

Legionnaires' disease caused by *Legionella dumoffii* in distilled water

Jean R. Joly, MD
Pierre Déry, MD
Léo Gauvreau, MD
Louise Coté, MD
Colette Trépanier, RN

Five cases of Legionnaires' disease caused by *Legionella dumoffii* were identified within an 11-month period in a hospital in the Quebec City area. In four cases bacterial isolates were obtained from clinical specimens, and in one case seroconversion was demonstrated. All the patients had been admitted to hospital within 10 days before diagnosis. Two of the patients were immunosuppressed children. Only 1 of the 40 hot water samples from the hospital yielded *L. dumoffii*; however, 6 of 11 distilled water samples contained the bacterium. All the patients had been exposed to distilled water, four through respiratory therapy equipment and one through a room humidifier. Following the use of sterile distilled water in the apparatus, no further cases were identified. This is the first reported outbreak of Legionnaires' disease caused by *L. dumoffii*, and it is the first time that nosocomial legionellosis has been linked to contaminated distilled water in Canada.

En 11 mois on reconnaît cinq cas de maladie du légionnaire par *Legionella dumoffii* dans un hôpital de la région de Québec. On isole le microbe quatre fois et démontre une fois la séroconversion. Tous les malades, y compris deux enfants en immunosuppression, ont été

From the Département de microbiologie, Faculté de médecine, université Laval, Quebec, the Service de microbiologie, Hôtel-Dieu de Québec, Quebec, and the Service de microbiologie and the Département de pédiatrie, Centre hospitalier de l'université Laval, Quebec

Reprint requests to: Dr Jean R. Joly, Service de microbiologie, Hôtel-Dieu de Québec, 11, Côte du Palais, Québec, PQ G1R 2J6

hospitalisés moins de 10 jours avant que le diagnostic soit posé. On trouve le germe causal dans seulement 1 échantillon d'eau chaude sur 40, mais dans 6 échantillons sur 11 d'eau distillée. Celle-ci a servi à traiter tous les malades: quatre d'entre eux par l'appareillage de thérapeutique respiratoire, un autre par un humidificateur de l'air ambiant. Dès remplacement de l'eau distillée par l'eau stérilisée, on cesse d'observer des cas de maladie du légionnaire. Il s'agit de la première épidémie de cette maladie causée par ce germe et de la première démonstration au Canada de transmission nosocomiale d'une légionellose par l'eau distillée.

Numerous outbreaks of nosocomial Legionnaires' disease have been reported in the last few years.¹⁻⁴ In most instances the infections were caused by a serogroup of *Legionella pneumophila*. However, *L. micdadei* has occasionally been found to be responsible for such outbreaks.⁵ In most cases the infections were linked to contaminated heat-exchange apparatus (e.g., cooling towers and evaporative condensers)¹ or potable water distribution systems.²⁻⁵

Cases of Legionnaires' disease caused by species other than *L. pneumophila* or *L. micdadei* are rare.^{6,7} We identified five cases of nosocomial legionellosis caused by *L. dumoffii* within an 11-month period in one institution. All five patients were exposed to contaminated distilled water.

Methods

Patients were considered to have Legion-

naires' disease caused by *L. dumoffii* if they had signs and symptoms of pneumonia and fulfilled at least one of the following criteria: isolation of *L. dumoffii* from tracheobronchial secretions or lung tissue; positive results of direct fluorescent antibody testing of sputum samples or lung tissue homogenates for *L. dumoffii*; or a fourfold or greater increase in the titre of antibody to *L. dumoffii* (to at least 1:128) between acute-phase and convalescent-phase serum samples, as determined by indirect immunofluorescence.

Sputum specimens and lung tissue homogenates were cultured on buffered charcoal yeast extract (BCYE) medium supplemented with 1% α -ketoglutarate and on BCYE medium supplemented with cefamandole (Mandol), polymyxin B and anisomycin (BMPA), a semiselective medium for Legionellaceae.^{8,9} Plates were incubated for 10 days at 37°C and examined daily. Colonies with morphologic features similar to those of Legionellaceae were cultured on BCYE medium without L-cysteine. Those that failed to grow on this medium were examined by direct immunofluorescence with monospecific antisera. The *L. dumoffii* isolates stained intensely with an anti-*L. dumoffii* antiserum but not with antibody conjugates to *L. pneumophila* serogroups 1 to 8, *L. micdadei*, *L. bozemanii*, *L. gormanii*, *L. longbeachae* serogroups 1 and 2 and *L. jordanis*. The isolates were further typed by multilocus enzyme electrophoresis.¹⁰ They had an identical enzyme pattern (Toni C. Woods, PhD, and Roger M. McKinney, PhD, Centers for Disease Control, Atlanta: personal communication, 1986).

The direct immunofluorescence procedure described by Cherry and colleagues¹¹ was performed retrospectively with an anti-*L. dumoffii* antiserum on clinical samples, which had been stored at -70°C, from the patients with *L. dumoffii* pneumonia and from 10 patients with other types of pneumonia. The samples were coded and were examined by two observers.

Titres of antibody to *L. dumoffii* were determined by the indirect immunofluorescence proce-

dures of Wilkinson and associates¹² with heat-killed antigens. Early- and late-phase serum samples from a patient with culture-proven *L. dumoffii* infection were used as positive and negative controls. All serum specimens were run in parallel.

Hot water samples were collected from various areas of the hospital, including rooms where patients with legionellosis had stayed. Distilled water samples were collected from the reservoir that supplied the respiratory therapy equipment and from various outlets in the hospital. The samples were cultured on BCYE medium and BMPA and were concentrated 100-fold by centrifugation at 5000 \times g for 30 minutes and resuspension of the sediment in an aliquot of the supernatant. Incubation of plates and identification of *Legionella* were performed as for the clinical samples.

