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Control of endemic nosocomial Legionnaires' disease by using sterile potable water for high risk patients

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SUMMARY

In a setting where potable water is contaminated with *Legionella pneumophila* serogroup 1, we performed two case control studies. The first case control study consisted of 17 cases of nosocomial Legionnaires' disease (LD) and 33 control (the patients who were admitted to the ward where the case was admitted immediately before and after the case) subjects. Cases had a higher mortality rate 65% vs 12% ($P < 0{\cdot}004$); were more likely to have received assisted ventilation ($P < 0{\cdot}00001$); to have nasogastric tubes ($P < 0{\cdot}0004$) and to be receiving corticosteroids or other immunosuppressive therapy ($P < 0{\cdot}0001$). Based on the results of this study, sterile water was used to flush nasogastric tubes and to dilute nasogastric feeds. Only 3 cases of nosocomial LD occurred during the next year compared with 12 the previous year ($P < 0{\cdot}0001$). Nine cases subsequently occurred and formed the basis for the second case-control study. Eighteen control subjects were those patients admitted to the same unit where the case developed LD, immediately before and after the case. The mortality rate for the cases was 89% vs 6% for controls ($P < 0{\cdot}00003$). The only other significant difference was that cases were more likely to be receiving corticosteroids or other immunosuppressive therapy 89% vs 39% ($< 0{\cdot}01$). We hypothesized that microaspiration of contaminated potable water by immunocompromised patients was a risk factor for nosocomial Legionnaires' disease. From 17 March 1989 onwards such patients were given only sterile potable water. Only two cases of nosocomial LD occurred from June 1989 to September 1990 and both occurred on units where the sterile water policy was not in effect. We conclude that aspiration of contaminated potable water is a possible route for acquisition of nosocomial LD in our hospital and that provision of sterile potable water to high risk patients (those who are receiving corticosteroids or other immunosuppressive drugs; organ transplant recipients or hospitalized in an intensive care unit) should be mandatory.

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INTRODUCTION

Legionella pneumophila, the aetiologic agent of the 1976 epidemic of community-acquired pneumonia at the American Legion Convention in Philadelphia [1] also caused an outbreak of nosocomial pneumonia at St Elizabeth's Hospital in Washington, D.C. in 1965 [2]. Since then *L. pneumophila* has emerged as an important cause of nosocomial pneumonia. Korvick and colleagues [3] summarized (1965–83) 16 reports of nosocomial legionellosis affecting 532 patients with a mortality rate ranging from a low of 17% to a high of 66% per report. *Legionella* species were isolated from the environment in 13 of these 16 reports; contaminated cooling tower water was implicated as a source in 5 and contaminated potable water was implicated in 12 [3]. Careful epidemiological studies have implicated the potable water supply as the cause of nosocomial legionellosis in many hospitals [4, 5]. Despite the cessation of outbreaks following eradication of *Legionella* sp. from the potable water system, the mode of transmission of *Legionella* sp. from the potable water to the patient is largely unknown [6]. We have identified *L. pneumophila* in the potable water of our hospital since 1981, and cases of nosocomial legionella pneumonia have occurred sporadically since then. From August 1983 we have prospectively studied all patients with nosocomial pneumonia to identify nosocomial Legionnaires' disease and since August 1985 we have used case-control methodology to determine risk factors for acquisition of this infection.

METHODS

The hospital

The Victoria General Hospital is an 800 bed tertiary care centre. It has the only organ (renal, hepatic, cardiac) transplantation and cardiac surgery units in Maritime Canada. The hospital has a cooling tower and its potable water supply is from a lake, the same source as the rest of the city of Halifax. The cooling tower operates from June to September and is used to cool the air supply of the operating rooms and the burns unit.

Prospective nosocomial pneumonia study

From 15 August 1983 to the present, all patients in our hospital have been followed for the development of nosocomial pneumonia. The diagnostic criteria of the National Nosocomial Infection Study are used [7]. An acute phase blood sample was obtained on enrolment and convalescent samples were obtained at 2, and 4–6 weeks later. These samples were tested for antibodies to *Legionella pneumophila* using an indirect immunofluorescence test. From April 1989 onwards, routine serological testing was discontinued and was carried out only at the direction of the attending physician and not for purposes of the study. Most intubated patients with nosocomial pneumonia had endotracheal secretions cultured for *Legionella* sp. The onset of pneumonia was defined as the day pneumonia was diagnosed – either clinically or radiographically. Some aspects of the first 4 years of this study have been previously reported [8].

Case-control studies

Study number 1. From 28 August 1985 to 20 May 1987 a case-control study was carried out. For each case of legionella pneumonia the patients admitted to the ward where the case was admitted (immediately before and after the case) served as controls. None of the control subjects developed pneumonia and they were not tested serologically for legionella infection. Risk factor analysis included those events which had occurred prior to the development of pneumonia – e.g. surgery, placement of nasogastric tubes, intubation. The APACHE II scoring system [9] was used to categorize the severity of disease.

Study number 2. A second case control study was begun in December 1988 and continued through May 1989. Controls were the patients who were admitted to the *same unit* (where the case developed Legionnaires' disease) immediately before and after the case. Data collection was as for study number one but, in addition, the amount of potable water ingested by each subject was noted.

