

## Legionellaceae in the potable water of Nova Scotia hospitals and Halifax residences

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*(Accepted 13 August 1993)*

### SUMMARY

Water was cultured from 39 of 48 hospitals (7 Halifax hospitals and 32 non-Halifax hospitals) in the province of Nova Scotia and from 90 residences (74 private dwellings, 16 apartments) in Halifax to determine the frequency of legionella contamination. Six of seven Halifax hospitals had Legionellaceae isolated from their potable water compared with 3 of 32 non-Halifax hospitals ( $P < 0.0001$ ). Overall, 19 of 59 (32%) of the water samples from Halifax hospitals were positive for legionellae compared with 5 of 480 (1%) samples from non-Halifax hospitals ( $P < 0.0000$ ). Five of the six positive Halifax hospitals had *Legionella pneumophila* serogroup 1 and 1 had *L. longbeachae* serogroup 2 recovered from their potable water. Legionella contamination was associated with older, larger ( $\geq 50$  beds) hospitals with total system recirculation. These hospitals also had water with a higher pH and calcium content but lower sodium, potassium, nitrate, iron and copper content.

Fourteen of the 225 (6.2%) water samples from Halifax residences were positive for legionellae – 8% (6/74) of the single family dwellings were positive, compared with 25% (4/16) apartments. The positivity rate of 15.7% for the 19 electric hot-water heaters in Halifax homes was not significantly different from the 32% positivity for Halifax hospitals. *L. longbeachae* accounted for 2 of the 14 isolates of legionellae from Halifax homes.

### INTRODUCTION

Contamination of the potable water of a hospital by members of the family Legionellaceae has been linked to cases of nosocomial Legionnaires' disease [1–3]. However Legionellaceae can be present in the potable water of an institution without causing disease [4].

In our previous study [5] the potable water from five of six health-care institutions in the city of Halifax were found to be contaminated with *Legionella pneumophila*. Despite the fact that all the institutions received their water from the same source and were clustered geographically within the city of Halifax, each

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institution had its own unique population of *L. pneumophila* as defined by plasmid content, monoclonal antibody type and restriction enzyme analysis [5].

In this study our goals were to determine if Legionellaceae were present in the water supply of hospitals outside the Halifax area, and if residences in the city of Halifax were colonized with legionellae to the same extent as Halifax hospitals.

## MATERIALS AND METHODS

### *Recruitment of institutions*

In March 1992 all 48 hospitals in Nova Scotia were invited by letter to participate in the study. Anonymity of the results in any resulting publication was a condition of participation.

### *Recruitment of Halifax homeowners*

A notice was placed in the Victoria General Hospital Newsletter (The Vine) explaining the nature of our study and inviting employees who lived within the city of Halifax to submit water samples from their homes. In addition, the investigators invited neighbours and friends to submit water samples.

### *Collection of water samples for culture and analysis*

#### *Non-Halifax hospitals*

Generally ten potable water samples from randomly selected sink faucets were collected from each institution.

Sampling was performed as previously described [5]. In brief, water samples were obtained by turning on the hot and cold water taps so that the water flowed slowly. The hot and cold water systems were not sampled separately. Two hundred millilitres of water was collected into a sterile bottle containing 0.1 ml of a 10% solution of sodium thiosulphite. Sampling began on 8 June 1992, and finished on 28 July 1992. Fourteen hospitals were sampled on two different occasions. All samples were collected by the same individual (P.G.).

After the water sample was obtained, the hot water was allowed to run freely for approximately 2 min and the temperature was taken. One sample of potable water from each institution was submitted to the Environmental Chemistry Laboratory of the Victoria General Hospital for chemical analysis [5].

#### *Halifax residences*

Water was collected from 74 private homes and 16 apartments as outlined above. Three sites were sampled – kitchen, bathroom, and the hot-water heater. If a hot-water heater was not accessible only two sites were sampled.

#### *Halifax hospitals*

Water samples were obtained from all seven Halifax hospitals by infection control nurses at each site using the protocol described above.

### *Culture of water for legionella*

Water samples (50 ml) were centrifuged at 1200 g for 20 min. The supernatant was aspirated leaving approximately 10% of the original volume in which the sediment was resuspended. With a sterile pipette tip 0.1 ml of the suspension was

removed and used to inoculate the surface of the following media: 5% sheep blood agar, buffered charcoal yeast extract (BCYE) agar containing 0.1% alphaketoglutarate (GIBCO Labs., Madison, WI) and BCYE agar containing cefamandole, polymyxin B and anisomycin (MPA).

