

# Molecular epidemiology of *Legionella pneumophila* infection at a Canadian tertiary care institution

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LE NICOLLE, H BIALKOWSKA-HOBRZANSKA, B DYCK, L SEKLA, S PARKER. **Molecular epidemiology of *Legionella pneumophila* infection at a Canadian tertiary care institution.** *Can J Infect Dis* 1994;5(4):157-162.

**OBJECTIVE:** To characterize the molecular epidemiology of *Legionella* species infection at one Canadian tertiary care centre.

**DESIGN:** Twenty-eight clinical isolates and 12 environmental isolates obtained over a six-year period were analyzed by restriction fragment length polymorphism (RFLP) of chromosomal DNA. Isolates included 15 from 12 patients with hospital acquired illness and 13 from nine patients with community acquired infection.

**RESULTS:** One nosocomial strain was *Legionella micdadei* and one community strain was *Legionella pneumophila* serotype 6; all others were *L pneumophila* serotype 1. RFLP typing revealed one clone for all cases of a 1985 single-ward outbreak and five of six nonoutbreak *L pneumophila* nosocomial cases. An RFLP pattern identical or highly related to that of the nosocomial clonal type was noted among nine of 12 *L pneumophila* serotype 1 community isolates. The remaining three isolates had two related RFLP patterns distinct from the institutional strain. The nosocomial and community strains were isolated from multiple institutional water samples in the institution. For the environmental isolates, monoclonal antibody typing was more discriminating than RFLP typing: seven monoclonal antibody subtypes were distinguished among 12 environmental isolates comprising three distinct RFLP patterns.

**CONCLUSIONS:** Despite multiple *L pneumophila* serotype 1 strains isolated in the authors' institutional water, a single clone of *L pneumophila* produced most disease. Community acquired disease was caused by a wider variety of strains. (*Pour résumé, voir page 158*)

**Key Words:** *Legionella pneumophila*, Molecular epidemiology, Nosocomial infection

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Received for publication June 29, 1993. Accepted December 13, 1993

## Épidémiologie moléculaire de l'infection à *Legionella pneumophila* dans un établissement canadien de soins tertiaires

**OBJECTIF :** Caractériser l'épidémiologie moléculaire de l'infection à *Legionella* dans un établissement de soins tertiaires au Canada.

**MODÈLE :** Vingt-huit isolats cliniques et douze isolats environnementaux obtenus sur une période de six ans ont été analysés par polymorphisme de restriction de la longueur d'un fragment (RFLP) d'ADN chromosomique. Parmi les isolats, 15 provenaient de douze patients porteurs d'une maladie nosocomiale et 13 provenaient de neuf patients porteurs de maladie extra-hospitalière.

**RÉSULTATS :** Une souche nosocomiale a été *Legionella micdadei* et une souche extra-hospitalière a été *Legionella pneumophila* de sérotype 6; tous les autres étaient *L pneumophila* de sérotype 1. Le typage par RFLP a révélé un clone pour tous les cas d'une épidémie restreinte à une unité en 1985 et cinq des six cas nosocomiaux de *L pneumophila* indépendants de l'épidémie. Un mode RFLP identique ou très similaire à celui du type nosocomial cloné a été noté parmi neuf isolats de *L pneumophila* de sérotype 1 sur 12 isolats non hospitaliers. Les trois isolats restants présentaient deux modes de RFLP distincts de celui de la souche nosocomiale. Les souches nosocomiales et extra-hospitalières ont été isolées à partir de multiples échantillons d'eau prélevés dans l'établissement même. Quant aux isolats environnementaux, le typage par anticorps monoclonaux a été plus discriminatoire que le typage par RFLP : sept sous-types d'anticorps monoclonaux ont été identifiés parmi les 12 isolats environnementaux répondant à trois modes de RFLP distincts.

**CONCLUSIONS :** Malgré les souches multiples de *L pneumophila* de sérotype 1 dénombrées dans les échantillons d'eau prélevés dans l'établissement des auteurs, un seul clone de *L pneumophila* a provoqué la majeure partie des cas de morbidité. La maladie extra-hospitalière a été causée par une plus grande variété de souches.