Environmental surveillance

From 6 February 1986 to 8 May 1990 water was obtained from the same 20 sites (one site per unit) approximately every 2 weeks for culture for *Legionella* sp; thereafter samples were collected once monthly. Water samples were obtained by turning on the hot and cold water taps so that the water flowed slowly. Two hundred ml of water was then collected into a sterile bottle containing sodium thiosulphite. In addition, when *Legionella pneumophila* was isolated from a patient, the water source nearest the patient was cultured. Water samples were also obtained from 10 oxygen bubblers and from 20 ventilator humidifiers that were in use. Serial samples (once weekly while a patient was being ventilated with the same machine) were obtained from the ventilator humidifier.

Culture for Legionella pneumophila

Respiratory specimens. Material for culture (sputum, endotracheal secretions, pleural fluid or lung tissue) was inoculated onto 5% sheep blood agar (BA), buffered charcoal yeast extract agar (BCYE) containing 0·1% alpha-ketoglutarate and two selective media: one BCYE containing cefamandole, polymyxin B and anisomycin (MPA) and the other BCYE containing polymyxin B, anisomycin and vancomycin [10] (PAV) [Gibco Laboratories, Madison, WI]. 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 cultured onto blood and BCYE agar. Those that failed to grow on blood agar were examined by a direct fluorescent antibody technique [11] using *L. pneumophila* serogroups 1–6 antisera (Centers for Disease Control, Atlanta GA).

Water specimens. Water samples (50 ml) were centrifuged at 3000 rpm for 20 min. The supernatant was removed, leaving approximately 10% of the original volume in which the sediment was resuspended. A sterile cotton tipped swab (Solon Manufacturing Company, Solon MN) was then used to inoculate the surface of BA,

BCYE, PAV and MPA plates. Plates were incubated and organisms identified as outlined above.

Direct fluorescent antibody studies for Legionella pneumophila

Endotracheal secretions, lung tissue and pleural fluid were examined for *Legionella pneumophila* using the direct fluorescent antibody technique as described by Cherry and colleagues [11]. In brief, the material to be examined is fixed onto a glass slide and overlaid with polyclonal fluorescein-isothiocyanate conjugated rabbit immunoglobulin directed against *L. pneumophila* serogroup 1 (Knoxville strain) [Centers for Disease Control, Atlanta, GA]. Negative controls were included with each test. The specimen was interpreted as positive if $\geqslant 5$ strongly fluorescing bacteria were present/smear.

Antibody titres to Legionella pneumophila

Antibody titres to *L. pneumophila* serogroup 1 were performed on acute and convalescent serum samples using an indirect fluorescent antibody technique [12]. A positive and negative control was included with each run. All reagents for this test were obtained from the Centers for Disease Control, Atlanta, GA. A fourfold rise in antibody titre to $\geqslant 128$ was considered evidence of recent *L. pneumophila* infection.

Monoclonal antibody typing

Patient isolates and selected environmental isolates were typed by monoclonal antibody reactivity patterns [13]. These isolates were typed by Dr Joly, Université Laval, Quebec City, who was unaware of the source of the isolates.

Plasmid profiles

Portions of the growth achieved after 48 h incubation of the isolates on BYCE agar were suspended in 0·5 ml of TE buffer (0·5 M Tris-HCl pH 8·0, 0·02 M EDTA). After pelleting and resuspending in 25 μl of TE, plasmid DNA was extracted from the cells using a modified alkaline SDS procedure [14]. The contents of the extracts were determined by electrophoresis in vertical 0·75% agarose gels followed by ethidium bromide staining. Strains with no detectable plasmids constituted profile 0; those carrying a 20 Md plasmid were profile II. Profiles III and VI were comprised of 96 and 72 Md and 100 Md plasmids respectively.

Endonuclease restriction analysis

Chromosomal DNA served as substrate for restriction endonuclease digestions. Its recovery from pelleted cells was achieved using a modified Roussel–Chabbert procedure [15]. The restriction enzymes *Eco*RI and *Bgl*II were used to differentiate the isolates. Digestion was continued for 8 h at 37 °C in buffers provided by the enzyme's supplier (Boehringer Mannheim, Dorval, Quebec). Restriction fragments were separated in vertical, 0·75% agarose gels and visualized after ethidium bromide staining by ultraviolet irradiation. Resultant distinct patterns were assigned letter codes a, b, c, or d.

Statistical analysis

The Chi square test was used to test for differences in proportions between cases and controls and the Students *t*-test was used to test the differences between

means. The Poisson probability distribution (one-tailed) was used to determine if there was a difference in the number of cases observed in the time intervals following institution of control measures compared with the previous year. In order to determine the factors important in predicting the acquisition of nosocomial Legionnaires' disease, factors found to be significant in the univariate analysis were entered into a logistic regression analysis with the use of the computer package GLIM (generalized linear interactive modelling) [16]. A logistic regression model was used because the response variable was a binomial random variable, i.e. the patient either did or did not develop Legionnaires' disease.