Initially preparations were plated only onto BCYE and blood agar. On 6 July a switch was made from BCYE to MPA to reduce higher numbers of contaminating bacteria. All plates were incubated at 37 °C in a humidified atmosphere containing 5% carbon dioxide for 7 days and examined daily. Colonies that morphologically resembled legionella were subcultured onto blood and BCYE agar. Representative colonies of those organisms that failed to grow on blood agar were examined by a direct fluorescent antibody technique [5] employing *L. pneumophila* serogroup 1–6 antisera (Mardx, Scotch Plains, NJ).

Colonies that did not exhibit fluorescence with *L. pneumophila* serogroup 1–6 antisera, but were suspected to be Legionellaceae, were examined for the presence of the 60 kDa legionella common antigen using a monoclonal antibody [6].

These isolates were then sent to Dr J. Joly, Université Laval, Quebec City, where a panel of monoclonal antibodies to *L. pneumophila* serogroup 1–8, *L. micdadei*, *L. dumoffi*, *L. bozemanii*, *L. gormanii*, *L. longbeachae* 1, 2, *L. jordanis*, *L. oakridgensis* was used to speciate the isolates.

#### Plasmid profiles

Portions of the growth achieved after a 48 h incubation of isolates on BCYE and MPA agar were suspended in 0.5 ml of TE buffer (0.5 Tris-HCl; pH 8.0, 0.02 EDTA). After pelleting and resuspending in 25 µl of TE buffer, plasmid DNA was extracted from the cells by a modified alkaline sodium dodecyl sulphate procedure [7]. The contents of the extracts were determined by electrophoresis with vertical 0.75% agarose gels followed by ethidium bromide staining. Strains with no detectable plasmids constituted plasmid type 0, while those carrying a 20-MDa plasmid were plasmid type II. Types III and VI isolates carried 96 and 72 MDa plasmids and 100 MDa plasmids respectively [5].

## RESULTS

### Hospitals

Thirty-nine (81.3%) of the 48 hospitals in Nova Scotia participated in our study. Nine of the 39 (23%) had *Legionella* species in their potable water at the time of our sampling. Six of the seven Halifax hospitals were positive for *Legionella* species compared with 3 of the 32 non-Halifax hospitals ( $P < 0.0001$ ). The number and proportion of samples positive for legionella in these hospitals is given in Table 1. Overall, 19 of 59 (32%) of the samples from Halifax hospitals were positive for Legionellaceae compared with 5 of the 480 (1.04%) samples from non-Halifax hospitals ( $P < 0.00001$ ).

Table 2 compares some relevant features of the hospitals that were positive for Legionellaceae with those that were negative. The positive hospitals were older, and had total system recirculation of their water. There was also an association between positive water samples with hospital size (Table 2). Eight of the nine hospitals with *Legionella* spp. in their water were  $\geq 50$  beds in size *v.* 15 of the 30

Table 1. Isolation rates and plasmid types of legionella present in potable water samples obtained from Nova Scotia hospitals

Halifax hospitals	No. of samples	No. (%) positive	Legionella isolated	Plasmid type
1	5	2 (40)	<i>L. pneumophila</i>	VI
2	5	3 (60)	<i>L. pneumophila</i>	O
3	15	1 (7)	<i>L. pneumophila</i>	not done
4	15	7 (47)	<i>L. pneumophila</i>	not done
5	5	3 (60)	<i>L. pneumophila</i>	O, II, VI
6	9	3 (33)	<i>L. longbeachae</i> 2	not done
7	5	0		
Non-Halifax hospitals*				
1	30	1 (3.3)	<i>L. pneumophila</i>	II
2	20	2 (10)	<i>Legionella</i> species	not done
3	20	2 (10)	<i>L. longbeachae</i> 2	not done

\* 29 non-Halifax hospitals were culture negative.

