Genomic Stability of "Legionella pneumophila" Isolates Recovered from Two Cardiac Transplant Patients with Nosocomial Legionnaires' Disease

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Pulsed-field gel electrophoresis revealed that multiple consecutive isolates of "Legionella pneumophila" from two cardiac transplant patients remained genomically stable, despite exposure to host defenses and antimicrobial agents.

A number of phenotypic and genotypic methods of differentiating strains of "Legionella pneumophila" are available (1, 8, 10).

We previously examined 81 potable water and 34 patient L. pneumophila isolates using HpaI and HpaII digestion and then electrophoresis and found four distinct patterns: patterns a, b, c, and d (1). Pattern b accounted for 67% of the isolates, while pattern d was second most common, accounting for 29% of the isolates (1).

More recently, pulsed-field gel electrophoresis (PFGE) has been used to compare the restriction endonuclease patterns of L. pneumophila (12, 15). We screened 30 enzymes and found that BssHII, Sall, and SpeI gave the best results with our particular isolates (9). Schoonmaker et al. (15) speculated that minor changes in PFGE banding patterns over time may mean that L. pneumophila isolates are genetically unstable when exposed to host defenses or antimicrobial agents.

We recently treated two cardiac transplant patients who developed nosocomial Legionnaires' disease. L. pneumophila continued to be isolated from samples of respiratory secretions from the patients, despite treatment with antibiotics. This gave us an opportunity to determine whether L. pneumophila is genetically stable when exposed to host defenses or antimicrobial agents. We characterized these multiple isolates of L. pneumophila using plasmid profiling, conventional restriction endonuclease analysis, and PFGE.

A 54-year-old male (patient 1) who developed nosocomial pneumonia caused by L. pneumophila serogroup 1 following cardiac transplantation was successfully treated with erythromycin and rifampin but relapsed following treatment with antilymphocyte globulin for graft rejection. Isolates of L. pneumophila were recovered on seven different occasions over a 45-day period. Another 53-year-old male (patient 2) developed nosocomial pneumonia caused by L. pneumophila serogroup 1 following cardiac transplantation. L. pneumophila serogroup 1 was isolated on seven occasions over a 2-week period.

L. pneumophila was isolated from respiratory secretions of the patients and from potable water in the microbiology laboratory of the Victoria General Hospital as described previously (1). Plasmids were extracted from buffered charcoal yeast extract-grown cells by a modified alkaline detergent procedure (4). Genomic DNA for use as a substrate for the generation of small fragments of restriction endonucleases was prepared by the procedure of Pitcher et al. (13). The endonucleases HpaI and HpaII were used as recommended by the manufactures. Electrophoretic separations were completed in vertical 0.75% agarose slab gels at 25 V for 18 h.

Growth from a single plate was used to prepare high-molecular-weight DNA in agarose plugs for each strain (9). Agarose plugs were digested overnight with BssHII, Sall, or SpeI as recommended by the manufacturer (Stratagene, Professional Diagnostics Inc., Edmonton, Alberta, Canada). Electrophoresis was performed in 1% agarose gels for 12 h with a 5-s pulse and then 12 h with a 10-s pulse with a contour clamped homogeneous electric field system (Pulsaphor Plus; Pharmacia LKB, Uppsala, Sweden). Gels were stained with ethidium bromide and were photographed under UV illumination. Unique large restriction fragment patterns were given arbitrary numerical designations within each enzyme category.

The MICs of ciprofloxacin, doxycycline, rifampin, and erythromycin for four isolates from patient 1 and six isolates from patient 2 were determined by a broth dilution technique described by Edelstein et al. (7).

Only pulsed-field analysis provided strain differentiation. Typical electrophoretic patterns are shown in Fig. 1. Multiple isolates from the same patient were identical; however, isolates from the two patients differed. One environmental isolate (lane 5) was different from the isolates from patient 1, whereas the other environmental isolate (lane 6) was similar to the isolates from patient 1. The environmental isolate, recovered from the potable water in a room in which patient 2 stayed, was different from the isolates recovered from the respiratory secretions of patient 2 (lane 8). All isolates recovered from patients 1 and 2 had the same plasmid and restriction endonuclease analysis patterns (see legend to Fig. 1). Likewise, the environmental isolates shown in lane 6 (patient 1) and lane 8 (patient 2) were identical. These isolates were obtained from water collected from faucets in the cardiovascular intensive care unit. The isolate associated with patient 1 was recovered from water sampled in September 1991, and the isolate associated with patient 2 was from water collected in April 1992.

The four isolates from patient 1 and the six isolates from patient 2 were all susceptible to ciprofloxacin, doxycycline, rifampin, and erythromycin. There was no change in the MICs

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for the first and the last isolate in each case. The MICs for the environmental isolates associated with these patients were the same as those for the clinical isolates.

These data suggest that *L. pneumophila* is genetically stable, despite prolonged exposure to antimicrobial agents and host defenses. Relapse of infection is not uncommon among cardiac transplant patients with nosocomial Legionnaire's disease (3), and prolonged treatment with antibiotics is necessary for cure. Patient 1 in the present study had such a relapse, and the similar PFGE patterns of the isolates recovered before and after the relapse of the infection in patient 1 suggest the presence of a single clone. Thus, it is likely that these organisms were still present, probably in reduced numbers, in his respiratory tract upon the initiation of antilymphocyte globulin treatment. The latter further impaired his cell-mediated immune response, allowing the organism to multiply and cause disease. Treatment failure and relapse of Legionnaires' disease have been reported previously (5, 11). Indeed, *L. pneumophila* has also been shown by others to persist in respiratory secretions despite treatment for prolonged periods (for a review, see reference 5). However, we are unaware of a report such as ours in which multiple isolates obtained over a period of weeks from the same patient have been examined and shown to be identical.

The range of concentrations of the various antibiotics required to inhibit our isolates was similar to those reported by others (2, 6, 14); thus, antibiotic resistance was not the reason for the persistence of the *Legionella* infections. Even though cell-mediated immunity is crucial to recovery from legionellosis (16), it is noteworthy that patient 1 did respond to treatment with doxycycline when all other treatments had failed.

An animal model of persistent *Legionella* infection is necessary to determine how long legionellae remain genetically stable in vivo. According to our data, legionellae remain stable for at least 80 days.

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REFERENCES


