

## Nosocomial legionella pneumonia: demonstration of potable water as the source of infection

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### SUMMARY

From January 1983 until December 1985, 35 cases of sporadic nosocomial legionella pneumonia, all caused by *Legionella pneumophila*, were diagnosed in a university hospital. *L. pneumophila* serogroup (SG) 1 was cultured from 12 of the 35 cases and compared to corresponding *L. pneumophila* SG 1 isolates from water outlets in the patients' immediate environment by subtyping with monoclonal antibodies. The corresponding environmental isolates were identical to 9 out of 12 (75%) of those from the cases. However, even in the remaining three cases identical subtypes were found distributed throughout the hospital water supply. From the hospital water supply four different subtypes of *L. pneumophila* SG 1 were isolated, three of which were implicated in legionella pneumonia. Of 453 water samples taken during the study 298 (65.8%) were positive for legionellae. Species of *Legionella* other than *L. pneumophila* have not been isolated. This may explain the exclusiveness of *L. pneumophila* as the legionella pneumonia-causing agent. Our results suggest that the water supply system was the source of infection.

### INTRODUCTION

Legionellae are found ubiquitously as contaminants of water supplies in communities and hospitals (Stout *et al.* 1985; Bartlett, Macrae & Macfarlane, 1986*a*). A number of studies have linked nosocomial *Legionella pneumonia* to air-conditioning systems and cooling towers (Badenoch *et al.* 1986; Bartlett, Macrae & Macfarlane, 1986*a*; Garbe *et al.* 1985; Timbury *et al.* 1986). In recent reports, however, the importance of hospital water supplies as a source of nosocomial infections has been emphasized (Hanrahan *et al.* 1987; Shands *et al.* 1985). Since legionellae are found widely distributed in aquatic habitats, the isolation from an environmental source is no proof as such of their role as infectious agents (Bartlett, Macrae & Macfarlane, 1986*b*; Edelstein, 1986). A number of techniques, e.g. plasmid analysis, subtyping with monoclonal antibodies (MAB), have been used to prove the identity of corresponding clinical and environmental legionella

isolates, and thus to localize the likely source of infection (Edelstein *et al.* 1986*b*; Meenhorst *et al.* 1985; Neil *et al.* 1985). In the Rudolf Virchow University Hospital (UKRV) legionellae were also found to be a cause of nosocomial pneumonia. In order to evaluate the role of the hospital water supply as the source of legionellosis we performed clinical and environmental studies over a 3-year period during which corresponding *L. pneumophila* SG 1 isolates from patients and from the water supply were subtyped using monoclonal antibodies.

#### MATERIALS AND METHODS

##### *Epidemiology studies*

(i) Cases. From January 1983 to December 1985 all patients with nosocomial pneumonia were screened for legionellosis. Pneumonias were considered nosocomial if the onset of the clinical symptoms occurred on or after the third day of hospitalization.

(ii) Laboratory methods. The indirect immunofluorescence (IFA) test against *L. pneumophila* SG 1–6, *L. micdadei* and *L. dumoffii* was used to detect serum antibodies (Wilkinson, 1987). Urine was tested for *L. pneumophila* SG 1 antigen by radioimmune assay (Fehrenbach *et al.* 1986; Kohler *et al.* 1984). Bronchial secretions and lung tissue obtained at autopsy were examined both by culture and by direct immunofluorescence (DFA). Material was inoculated onto BCYE agar (Edelstein, 1981) and onto two semiselective media described by Edelstein (1981) and by Wadowsky as modified by Dennis, Bartlett & Wright (1984). Antibodies prepared against *L. pneumophila*, SG 1–6, *L. gormanii*, *L. dumoffii*, *L. micdadei*, *L. longbeachae* SG 1–2 and *L. bozemanii* (kindly provided by the Center for Disease Control, Atlanta, USA) were used in the DFA (Cherry *et al.* 1978).

(iii) Criteria for confirmation of diagnosis. (a) A fourfold or greater rise of serum antibodies for confirmed cases, and a single or maintained titre  $\geq 256$  for presumptive cases (Wilkinson, 1987). (b) Positive culture or demonstration of legionellae by DFA in secretions or lung specimens. (c) The detection of antigen in urine.