**L**EGIONELLA PNEUMOPHILA IS AN IMPORTANT CAUSE OF both nosocomial and community acquired pneumonia worldwide (1). While *Legionella* species are ubiquitous in environmental water sources, relatively few strains, primarily *L pneumophila* serogroup 1, cause human disease. Several studies have reported that endemic and epidemic nosocomial infection in an institution is usually attributable to a single strain among many contaminating the potable water system, suggesting that certain strains are uniquely virulent (2-6). Identification and characterization of these virulent strains will contribute to our knowledge of the disease and could, potentially, have implications for management of contaminated institutional water sources.

The clinical microbiology laboratory at the Winnipeg Health Sciences Centre has cultured *L pneumophila* from clinical specimens since 1981. In 1985, an outbreak of *L pneumophila* occurred in renal transplant patients (7) and, subsequently, one to four cases per year of nosocomial *L pneumophila* have been identified (8). The institutional potable water is colonized with multiple different legionella strains.

Several approaches have been used to distinguish individual strains of *L pneumophila* in epidemiological investigations. These methods include monoclonal antibody subtyping (3,9), multilocus enzyme electrophoresis (2,10), plasmid profile analysis (11), restriction fragment length polymorphism (RFLP) typing of whole DNA (12-14) and rRNA genes (15,16). Greater discriminatory power has been noted with two or more typing methods combined (5,12). The current study was undertaken to describe the molecular epidemiology of *L pneumophila*, both nosocomial and community acquired, at the authors' institution using molecular typing and limited monoclonal antibody subtyping.

## PATIENTS AND METHODS

**Institutional characteristics:** The Winnipeg Health Sciences Centre is an 1100 bed acute care hospital which includes a pediatric hospital and programs for renal transplantation, bone marrow transplantation and oncology. It is one of two tertiary care referral hospitals serving the populations of Manitoba and northern Ontario. An outbreak of *L pneumophila* occurred in renal transplant patients on one ward in 1985, and extensive isolation of *Legionella* species from potable water was documented at that time (7). Attempts to limit water colonization subsequent to that outbreak included intermittent shock chlorination and superheating. These interventions had limited utility in maintaining the water system free from *Legionella* species. Further cases of *L pneumophila* in the hospitalized renal transplant population did not occur, however, after institution of trimethoprim/sulfamethoxazole prophylaxis.

Cases of endemic nosocomial legionella pneumonia have continued to occur in the hospital population, with one to four cases identified each year (8). These occur in all areas of the main hospital, but none in the women's and children's hospitals. Nosocomial cases are generally, but not uniquely, identified in patients receiving high dose steroid therapy. A similar number of cases are admitted from the community or transferred from other institutions and diagnosed with *L pneumophila* pneumonia at admission each year.

**Microbiological methods:** Clinical specimens for *L pneumophila* were inoculated onto buffered charcoal yeast extract (BCYE) agar for isolation and identified using standard methods (17). Serotyping was performed using commercially prepared antisera. Isolates from patients were stocked in skim milk at -70°C. All patient isolates identified between 1985 and 1991 were re-