RESULTS

The number of cases of nosocomial pneumonia per year from 1983–90, the number of cases of nosocomial Legionnaires' disease and the number of patients who had respiratory secretions cultured for legionella are given in Table 1. The temporal occurrence of cases of Legionnaires' disease from September 1983 to September 1990 is given in Fig. 1. From 28 August 1985 to 20 May 1987 (period of case-control study number 1) there were 17 cases of nosocomial pneumonia due to *Legionella pneumophila* serogroup 1. The diagnosis of Legionnaires' disease was made by isolation of the organism (12 patients), seroconversion, (4 patients) and by a positive direct fluorescent antibody test on lung tissue in one patient.

The results of case control study number one are shown in Tables 2 and 3. The mean age of the case and control groups were similar. Those with Legionnaires' disease had a significantly longer hospital stay 43·4 days vs 14·2 days ($P < 0{\cdot}01$) and a significantly higher mortality rate – 65% vs 12% ($P < 0{\cdot}0004$). Patients who developed nosocomial Legionnaires' disease were hospitalized for a mean of 18·7 days prior to onset of pneumonia; a period that was longer than the entire stay of 14 days for the control patients. Cases were more seriously ill mean APACHE II score $19 \pm 5{\cdot}75$ compared with controls $9 \pm 7{\cdot}22$ $P < 0{\cdot}001$ (Table 2). Three risk factors for acquisition of Legionnaires' disease were identified – assisted ventilation, immunosuppressive therapy and nasogastric tubes (Table 3). These factors were still significant ($P < 0{\cdot}05$) when analysed using logistic regression.

When these results became available, 23 September 1987, the use of sterile water to flush nasogastric tubes and to dilute tube feedings was instituted as a control measure. From 23 September 1987 to 23 September 1988 there were 3 cases of nosocomial Legionnaires' disease compared with 12 cases in the year previously when sterile water was not used in nasogastric tubes ($P < 0{\cdot}001$ Poisson probability distribution). These 3 cases differed from most of the 17 in the first case control study. They did not receive assisted ventilation prior to onset of the pneumonia and they did not have nasogastric tubes. It is noteworthy that while 12 of the 31 cases from 1983 to August 1987 occurred in intensive care units, in the 3 years since then only 2 of 15 cases occurred in an intensive care unit. One of these patients had macroaspiration of gastric contents ($P = 0{\cdot}07$ Fisher's exact test).

Nine cases of nosocomial Legionnaires' disease occurred from 30 December 1988 to 14 May 1989. These cases were the subject of a second case control study, the results of which are given in Tables 4 and 5. *Legionella pneumophila* serogroup 1 was isolated from 8 patients and *L. micdadei* from 1. Four of the 9 developed Legionnaires' disease in the room to which they were admitted. An additional 2

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Table 1. *Selected features of nosocomial pneumonia at Victoria General Hospital per year (13 August to 14 August), 1983–90*

Year	No. of cases of nosocomial pneumonia	No. (%) of patients who had respiratory secretions cultured for legionella	No. of patients from whom legionella isolated	No. of cases of nosocomial L.D.*
1983–84	165	38 (23)	4	6
1984–85	191	44 (23)	5	6
1985–86	264	66 (25)	5	7
1986–87	193	62 (32)	9	12
1987–88	182	66 (36)	3	3
1988–89	267	75 (28)	9	9
1989–90	203	72 (35)	1	2

* L.D., Legionnaires' disease.

patients developed legionella pneumonia on the same floor but in a different room and 3 developed their nosocomial pneumonia on wards other than the one to which they were admitted. Eight of the cases (89%) and 16 of the controls (89%) ingested potable water (P – NS). The differences between cases and controls were: the number who were immunosuppressed, 8 of 9 vs 7 of 18, ($P < 0{\cdot}01$); the controls did not develop pneumonia; the mortality rate was much higher among the cases 89% vs 6% ($P < 0{\cdot}0003$); cases were more seriously ill mean APACHE II score $16{\cdot}67 \pm 5$ compared with controls mean APACHE II score $12{\cdot}11 \pm 5{\cdot}89$ ($P < 0{\cdot}05$).

As a result of this study, we hypothesized that microspiration of contaminated potable water by immunocompromised patients was the mechanism for acquisition of *Legionella* in our hospital. Starting 17 March 1989, sterile potable water was given to immunosuppressed patients on four nursing units where most (75%) of the cases of nosocomial Legionnaires' disease had occurred. On 1 June 1990, a policy of sterile potable water for organ transplant patients on units 4B, CCU, CVICU and SICU was instituted. These patients were also instructed to refrain from taking showers. Four cases occurred in April and May 1989; three occurred on units where the sterile water policy was not in effect and one case occurred on a unit where the policy was in effect but the patient was given potable tap water to drink. Only two cases of nosocomial Legionnaires' disease cases occurred from June 1989 to September 1990 and both cases occurred in wards where the sterile potable water policy was not in effect.