Table 2. A comparison of Nova Scotia hospitals that had Legionella species isolated from their potable water (positive) with those that did not (negative)

Characteristic	Positive (n = 9)	Negative (n = 30)	P
Mean age ( $\pm$ SD) of plant - years	29 $\pm$ 18	17 $\pm$ 13	< 0.04
No. (%) with horizontal water tank	2 (22%)	6 (20%)	NS
No. (%) total system recirculation	6 (67%)	2 (7%)	< 0.001
Mean temperature ( $\pm$ SD) of hot water at faucet ( $^{\circ}$ C)	52.54 $\pm$ 5.86	54.98 $\pm$ 6.49	NS
No. (%) with $\geq$ 200 beds	4 (44%)	2 (6.6%)	< 0.02
No. (%) with $\geq$ 100 beds	7 (78%)	9 (30%)	< 0.03
No. (%) with $\geq$ 50 beds	8 (89%)	15 (50%)	$\leq$ 0.05

with negative water samples ( $P \leq 0.05$ ). There were 15 hospitals with  $\leq 30$  beds and only one of these was positive compared with 8 of 24 larger than this ( $P = 0.11$  NS). The smallest positive hospital was 24 beds. The other positive hospitals ranged in size from 150 to 793 beds.

There were marked differences in the chemical composition of the water from the six legionella positive Halifax hospitals compared to the non-Halifax hospitals that were negative for legionella (Table 3). Calcium content and the pH were significantly higher in the Halifax hospital waters while concentration of sodium, chloride, nitrate plus nitrite, iron and copper were significantly lower.

### Homes

Six of the 74 (8%) single family dwellings were contaminated with legionella and 4 of the 16 apartments sampled were positive for legionella.

Fourteen of the 225 water samples (6.2%) obtained from 90 Halifax residences were positive for Legionellaceae. The rate of positivity from the various sampling sites is given in Table 4. Fifteen percent of the samples from electric hot-water heaters were positive for Legionellaceae. None of the homes was positive at all 3 sampling sites; 4 homes were positive at 2 sites.

Table 3. Comparison of chemical analysis of water from six Halifax hospitals positive for legionellae with the 30 non-Halifax hospitals that were negative on culture for legionellae

Mean $\pm$ SD of	Positive hospitals (n = 6)	Negative hospitals (n = 30)	P
Sodium*	3.7 $\pm$ 0.06	21.7 $\pm$ 37.6	< 0.05
Potassium	0.4 $\pm$ 0	0.54 $\pm$ 0.53	NS
Calcium	14.72 $\pm$ 1.6	9.6 $\pm$ 9.9	< 0.05
Chloride	7.8 $\pm$ 0.2	21.8 $\pm$ 29.3	< 0.05
Nitrate plus nitrite	0.05 $\pm$ 0	0.2 $\pm$ 0.4	< 0.05
Iron	0.02 $\pm$ 0	0.15 $\pm$ 0.13	< 0.01
Copper	0.04 $\pm$ 0.02	0.64 $\pm$ 0.82	< 0.01
Conductivity (u $\Omega$ /cm)	95.3 $\pm$ 7.6	157 $\pm$ 172	NS
pH	7.42 $\pm$ 0.28	6.8 $\pm$ 0.44	< 0.01
Total organic carbon	3.73 $\pm$ 5.1	2.02 $\pm$ 1.0	NS

\* Unless otherwise indicated (and except for pH), values are reported in milligrams per litre.

Table 4. Results of cultures of water samples obtained from 90 Halifax homes

Site	No. samples	No. (%) positive for Legionellaceae
Kitchen	89	4 (4.5)
Bathroom	90	6 (6.7)
Hot-water heaters	46	4 (8.6)
electric	19	3 (15.7)
oil	24	1 (4.1)
unknown	3	0

Table 5. Speciation of Legionellaceae isolated from potable water of Halifax homes

Species	No.
<i>L. pneumophila</i> serogroup 1	3*
<i>L. pneumophila</i> serogroup 2	1
<i>L. longbeachae</i> 2	2
<i>L. micdadei/bonzemaniai</i>	2
Ungroupable	2
Not typed	4

\* Two isolates were plasmid type VI and 1 was plasmid type III.

Ten of the 14 isolates were legionellae other than pneumophila. The results of speciation of these isolates are shown in Table 5.

The 32% contamination rate of samples from Halifax hospitals was not significantly different from the 15.7% (3/19) rate of domestic electric hot-water heaters in the city of Halifax.

## DISCUSSION

The major findings from this study were firstly that Halifax hospitals were contaminated with Legionellaceae at a significantly higher rate than hospitals in the rest of Nova Scotia. Indeed only 1 of 32 non-Halifax hospitals was positive for *L. pneumophila* serogroup 1. Secondly electric hot-water heaters in Halifax homes were contaminated by Legionellaceae just as frequently as were Halifax hospitals.