TABLE 1  
Molecular types of *Legionella pneumophila* and *Legionella micdadei*

| Organism | Isolate number | Date | Nosocomial (N) or Community (C) | Plasmid type | RFLP type |        |       | Clonal group |
|----------|----------------|------|---------------------------------|--------------|-----------|--------|-------|--------------|
|          |                |      |                                 |              | HindIII   | HindII | EcoRI |              |
| Lpn1     | L2             | 1985 | N*                              | np           | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L4             | 1985 | N*                              | np           | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L5             | "    |                                 | a            | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L6             | "    |                                 | a            | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L7             | 1985 | N*                              | np           | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L11            | 1985 | N*                              | a            | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L12            | "    |                                 | a            | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L14            | 1985 | N*                              | a            | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L16            | 1986 | N*                              | np           | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L17            | 1986 | C                               | np           | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L18            | "    |                                 | a            | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L21            | 1988 | N                               | np           | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L22            | 1988 | N                               | np           | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L25            | 1989 | C                               | np           | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L26            | 1989 | C                               | np           | 1.1       | 1.2    | 1.2   | 1            |
| Lpn1     | L28            | 1990 | N                               | a            | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L29            | 1991 | N                               | a            | 1.1       | 1.1    | 1.1   | 1            |
| Lpn1     | L8             | 1985 | C                               | a            | 1.2       | 1.2    | 1.2   | 1            |
| Lpn1     | L9             | "    |                                 | a            | 1.2       | 1.2    | 1.2   | 1            |
| Lpn1     | L10            | "    |                                 | a            | 1.2       | 1.2    | 1.2   | 1            |
| Lpn1     | L19            | 1988 | C                               | a            | 1.1       | 1.1    | 1.2   | 1            |
| Lpn1     | L20            | 1988 | C                               | a            | 1.1       | 1.1    | 1.2   | 1            |
| Lpn1     | L13            | 1985 | N                               | np           | 2.1       | 2.1    | 2.1   | 2            |
| Lpn1     | L27            | 1990 | C                               | np           | 2.1       | 2.1    | 2.1   | 2            |
| Lpn1     | L23            | 1989 | C                               | np           | 2.2       | 2.1    | 2.1   | 2            |
| Lpn1     | L24            | "    |                                 | np           | 2.2       | 2.1    | 2.1   | 2            |
| Lmic     | L15            | 1986 | N                               | np           | 3.1       | 3.1    | 3.1   | 3            |
| Lpn6     | L30            | 1991 | C                               | np           | 5.1       | 5.1    | 5.1   | 5            |

Lmic *L. micdadei*; Lpn1 *L. pneumophila* serogroup 1; Lpn6 *L. pneumophila* serogroup 6; \*Outbreak associated strains; " Isolates from the same patient. RFLP Restriction fragment length polymorphism

trieved for this study. Environmental isolates were identified by the Cadham Provincial Laboratory. These 12 *L. pneumophila* environmental isolates were obtained from water sources throughout the institution geographically linked to patients with documented nosocomial *L. pneumophila*. Methods for processing of environmental samples and isolation of strains have been previously described (7).

**Epidemiological typing:** For (RFLP) typing, cellular DNA was extracted using previously described procedures (18) with the following modifications: bacterial confluent growth from a single BCYE agar plate was resuspended in 2 mL of 50 mM Tris hydrochloride (pH 8) – 100 mM EDTA buffer to a final density of about 12 units, at an optical density of 600 nm. Lysozyme and RNase were added to a final concentration of 200 µg/mL, and the suspension was incubated for 60 mins at 37°C. Sodium dodecyl sulphate and proteinase K were used at a final concentration of 0.5% and 200 µg/mL, respectively, during 60 mins incubation at 50°C. Total DNA was digested with three restriction endonucleases individually (*Hind*III, *Hind*II, *Eco*RI), under conditions recom-