##### *Environmental studies*

(i) The hospital and its water supply. UKRV has 1538 beds with 19 clinical departments contained in 47 wards housed in 13 separate buildings. The hospital has its own potable water supply which is supplemented when necessary from the community supply.

Hot and cold water for the clinical departments is held in three tanks and is distributed to the 13 buildings through a central distributor by way of separate branches from one of three main circular circuits.

(ii) Water sampling. Samples were taken randomly over a 3-year period (1983–85) from the hot and cold water tanks, the piped mains supply, and from faucets and showers on the wards. Where legionellae were isolated from patients, water samples from the patients' immediate environment (room, anteroom, toilet) and from showers on the ward were taken. Such samples were taken from 1–16 weeks after the isolation of legionella from the patient.

The outlets were disinfected with a gas burner and the first litre was discarded.

Table 1: Detection of nosocomial *L. pneumonia* in 35 patients

Type of specimen	Diagnostic procedure	Result positive/total (%)
Serum	IFA*	6/22 (27.3)
Sputum	DFA†	4/14 (18.0)
	culture	0/12
Urine	RIA‡	12/13 (92.3)
Lung tissue	DFA	22/24 (91.7)
	culture	13/15 (86.7)

\* Indirect immunofluorescence.

† Direct immunofluorescence.

‡ Radioimmunoassay.

Cold (14–27 °C) and hot water (35–69 °C) samples of 100 to 1000 ml were taken. After filtration through polycarbonate membrane filters (diameter 50 mm, median pore size 0.2–0.4 µm), the filters were suspended in 1 ml of tap water and sonicated for 10 sec. Two semiselective agar media, described by Edelstein (1981) and by Wadowsky as modified by Dennis, Bartlett & Wright (1984), were inoculated with 0.1 ml of the sonicated suspension. Suspicious colonies were subcultured on BCYE (cystein free)-agar and BCYE-agar. Isolates which could be subcultured on BCYE-agar, but not on BCYE (cystein free)-agar were picked and further identified by biochemical characteristics (Wilkinson, 1987) and finally identified at species and serogroup level using DFA (Cherry *et al.* 1978).

Corresponding *L. pneumophila* SG 1 isolates from human specimens and from environmental water supplies were compared with a standardized subgrouping scheme of seven MAB using the indirect immunofluorescence assay according to Joly *et al.* (1986). MAB were kindly supplied by Dr McKinney (CDC, Atlanta, USA) (MAB 1–3), by Dr Tobin (Sir William Dunn School of Pathology, Oxford, UK) (MAB 4), and by Dr Joly (Université Laval, Quebec, Canada) (MAB 5–7).

## RESULTS

**Epidemiological findings.** From 1983 to 1985, 35 sporadic cases of nosocomial *L. pneumonia* were diagnosed. Pneumonia developed in all patients 8 days or more after admission (in 30 cases after at least 14 days). Legionellosis was confirmed in all 35 patients as shown in Table 1. Positive cultures were obtained from lung tissue specimens (*L. pneumophila* SG 1 in 12 cases and *L. pneumophila* SG 5 in 1 case) from 13 patients who died. The only *Legionella* species involved in all cases was *L. pneumophila*, with *L. pneumophila* SG 1 in 29/35 cases (82.8%). The SG distribution of the clinical isolates is presented in Table 2. The affected patients were housed in 9 of the 13 clinical buildings and there was no accumulation of cases in respect to certain buildings or to certain periods of time.

**Environmental findings.** A total of 453 water samples were taken. Of these 298 (65.8%) samples yielded growth of legionellae. Legionellae were not isolated from water samples from the central hot and cold water tanks. Approximately the same proportion of samples from water circuits, their branches supplying the buildings, and outlets (faucets and showers) were positive. Seventy-four out of 171 (43.2%) of

Table 2. *Distribution of L. pneumophila SG in 35 nosocomial pneumonias and 298 hospital water samples*

<i>L. pneumophila</i> SG	No. (%)	
	Environment	Patients
1	166 (43.8)	29 (82.8)
4	5 (2.1)	1 (2.9)
5	58 (24.7)	3 (8.6)
6	69 (29.4)	2 (5.7)
Total	298 (100)	35 (100)

Table 3. *Isolation of legionella from water samples of the hospital water supply*