Results

Five patients fulfilled the diagnostic criteria for *L. dumoffii* infection. Four had positive results of culture, and in one seroconversion occurred and direct fluorescent antibody testing gave a positive result. In all five cases at least two of the three laboratory procedures gave positive results (Table I). Four of the patients were male, and two of the five were under 2 years of age. Four patients (including the two children) were receiving high doses of oral or parenteral steroids for underlying conditions. The remaining patient had gastric carcinoma, and Legionnaires' disease developed after surgery while the patient was receiving assisted ventilation. The signs, symptoms and radiologic features of the patients were similar to those found in patients with Legionnaires' disease caused by other species of *Legionella*. In three patients (including one child) legionellosis contributed to death despite adequate treatment with erythromycin. All three had severe underlying diseases (selenium intoxication, gastric carcinoma and severe emphysema).

Table I — Results of laboratory tests in patients with *Legionella dumoffii* infection

Patient	Date of onset of disease	Type of sample	Direct fluorescent antibody test	Culture		Titre of antibody to <i>L. dumoffii</i>	
				Buffered charcoal yeast extract (BCYE)	BCYE with cefamandole, polymyxin B and anisomycin	Acute-phase serum	Convalescent-phase serum
1	21/03/84*	Bronchial aspirate	+	+	+	-	-
2	26/04/84	Sputum	+	-	-	1:32	1:128
3	27/12/84	Bronchial aspirate	+	+	+	-	-
4	11/01/85	Bronchial aspirate	-	+	+	1:128	1:512
5	15/01/85	Bronchial aspirate	+	-	+	1:128	1:512

*Probable date.

Of the 40 hot water samples collected in various areas of the hospital, all but 1 gave negative culture results. The sample with a positive result yielded only two colonies of *L. dumoffii* and came from an area where no cases had been identified. Distilled water was collected from the reservoirs of two nebulizers, and *L. dumoffii* was isolated in both cases. Distilled water samples were then collected at 11 sites, and six were found to contain *L. dumoffii*.

Discussion

The occurrence of five cases of Legionnaires' disease in a single hospital strongly suggested a common source. The isolated organism, *L. dumoffii*, is usually a rare cause of legionellosis, and most reported cases are sporadic and community-acquired. In outbreaks of Legionnaires' disease caused by other species of *Legionella*, hospital ventilation or potable water systems have been implicated as sources of infection. In this outbreak the air conditioning system could not be implicated since it was not in use when the cases occurred. Following the first two cases, in the spring of 1984, we initially focused our attention on the potable water system. The isolation of *L. dumoffii* from only one outlet was not considered sufficiently conclusive to initiate eradication procedures. Indeed, Best and colleagues¹³ have suggested that nosocomial legionellosis occurs only when over 30% of potable water samples are contaminated with *Legionella*.

Between April and December 1984 no further cases were diagnosed despite continued surveillance. The diagnosis of three additional cases of Legionnaires' disease caused by *L. dumoffii*, in late December 1984 and January 1985, prompted intensive investigation into the source. The fact that all three patients had been exposed to respiratory therapy equipment led to the hypothesis that aerosolized water from the equipment could be implicated. Aerosolized tap water from respiratory devices has previously been reported to cause Legionnaires' disease,^{14,15} but no documented cases had previously been linked to aerosolized distilled water. The isolation of *L. dumoffii* from the nebulizer reservoir in two cases strongly suggested a causal relation. Further investigation revealed that unsterile distilled water had been used in this apparatus.

In this institution distilled water is produced locally and is distributed to laboratories and other hospital facilities by an independent plumbing system. Hospital wards and units receive bottled distilled water from a central distribution unit, where all bottles are filled at a single tap. Water from this outlet was found to be contaminated by *L. dumoffii*. Review of the clinical records of the patients with Legionnaires' disease revealed that all had been exposed to aerosolized distilled water, four through respiratory therapy equipment and

one through a bedside room humidifier.

The presence of *L. dumoffii* in the hospital distilled water supply was further confirmed by the isolation of the bacterium from 6 of the 11 cultured samples. The origin of the contamination was obscure. Maintenance employees did, however, confirm that the water distillation system occasionally malfunctioned. When it did, potable hot water could overflow in either of the two reservoirs located on the top of the building, where the ambient temperature is between 35°C and 40°C. Although we were unable to isolate *L. dumoffii* from either of the reservoirs, we postulated that when a malfunction occurred the distilled water was seeded with *L. dumoffii* present in the tap water. The bacteria then multiplied and colonized the distribution system.

As soon as the origin of the bacterium was found, sterile distilled water was distributed throughout the hospital. Surveillance for additional cases of legionellosis was continued, but no further cases have been identified, despite the fact that the hospital's distilled water is still contaminated by *L. dumoffii*.

The diagnosis of Legionnaires' disease was easily made in all five patients. The importance of bacterial culture for *Legionella* cannot be overemphasized. In the first case of Legionnaires' disease in this outbreak culture gave positive results. Since this patient clearly had a nosocomial infection, serologic testing for *L. dumoffii* was performed in all further cases of atypical nosocomial pneumonia. This led to the detection of the second case. Although the initial environmental investigation was unsuccessful, the second cluster of cases was more rapidly identified, and this led to identification of the source of infection.

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The mind may undoubtedly affect the body; but the body also affects the mind. There is a re-action between them; and by lessening it on either side, you diminish the pain on both.

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