Environmental studies

Twenty-two hundred samples of potable water were cultured from 20 separate sites throughout the hospital from 1986 through 1989. During the period of continuous hyperchlorination (6 February 1986 until 8 October 1986) only 7·9% (57/714) of the water samples grew *L. pneumophila* while during 1989 when there was no hyperchlorination, 41·6% (175/420) of the samples yielded this microorganism. Fifty-four percent of the 180 isolates that were typed using monoclonal antibodies were subtype Olda. The remainder were subtype Oxford. A temporal shift in the two subtypes was evident with most of the Oxford isolates present from November 1987 to July 1988 – the 7 months immediately following

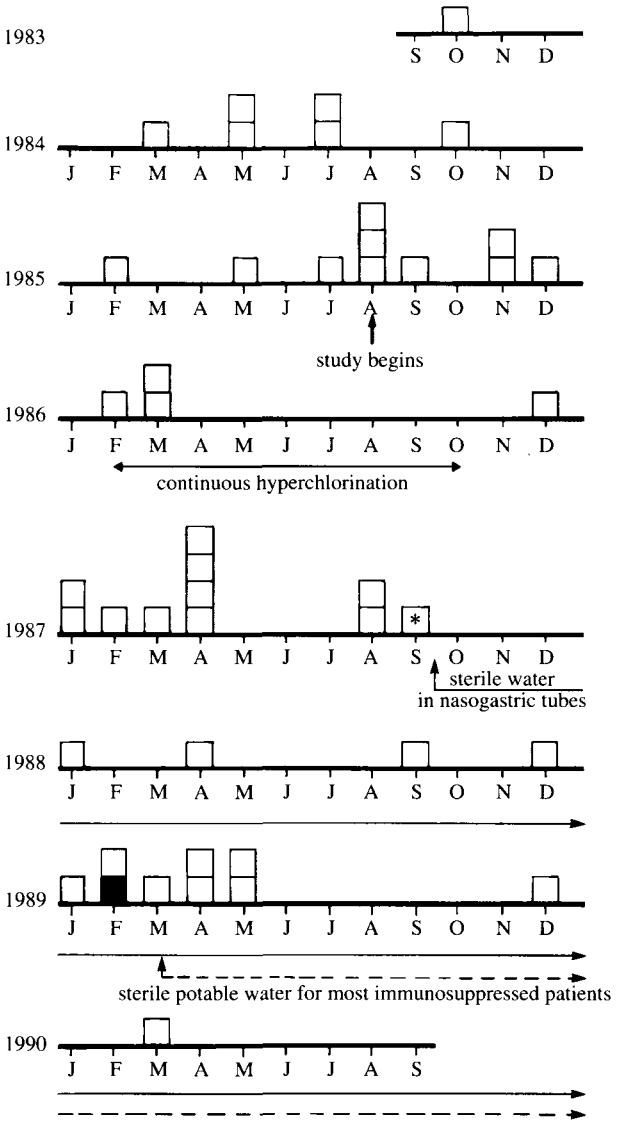

Fig. 1. Temporal occurrence of cases of nosocomial Legionnaires' disease, Victoria General Hospital, 15 September 1983 to 15 September 1990. The * indicates that *Legionella pneumophila* serogroup six was isolated; shaded block indicates *L. micdadei* was isolated; all other isolates were *L. pneumophila* serogroup 1. Each block represents one patient. Letters indicate months of the year.

cessation of hyperchlorination. Indeed, 76 of the 82 (92%) Oxford isolates were recovered during this time period. Only six isolates from 1989 were typed and all were subtype Oxford.

Samples were also collected in 11 instances from the water source closest to the patient from whom *L. pneumophila* was isolated. The resultant nine sets of patient-environmental isolates were compared with regard to their monoclonal subtype, plasmid complement and endonuclease fragmentation patterns of their

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Table 2. *Case-control study number 1. Demographic features of 17 cases of nosocomial Legionnaires' disease and 33 control patients. Control subjects were the patients admitted to* the ward *immediately before and after the case*

	Cases (17)	Controls (33)	*P*
No. males/females	11/6	22/11	
Mean age (years)	62·2	55·8	NS
Mean length of stay (days)	43·4	14·2	< 0·01
Mean no. days after admission until pneumonia developed	18·7	0	
No. (%) died	11 (65)	4 (12)	< 0·0004*
APACHE II score (mean ± SD)	19 ± 5·75	9 ± 7·22	< 0·0001

* Odds ratio 13·3; 95% confidence intervals 2·6, 75.

Table 3. *Case-control study number 1. A comparison of cases and controls for selected factors that might predispose to acquisition of nosocomial Legionnaires' disease*

Factor	Cases (17) No. (%)	Controls (33) No. (%)	*P*	Odds Ratio	CI*
Assisted ventilation	12 (71)	1 (3)	< 0·00001	76·8	7·1, 448·9
Assisted ventilation prior to developing pneumonia	11 (65)	0			
Aerosol therapy	6 (35)	4 (12)	NS		
corticosteroid or other immunosuppressive therapy	14 (82)	6 (18)	0·0008	10·8	2·3, 55·39
Nasogastric tubes	15 (88	1 (3)	< 0·00001	240	16, 1530
Nasogastric tubes prior to onset of pneumonia	8 (47)	0	< 0·0004	unbounded	
Mean duration (days) nasogastric tube in place prior to development of pneumonia	14·7 ± 13·8	0			
Tobacco smoker	6 (35)	5 (15)	NS	3·0	0·64, 15
Surgery prior to onset of pneumonia (cases) or during hospital stay (controls)	12 (71)	16 (49)	NS	2·5	0·63, 10·7
H_2 blockers	5 (29)	9 (27)	NS	1·1	0·25, 4·8
Bedside humidifier	1 (6)	1 (3)	NS	2·0	0·01, 283
Mean distance from head of bed to sink (cms)	281·6	301·48	NS		
Showers	0	0			