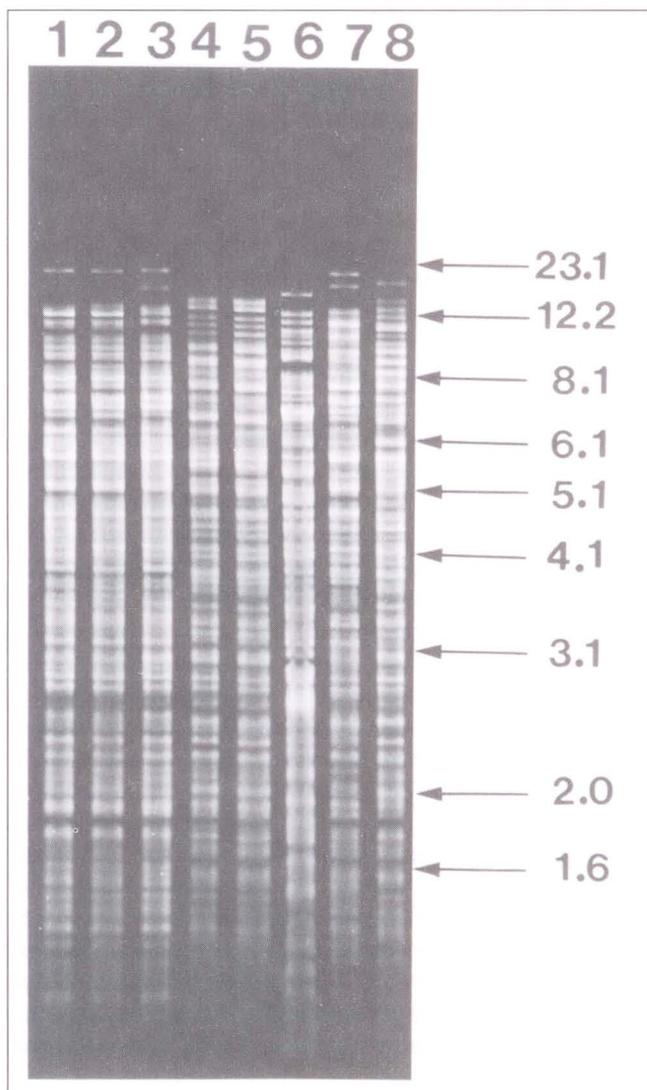
mended by the supplier (Pharmacia Fine Chemicals, New York). Agarose gel electrophoresis and numerical analysis of RFLP patterns were carried out as previously described (19). The criteria for the identity of isolates were based on the relatedness of chromosomal DNA banding patterns. Isolates with more than 95% DNA banding similarity were considered to be identical strains, and those with 85 to 95% similarity were considered to be related strains. RFLP types were designated .1 for more than 95% DNA banding similarity and .2 for more than 85% and up to 95% similarity. Both identical and related strains were considered to be from the same clonal group.

For plasmid profile analysis, undigested total DNA was separated by agarose gel electrophoresis as described above. The molecular size of the plasmid was determined according to previously reported procedures (20). Monoclonal antibody typing was previously performed on *L. pneumophila* isolated from patients during the 1985 outbreak and was routinely performed for environmental isolates. Methods have been previously described (7). Monoclonal antibody typing was

**TABLE 2**  
Distribution of patient isolates of different RFLP type by nosocomial and community acquisition

|                    | Number of isolates | Number of patients | RFLP type                  | Plasmid present (number) |
|--------------------|--------------------|--------------------|----------------------------|--------------------------|
| Hospital acquired  | 13                 | 10                 | 1.1                        | 7                        |
|                    | 1                  | 1                  | 2.1                        | —                        |
|                    | 1                  | 1                  | <i>Legionella micdadei</i> | —                        |
| Community acquired | 3                  | 3                  | 1.1                        | 1                        |
|                    | 6                  | 3                  | 1.2                        | 5                        |
|                    | 1                  | 1                  | 2.1                        | —                        |
|                    | 2                  | 1                  | 2.2                        | —                        |
|                    | 1                  | 1                  | Lpn 6*                     | —                        |

\**Legionella pneumophila* serogroup 6; RFLP Restriction fragment length polymorphism



**Figure 1** Agarose gel electrophoresis of *Hind*III digested total DNA from nosocomial (N), community (C) and environmental (E) isolates of *Legionella pneumophila*. Lanes 1 to 5, *L pneumophila* serogroup 1 isolates L7 (N restriction fragment length polymorphism [RFLP] 1.1), L17 (C RFLP 1.1), L26 (C RFLP 1.2), L13 (N RFLP 2.1), L27 (C RFLP 2.1), respectively; lane 6, *Legionella micdadei* isolate L15 (N RFLP 3.1); lane 7, *L pneumophila* serogroup 6 isolate L30 (C RFLP 5.1); and lane 8 *L pneumophila* serogroup 1 of unknown monoclonal antibody subtype #2 (E RFLP 4.1). Numbers to the right of the gel are molecular size standards (in kb)

not performed for other clinical isolates.