Alary and Joly found that 57/84 (67.9%) of hospitals in Quebec, Canada were contaminated by Legionellaceae [8]. Contamination was associated with location (Montreal 26/29 (89.7%) of hospitals positive compared with 31/55 (56.4%) hospitals elsewhere in the province of Quebec), and number of hospital beds. They also found that heavy contamination was associated with large volume hot-water tanks and a low water temperature at the faucet. We also noted an association between hospital size and contamination of the water with *Legionella* spp. Only 1 of the 15 hospitals with  $\leq 30$  beds had positive cultures. Vickers and colleagues reported that 9 of 15 (60%) hospitals in Western Pennsylvania were contaminated with *L. pneumophila* [9]. They found that a temperature  $< 60$  °C, vertical configuration of the hot-water tanks, older tanks and elevated calcium and magnesium concentrations were associated with contamination [9].

Our findings showed that there was an increased concentration of calcium in the water and that a higher pH was associated with contamination by Legionellaceae. In addition lower levels of iron were associated with contamination as has been found by others [10, 11]. High concentration of copper in conjunction with silver is toxic to legionellae [12]. It is likely that the chemical composition of some waters is more conducive to the growth of legionella than others. The heavy contamination of potable water in Halifax area hospitals would appear to support this view. However, this is not the only factor that influences colonization. One of the Halifax hospitals, a 38-year-old maternity hospital, has remained free of legionella even though it receives water from the same source as the contaminated hospitals and is part of the same distribution system [5].

Somewhat surprising was the finding that the rate of colonization of the domestic electric hot-water heaters in Halifax was not significantly different from that of the Halifax hospitals. In other studies electric hot-water heaters were much more likely to be contaminated with legionellae than oil or gas-fired heaters [13, 14]. De Waelly and Joly found that 84 of 255 (33%) of domestic hot-water heaters in three towns in the Quebec City area were contaminated with legionellae [14]. In another study Alary and Joly found that 69 of 178 (39%) of Quebec City homes with electric hot-water heaters were colonized by legionellae [13]. Two studies in the Pittsburgh area found that 6 of 55 (11%) homes [15] and 14 of 218 (6%) water samples yielded *L. pneumophila* [16]. In the latter study the proportion of homes colonized varied from 0 to 22% according to geographical area (postal code) within the city [16].

Quite unexpected was the isolation of *L. longbeachae* from one Halifax hospital, one Halifax home and one non-Halifax hospital. *L. longbeachae* was first isolated by McKinney and colleagues from a 59-year-old man admitted to a Long Beach, California hospital with pneumonia [17]. Bibb and colleagues in 1981 reported *L. longbeachae* serogroup 2 as a cause of fatal pneumonia [18], and the organism accounted for 17 of the 108 (16.7%) cases of Legionnaires' disease diagnosed in South Australia between 1979 and 1988 [19]. Indeed this microorganism was isolated from 33 of 45 potting soils in Australia [20]. *L. longbeachae* 1 and 2 are relatively uncommon isolates from potable water. Bartlett and colleagues [21] examined 1538 water samples from hospitals and hotels in London, England. They found that 314 were positive for legionella, 3 of which were *L. longbeachae* (1 serogroup 1, 2 serogroup 2).

Alary and Joly [13] recovered *L. longbeachae* – 2 from the water of 1 of 84 Quebec hospitals tested. They also found 1 isolate of *L. longbeachae* (serogroup not stated) among 105 legionellae isolates obtained from domestic water supplies [13].

The number of infections due to *L. longbeachae* remains low. A total of 30 cases were reported from Australia up to 1990 [22]. Individual cases have been reported from Vancouver, Canada [23]; Sweden [24], Germany [25].

In conclusion *Legionella pneumophila* is rarely found in the potable water of hospitals outside the city of Halifax in contrast to the finding that the water of 6 of 7 Halifax hospitals is so contaminated. *Legionella longbeachae* 2 is present in the potable water of a Halifax hospital, a Halifax home and in one non-Halifax hospital.

#### ACKNOWLEDGEMENTS

This research was supported by a grant, MT-10577, from the Medical Research Council of Canada. We wish to thank the administration and staff of the participating hospitals without whom this study would not have been possible. We are grateful to Dr Jean Joly, Université Laval, Quebec City, for identifying some of our isolates.

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