* 95% confidence interval.

chromosomal DNA (Table 6). There was concordance in the monoclonal subtypes of the isolates from patients and the environment for 6 of the 9 sets. Patient and environmental isolates displayed the same plasmid pattern in 7 of the sets and identical fragmentation (REA) patterns in 8 sets. Concordance in all three parameters was achieved in 5 sets. By assuming the monoclonal subtype to be a

Table 4. *Case-control study number 2. Demographic features of 9 cases of nosocomial Legionnaires' disease and 18 control patients. Control subjects were the patients who were admitted to the* same unit *where the case developed Legionnaires' disease immediately before and after the case*

	Cases (9)	Controls (18)	*P*
No. males/females	7/2	10/8	
Mean age (years)	58·9	54·6	NS
Mean length of stay (days)	56·3	66·6	NS
No. (%) died	8 (89	1 (6)	< 0·0003
Mean number of days after admission until pneumonia developed	26·2	0	not tested
No. (%) with malignancy	3 (33)	4 (22)	
APACHE II Score (Mean ± SD)	16·67 ± 5	12·11 ± 5·89	< 0·05

Table 5. *Case-control study number 2. Comparison of cases and controls for selected factors that might predispose to acquisition of nosocomial Legionnaires' disease*

Factor	Cases (9) No. (%)	Controls (18) No. (%)	*P*
Assisted ventilation during hospital stay	7 (78)	8 (44)	NS
Assisted ventilation prior to onset of pneumonia	7 (78)	N.A.*	
Aerosol therapy	5 (56)	3 (17)	NS
Corticosteroid or other immunosuppressive therapy	8 (89)	7 (39)	0·01
Aspirated gastric contents	2 (22)	0	NS
Nasogastric tube prior to onset of pneumonia (cases) or during hospital stay (controls)	3 (33)	4 (22)	NS
Drank tap water	8 (89)	16 (89)	NS
Mean amount of tap water ingested (litres)	29·3	16.5	NS
Altered level of consciousness	1 (11)	4 (22)	NS
Difficulty swallowing	2 (22)	4 (22)	NS
Surgery prior to the onset of pneumonia (cases) or during hospital stay (controls)	3 (33)	4 (22)	NS

* N.A., not applicable. Controls did not have nosocomial pneumonia.

phenotypic trait subject to variable expression, the proportion of complete concordance is increased to 7 of the 9 sets using plasmid and REA patterns (genotypes) as parameters.

There was no isolate obtained from the water supply nearest the patient from whom we recovered *L. pneumophila* serogroup 6. This patient had been transferred

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Table 6. *Properties of* L. pneumophila *recovered from patients and potable water during case control studies 1 and 2. Isolates were matched for room number and isolation date*

Case control study	Set No.	Source	MAB* subtype	Plasmid pattern	REA† pattern
1	1	Patient	OLDA	II	b
		Water (sink)	OLDA	II	b
		Water (respirator)	OLDA	II	b
		Water (dehumidifier)	OLDA	II	b
	2	Patient	OLDA	II	d
		Water‡	OLDA	II	d
	3	Patient	OLDA	V	d
		Water	OLDA	V	d
	4	Patient	OLDA	III	d
		Water	OLDA	II	b
2	1	Patient	OLDA	II	b
		Water	OLDA	II	b
	2	Patient	OXFORD	II	b
		Water	OXFORD	II	b
	3	Patient	nd§	II	b
		Water	nd	II	b
	4	Patient	OLDA	VI	b
		Water	OXFORD	VI	b
	5	Patient	OLDA	VI	b
		Water	OXFORD	II	b

* Monoclonal antibody.
† Restriction endonuclease analysis.
‡ Taken from nearest sink unless otherwise indicated.
§ Not done.

from another hospital. Water samples from his room in the referring hospital were negative for *Legionella* spp. We have never isolated *L. micdadei* from our potable water but we did have one such patient isolate. Water from the oxygen bubblers was negative for legionella. Ten patients who were receiving assisted ventilation had 43 cultures of the ventilator humidifier water and all except one sample were negative. The one positive sample was from a ventilator that was in use for a patient with nosocomial Legionnaires' disease. Samples of water from the afferent and efferent limbs of the respiratory tubing also grew *L. pneumophila*.

Cost of sterile potable water

The cost of providing sterile potable water to the immunocompromised patients is $12 151·50 per year.

