 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 NotI 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 NotI patterns of L. pneumophila Philadelphia 1 (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 (U2156 and MSP1951; Fig. 1A, lanes 2 and 3) also showed totally different NotI 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 SfiI. Figure 1B shows that SfiI cleavage led to a larger number of fragments.
fragment patterns (cf. Fig. 1A, lanes 4 to 9), and the strains from a water tap in the care unit where the patients were cleaved genomic DNA of the infection by comparing those obtained by orthogonal field alternation gel electrophoresis for analyzing L. pneumophila.

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 NotI digestions only (A).

In a previous report, we demonstrated the usefulness of NotI restriction fragment analysis. Those strains, however, which exhibited identical NotI fragments also displayed highly related SfiI restriction fragment patterns (cf. Fig. 1A, lanes 4 to 9), and the strains which had different NotI patterns showed differences in their SfiI profiles (Fig. 1A, lanes 1 to 3 and 10).

In a previous report, we demonstrated the usefulness of the genomic NotI restriction fragment patterns 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 NotI 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 NotI 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.

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