**Definitions and data analysis:** Pneumonia developing in a patient after 72 h of hospitalization was considered to be nosocomial infection; all others were community acquired. Two cases were transferred to the authors' institution from other hospitals in the province and diagnosed with Legionnaire's disease on admission. Both cases had been admitted to the peripheral hospital with pneumonia and, thus, were considered to be community acquired. Comparisons of distinct strains identified by molecular typing were made of epidemic with endemic isolates for nosocomial cases, of nosocomial with community isolates, and of nosocomial clinical isolates with environmental isolates.

## RESULTS

**Bacterial strains:** Twenty-eight isolates were available from 21 patients (Table 1). These included eight isolates from five patients in the 1985 outbreak, seven isolates from seven patients with hospital acquired infection isolated between 1985 and 1991, and 13 isolates from nine patients with community acquired infection admitted between 1986 and 1991. One nosocomial strain was *Legionella micdadei* and one community acquired strain was *L pneumophila* serogroup 6. All other strains were *L pneumophila* serogroup 1.

**RFLP and plasmid typing:** RFLP typing identified one dominant clone with RFLP type 1.1, which included 16 of 28 isolates (57%), and six isolates (21%) characterized by a highly related RFLP type 1.2 (Table 1). The *L micdadei* and *L pneumophila* serogroup 6 strain had distinct RFLP types. The remaining four isolates had two highly related RFLP types 2.1 and 2.2 (clonal group 2) that were distinct with respect to the major clone (Figure 1).

In every case where multiple isolates were obtained from the same patient, the isolates were identical. Thirteen of 15 nosocomial acquired isolates from 10 patients, including all the epidemiologically linked outbreak strains, derived from a single strain characterized by the RFLP type 1.1 (Table 2). Three isolates from three patients of 13 community acquired isolates had RFLP patterns identical to this strain. A second strain, including six isolates identified in three of the

TABLE 3

Molecular types and monoclonal antibody subtypes of environmental isolates\* of *Legionella pneumophila* serogroup 1

| Source     | Year | Monoclonal antibody subtype | RFLP type |        |       | Clonal group |
|------------|------|-----------------------------|-----------|--------|-------|--------------|
|            |      |                             | HindIII   | HindII | EcoRI |              |
| CCU        | 1988 | Philadelphia 1              | 1.1       | 1.1    | 1.1   | 1            |
| Ward water | 1988 | Philadelphia 1              | 1.2       | 1.3    | 1.3   | 1            |
| Ward water | 1985 | Camperdown 1                | 1.2       | 1.3    | 1.3   | 1            |
| Dialysis   | 1985 | Camperdown 1                | 1.2       | 1.3    | 1.3   | 1            |
| Ward water | 1987 | Philadelphia 1              | 1.1       | 1.1    | 1.1   | 1            |
| Ward water | 1987 | Bellingham 1                | 2.1       | 2.1    | 2.1   | 2            |
| CCU        | 1988 | Philadelphia 1              | 1.1       | 1.1    | 1.1   | 1            |
| Recovery   | 1988 | Oxford 4032E                | 1.1       | 1.1    | 1.1   | 1            |
| Ward water | 1988 | Unknown #1                  | 1.1       | 1.1    | 1.1   | 1            |
| Recovery   | 1988 | Unknown #2                  | 4.1       | 4.1    | 4.1   | 4            |
| Ward water | 1988 | Olda or Heysham             | 2.1       | 2.1    | 2.1   | 2            |
| SICU       | 1988 | Oxford 4032E                | 1.1       | 1.1    | 1.1   | 1            |

\*None of the isolates had plasmids. CCU Coronary care unit; SICU Surgical intensive care unit

nine community acquired pneumonia patients, was highly related to the predominate nosocomial strain. The single nosocomial isolate of *L pneumophila* serotype 1 with RFLP type 2.1 differing from other nosocomial isolates was similar to three community isolates (with RFLP type 2.1 and RFLP type 2.2). The RFLP types for the two community acquired strains isolated from patients transferred from other facilities were 1.1 and 1.2.