DISCUSSION

One of the unanswered questions in the epidemiology of nosocomial legionellosis in the setting of contaminated potable water is the route of transmission from the water to the respiratory tract. There is little doubt that potable water is the source

of the legionella in our hospital. The water is contaminated and there is no other known source of legionella. Furthermore, the monoclonal subtypes, plasmid and restriction endonuclease fragmentation patterns of *L. pneumophila* recovered from the water source closest to the patient were the same as those detected in the patient isolates in most instances. Finally, the ultimate test; when sterile water was given to patients at the highest risk for developing nosocomial Legionnaires' disease, cases no longer occurred in areas where this policy was followed. We now define high risk patients as those who are receiving corticosteroid or other immunosuppressive therapy, or who have had an organ transplant or who are hospitalized in an intensive care unit.

Our first case control study showed that cases differed from controls in three of the factors analyzed: they were more likely to have received assisted ventilation prior to the onset of pneumonia, to be immunosuppressed and to have had nasogastric tubes. The immunosuppression predisposes to infection but does not explain transmission of the infection. In contrast, ventilation therapy and nasogastric tubes may be important in this regard. We were told that on occasion nurses rinsed ventilator tubing and 'T' pieces in tap water to remove secretions. We were not able to determine how often this occurred and whether it applied to any of the patients with Legionnaires' disease. Woo and colleagues [17] showed that rinsing a ventilator bag with contaminated tap water led to the isolation of *L. pneumophila* from culture plates after the bags were squeezed. They suggested that *L. pneumophila* could be aerosolized into the bronchial tree in this manner. Two other studies support our findings of the importance of ventilator therapy as a risk factor for nosocomial Legionnaires' disease. Muder and co-workers [18] found that patients with nosocomial legionellosis were more likely to have been intubated and Markowitz and colleagues [19] found that heart transplant patients who developed Legionnaires' disease were intubated longer than controls. They suggested that rinsing ventilation bags with contaminated tap water may have been important in the acquisition of nosocomial legionella infection. In our hospital ventilation bags were not rinsed with tap water. More recently acquisition of nosocomial Legionnaires' disease has been associated with rinsing nebulizers used to deliver medication with contaminated tap water [20]. There was no association with nebulizer use and nosocomial Legionnaires' disease in our patients (Tables 3 and 5). Furthermore sterile water was used to rinse the nebulizers.

One of our patients probably acquired legionellosis from a contaminated bedside humidifier. This young female with acute myelogenous leukaemia and asthma had a bedside humidifier which she filled with tap water. This tap water grew *L. pneumophila* of the same monoclonal antibody type as isolated from the patient. There have been two other reports of nosocomial Legionnaires' disease following exposure of contaminated humidifiers [21, 22]. Bedside humidifiers are ordinarily not allowed in our hospital.

In the first case control study there was a strong association with legionellosis with the use of nasogastric tubes. Eight (47%) of the patients had such tubes in place a mean of 2 weeks prior to the diagnosis of pneumonia. Eventually 15 of the 17 patients with nosocomial Legionnaires' disease had such tubes. Nasogastric tubes were frequently flushed with tap water. if the tube was to be used for

feeding, tap water was used first and then the feeds were introduced; also, nasogastric feedings were diluted to the desired strength with tap water. There is mounting evidence that the majority of nosocomial pneumonia results from aspiration [23–27]. Patients who are intubated are at even greater risk for aspiration as oropharyngeal secretions commonly leak around the endotracheal tube cuff [23]. The stomach is a reservoir for aerobic Gram-negative rods that are aspirated to cause pneumonia [24–26]. Typically, the stomach is colonized and 1–2 days later the respiratory tract is colonized with the same bacteria. In Craven's [24–25] and other studies [26–27] intubated patients who received H_2 blockers had a high rate of nosocomial pneumonia compared with patients who did not receive these agents. Elevation of the gastric pH allows aerobic Gram-negative rods to survive. H_2 blockers were not a risk factor for nosocomial legionellosis in our study. *Legionella pneumophila* can survive for 1 month in tap water varying in pH from 4 to 7 [28].

Logistic regression analysis of the data from the first case-control study revealed that immunosuppression, ventilator use and the presence of a nasogastric tube were all highly correlated with the acquisition of nosocomial Legionnaires' disease. Use of sterile water to flush nasogastric tubes led to a transient decrease in the number of cases of nosocomial Legionnaires' disease and a sustained decrease in cases in our intensive care units. There were only two cases from September of 1987 to date and one of these patients, a liver transplant patient, had macroaspiration of stomach contents. Thus, only one patient with a nasogastric tube acquired Legionnaires' disease in our intensive care units in the last 3 years. Why using sterile water in nasogastric tubes and to dilute nasogastric feedings led to a transient decrease in cases of nosocomial Legionnaires' disease throughout the hospital is unknown.

The continuation of cases (not surprising since nasogastric tubes were implicated in only 47% of the cases in the first study) led to the second case control study. This time the only significant differences between cases and controls were the high rate of immunosuppression among the cases, a higher mortality rate and a higher APACHE II score. We hypothesized that microaspiration of contaminated potable water could lead to Legionnaires' disease in highly immunosuppressed patients therefore we instituted a policy of sterile potable water for immunosuppressed patients on units where these patients are usually hospitalized. Only one case of Legionnaires' disease has occurred on the units where our sterile potable water policy was in effect and this represented a failure of the policy in that the patient was given tap water to drink.