Fifteen isolates had no plasmid, and the remaining 13 had a similar large plasmid of size 21.6 MDa (Table 2). The presence of a plasmid was not unique to either nosocomial or community isolates, and was not consistent for different isolates from the same patient. No environmental isolates had a plasmid.

**Monoclonal antibody typing:** Strains from the 1985 outbreak were all monoclonal antibody type Philadelphia 1 (7). Among the environmental isolates, five monoclonal antibody types and two unknowns were identified (Table 3). The monoclonal antibody typing appeared to be more discriminating than RFLP typing for identifying strain differences. RFLP type 1.1 included both Philadelphia 1 and Oxford 4032E monoclonal types, and RFLP type 2.1 included both Bellingham and Olda or Heysham monoclonal types.

### DISCUSSION

In 1985 an outbreak of *L pneumophila* pneumonia occurred in renal transplant patients at our facility (7). The RFLP typing performed in this study is consistent with the previously reported monoclonal antibody typing showing that a single strain was responsible for this outbreak. After this outbreak, sporadic cases of nosocomial Legionnaire's disease occurring throughout our institution have, with few exceptions, been of the same molecular type. The only exceptions are one nosocomial strain that occurred the same year as the outbreak and an *L micdadei* infection that occurred the next year. Thus, for our institution, a single strain of *L pneumo-*

*phila* is responsible for most nosocomial Legionnaire's disease.

There was a wider molecular variety of strains causing legionellosis in patients admitted from the community. While a third of the community isolates was identical to the nosocomial strain, a related strain was identified as frequently among community isolates, but not in any nosocomial isolates. This strain was, however, present in the institutional water system. Finally, one *L pneumophila* serogroup 6 and two other community strains were also isolated. One of these community isolates was similar to the single unique *L pneumophila* serotype 1 nosocomial isolate and was identified in the institutional environmental isolates. RFLP typing, plasmid typing and, for environmental isolates, monoclonal antibody typing were compared. Strains either had no plasmids or had a single plasmid of 21.6 MDa, and strains with a plasmid were isolated from both community and nosocomial cases. No environmental isolates, however, had plasmids. The presence of a plasmid was not associated with any single RFLP type. Thus, plasmid typing did not appear to be helpful in differentiating strains for epidemiological purposes. This is consistent with observations from other investigations (12,15,16).

Monoclonal antibody typing was not available for most clinical isolates. The monoclonal antibody typing of the environmental isolates, however, identified a greater number of unique strains than RFLP types. In particular, the single RFLP type identified in most nosocomial cases comprised two different monoclonal antibody types, and RFLP type 2.1 also comprised two different monoclonal antibody types. Struelens et al (21) also reported greater variation with monoclonal antibody typing. They suggested that phenotypic variation, in fact, compromised the utility of monoclonal antibody typing.

These observations of one predominant institutional strain causing disease are similar to those reported

from other centres (2,12,14,21). While multiple strains of *Legionella* species are identified in potable water sources, a restricted number of strains are isolated from patients with nosocomial disease. Community strains included isolates similar to both nosocomial *L pneumophila* serogroup 1 strains, but *L micdadei*, isolated from one patient with nosocomial disease, has not been isolated from any patients with community acquired disease. Most of the community strains were also isolated from the institutional water samples. Thus these strains caused disease in the community and

were present in the institution, but did not contribute to institutional acquired disease. The organism or environmental determinants of infection by a particular strain will need further clarification to explain these observations.

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**ACKNOWLEDGEMENTS:** We thank D Jaskot and D Milley for excellent technical assistance; the clinical microbiology laboratory at the Health Sciences Centre for their assistance with *L pneumophila* isolates; Dr J Jolly, who provided the monoclonals; and B DesRosiers for secretarial support.

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