Le Saux and colleagues [29] showed that increasing corticosteroid dosage was associated with lymphopenia and they speculated that these effects were the reasons for the enhanced risk of Legionnaires' disease. Guiget and co-workers [30] showed that exposure to corticosteroids prior to admission was a major risk factor for nosocomial legionellosis (relative risk 7·9). By way of comparison, malignant illness had a relative risk of 3·5 and ultimately fatal disease a risk of 2·6.

Are there other possible explanations for our findings? Certainly, cases of nosocomial Legionnaires' disease can be episodic (Table 1 and Fig. 1), so it is possible that our results may have occurred by chance alone. Against this is the consistent finding of 6–7 cases per year in the absence of any intervention (Fig. 1

– September 1983 to September 1987). It is possible that we may still encounter occasional cases of nosocomial Legionnaires' disease. Helms and colleagues [31] found four cases of nosocomial LD on a hematology-oncology ward (1/1000 admissions) during a 5-year-period when hyperchlorination was carried out and surveillance cultures of the water were consistently negative. Prior to hyperchlorination they encountered 16 cases among 456 patients (35/1000 admissions). They did not know the source of the cases that occurred during the period of hyperchlorination.

Another consideration is that the *Legionella* sp. in the potable water may have become avirulent. We have no evidence for or against this possibility. It is known that environmental isolates of *Legionella* sp. may differ in virulence [32] and that environmental temperature may modulate the virulence of this organism perhaps by affecting bacterial adherence to host cells [33]. The hot water temperature was not intentionally altered during the time of our studies.

It is unlikely that instructions to high risk patients not to have showers played any role in reducing cases of Legionnaires' disease since these patients did not shower. This instruction was given to avoid cases due to showering.

The final consideration is that we may not have detected all cases of nosocomial Legionnaires' disease from June 1989 onwards. This is unlikely since our Infection Control Practitioners monitor nosocomial pneumonia and request cultures for legionella. We have active Infectious Disease consultants who are fully aware of the epidemiology of legionella in our hospital. In a previous study [8] we found that we would miss only 13% of our case of nosocomial Legionnaires' disease by not doing serological testing. Also one of the two cases in the last year of the study (1989–90) was diagnosed serologically. Finally, the number of patients with nosocomial pneumonia who had respiratory secretions cultured was higher the last 2 years of the study than previously (Table 1).

The cost of providing sterile potable water to immunosuppressed patients in areas of the hospital where most of the cases of Legionnaires' disease occurred was $12151·00 per year – a figure that compares favourably with the $12500 annual operating expenses for continuous hyperchlorination reported by others [31]. Our method avoids the problems of hyperchlorination which include corrosion damage to the water distribution system and the production of triholmethanes which may be carcinogenic [31].

It is our hypothesis that ingestion and subsequent aspiration of contaminated potable water is the mode of transmission of nosocomial Legionnaires' disease in our hospital. The evidence for this is circumstantial, however, our study adds to several other suggestions that aspiration of contaminated potable water is an important mode of transmission of Legionnaires' disease. The outbreak that occurred at the 1976 Philadelphia Legionnaires convention showed a correlation of disease with drinking tap water in patients who were at risk for subclinical aspiration (those with alcohol and tobacco intake) [34].

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REFERENCES

1. McDade JE, Shepard CC, Fraser DW, et al: Legionnaires' disease. Isolation of a bacterium and demonstration of its role in other respiratory disease. N Engl J Med 1977; **297**: 1197–203.
2. Thacker SB, Bennett JV, Tsai T, et al. An outbreak in 1965 of severe respiratory illness caused by Legionnaires' disease bacterium. J Infect Dis 1978; **238**: 512–9.
3. Korvick, Yu VL, Fang G-D. Legionella species as hospital-acquired respiratory pathogens. Semin Respir Infect 1987; **II**: 34–47.
4. Stout JE, Yu VL, Vickers RM, et al. Ubiquitousness of *Legionella pneumophila* in the water supply of a hospital with endemic Legionnaires' disease. N Engl J Med 1982; **306**: 466–8.
5. Stout JE, Yu VL, Muraca P. Isolation of *Legionella pneumophila* from the cold water of the hospital ice machines. Implications for origin and transmission of the organism. Infect Control 1984; **6**: 141–6.
6. Fraser DW. Potable water as a source for Legionellosis. Environ Hlth Perspec 1985; **62**: 337–41.
7. Haley RW, Quade D, Freeman HE, Bennett JV. CDC SENIC planning committee: study on the efficacy of nosocomial infection control (SENIC-Project): Summary of study design. Am J Epidemiol 1980; **111**: 472–85.
8. Marrie TJ, MacDonald S, Clarke K, Haldane D. Nosocomial Legionnaires' disease – Lessons from a four year prospective study. Am J Infect Control 1991; **19**: 79–85.
9. Knaus WA, Draper EA, Wagner DP, et al. APACHE II: A severity of disease classification system. Crit Care Med 1985; **13**: 818–22.
10. Vickers RM, Stout JE, Yu VL, Rihs JD. Manual of culture methodology for Legionella. Semin Respir Infect 1987; **2**: 274–9.
11. Cherry WB, Pittman B, Harris PP, Hebert GA, Thomason BM, Weaver RE. Detection of Legionnaires' disease bacteria by direct immunofluorescent staining. J Clin Microbiol 1981; **14**: 298–303.
12. Wilkinson HW, Cruce DW, Fikes BJ, Yealy LP, Fashy CE. Indirect immunofluorescence test for Legionnaires' disease. p. 112–116. In Jones G, Herbert GA, eds 'Legionnaires'': the disease, the bacterium and methodology. Atlanta, GA: Centers for Disease Control, 1979.
13. Joly JR, Chen Y-Y, Ramsay D. Serogrouping and subtyping of *Legionella pneumophila* with monoclonal antibodies. J Clin Microbiol 1983; **18**: 1040–6.
14. Dillon JR, Bezanson GS, Yeung K-H, Basic techniques. In Dillon JR, Nasim A, Nestman E, eds. Recombinant DNA methodology. Toronto: J. Willey and Sons Inc. 1985: 1–125.
15. Rousell AL, Chabbert YA. Taxonomy and epidemiology of gram-negative bacterial plasmids studied by DNA-DNA hybridization in formamide. J Gen Microbiol 1978; **104**: 269–76.
16. McCullough P, Nelder IA. Generalized linear models. New York: Chapman and Hall, 1983: 72–100.
17. Woo AH, Yu VL, Goetz A. Potential in-hospital modes of transmission of *Legionella pneumophila*. Demonstration experiments for dissemination by showers, humidifiers and rinsing of ventilation bag apparatus. Am J Med 1986; **80**: 567–73.
18. Muder RR, Yu VL, McClure J, et al. Nosocomial Legionnaires' disease uncovered in a prospective pneumonia study: implications for underdiagnosis. JAMA 1982; **249**: 3184–92.
19. Markowitz L, Tompkins L, Wilkinson H, et al. Transmission of nosocomial Legionnaires' disease in heart transplant patients. Program and abstracts of the 24th Interscience Conference on Antimicrobial Agents and Chemotherapy 1984. American Society for Microbiology, Washington, D.C., 170.
20. Mastro TD, Fields BS, Breiman RF, Campbell J, Plikaytis BD, Spika J. Nosocomial Legionnaires' disease and use of medication nebulizers. J Infect Dis 1991; **163**: 667–71.
21. Arnow PM, Chou T, Weil D, Shapiro EN, Kretzschmar C. Nosocomial Legionnaires' Disease caused by aerosolized tap water from respiratory devices. J Infect Dis 1982; **146**: 460–7.
22. Kaan JA, Simoons-Smit AM, MacLaren DM. Another source of aerosol causing nosocomial Legionnaires' disease. J Infect 1985; **11**: 145–8.

23. Pierce AK, Sanford JP. Aerobic gram-negative bacillary pneumonias. Am Rev Respir Dis 1974; **110**: 647–58.
24. Craven DE, Kunches LN, Kilinsky V, et al. Risk factors for pneumonia and fatality in patients receiving continuous mechanical ventilation. Am Rev Respir Dis 1986; **133**: 792–6.
25. Craven DE, Driks MR. Nosocomial pneumonia in the intubated patient. Semin Respir Infect 1987; **II**: 20–33.
26. du Moulin GC, Paterson DG, Hedley-Whyte J, Lisbon A. Aspiration of gastric bacteria in antacid-treated patients: a frequent cause of postoperative colonization of the airway. Lancet 1982; **1**: 242–5.
27. Atherton ST, White DJ. Stomach as a source of bacteria colonizing respiratory tract during artificial ventilation. Lancet 1978; **2**: 968–9.
28. Katz SM, Hammel JM. The effect of drying, heat and pH on the survival of *Legionella pneumophila*. Ann Clin Lab Sci 1987; **17**: 150–6.
29. Le Saux NM, Sekla L, McLeod J, Parker S, Rush D, Jeffery JR, Brunham RC. Epidemic of nosocomial Legionnaires' disease in renal transplant recipients: a case-control and environmental study. Can Med Assoc J 1989; **140**: 1047–53.
30. Guiguet M, Pierre J, Burn P, Berthelot G, Gottot S, Gibert C, Valleron AJ. Epidemiological survey of a major outbreak of nosocomial legionellosis. International J Epidemiol 1987; **16**: 466–71.
31. Helms CM, Massanari RM, Wenzel RP, Pfaller MA, Moyer NP, Hall N, and the Legionella monitoring committee. Legionnaires' disease associated with a hospital water system. A five-year progress report on continuous hyperchlorination. JAMA 1988; **259**: 2423–7.
32. Bollin GE, Plouffe JF, Para MF, Prior RB. Difference in virulence of environmental isolates of *Legionella pneumophila*. J Clin Microbiol 1985; **21**: 674–7.
33. Edelstein PH, Beer KB, DeBoynton ED. Influence of growth temperature on virulence of *Legionella pneumophila*. Infect Immun 1987; **55**: 2701–5.
34. Fraser DW, Tsai TR, Orenstein W, et al. Legionnaires' disease: Description of an epidemic of pneumonia. N Engl J Med 1977; **297**: 1189–97.