Source	Temperature range (°C)	No. of samples	
		Positive	Total (%)
Cold water	14-27	74	171 (43.2)
Hot water	35-69	224	282 (79.4)
Total		298	453 (65.8)

Table 4. *Comparison of clinical and environmental L. pneumophila SG 1 isolates using monoclonal antibodies*

Case	Clinical isolate	No of outlets* positive/total	Water isolate†
1	+ + - - + - + (1)	5/10	+ + - - + - + (1)
2	+ + - - + - + (1)	3/6	+ + - - + - + (1)
3	+ + - - + - + (1)	4/6	+ + - - + - + (1)
4	+ + + - - + - (4)	2/4	+ + + - - + - (4)
5	+ - - + - - + (3)	3/5	+ - - + - - + (3)
6	+ + - - + - + (1)	5/9	+ + - - + - + (1)
			+ + - + + - + (2)
			+ - - + - - + (3)
7	+ + - - + - + (1)	6/10	+ + - - + - + (1)
			+ + - + + - + (2)
8	+ + + - - + - (4)	3/6	+ + + - - + - (4)
			+ + - - + - + (1)
			+ - - + - - + (3)
9	+ - - + - - + (3)	2/5	+ - - + - - + (3)
			+ + + - - + - (4)
10	+ + - - + - + (1)	4/6	+ - - + - - + (3)
11	+ + - - + - + (1)	2/5	+ - - + - - + (3)
12	+ + - - + - + (1)	3/9	+ - - + - - + (3)

Subtypes of *L. pneumophila* SG 1: (1) Benidorm, (2) Philadelphia, (3) Bellingham, (4) Knoxville.

\* Positive indicates isolation of *L. pneumophila* SG 1 only.

† Having several isolates of the same subtype of *L. pneumophila* SG 1 only one was listed.

cold water samples and 224 out of 282 (79.4%) of hot water samples were positive (Table 3). The distribution of *L. pneumophila* serogroups is listed in Table 2. *Legionella* species other than *L. pneumophila* were not found in hospital water.

Comparison of clinical and environmental isolates. Fifty-three of 81 (65.4%) water samples taken from the rooms or from adjacent locations of 12 patients with culture-proven *L. pneumophila* SG 1 infection were positive. *L. pneumophila* SG 1 was found in 42 (79.2%), *L. pneumophila* SG 5 in 6 (11.3%) and *L. pneumophila* SG 6 in 5 (9.4%) cases. The results of the MAB subtyping of corresponding *L. pneumophila* SG 1 isolates are listed in Table 4. In all cases water samples from at least two outlets were positive for *L. pneumophila* SG 1. Up to three different subtypes were found in water samples from various outlets in the close environment of individual patients. In each of nine cases the *L. pneumophila* SG 1 subtype was identical with at least one environmental isolate. In three cases the MAB pattern of the isolates from the direct environment in the patients' room was at variance, but the responsible subtype was found distributed throughout the hospital water supplies. Three different subtypes (Benidorm, Bellingham, Knoxville) of *L. pneumophila* SG 1 were found to be involved in nosocomial legionellosis. *L. pneumophila* SG 1 isolates from the hospital water supply were found to belong to four different subtypes (Benidorm, Bellingham, Knoxville, Philadelphia).

#### DISCUSSION

In prospective studies legionellosis has been reported to be the cause of 14% to 47% of cases of hospital-acquired pneumonias (Muder *et al.* 1983; Rudin *et al.* 1986; Yu *et al.* 1982) and from time to time particularly high incidences have resulted from epidemic or hyperendemic situations (Broome, 1984). Of the 35 nosocomial cases which were diagnosed over a period of 3 years in the UKRV, 16 were detected in a 1-year prospective pneumonia study, in which the frequency of legionellosis amongst nosocomial pneumonias was 6.8% (16/236 cases) (Ruf *et al.* 1988).

The water supply system of the hospital was proved to be intensely colonized (65.8% of samples positive). However, similar colonization frequencies have been reported from other studies (Johnson *et al.* 1985; Shands *et al.* 1985). All cases were caused by *L. pneumophila*, with *L. pneumophila* SG 1 being responsible for 82.8%. At the same time *L. pneumophila* SG 1 was the strain most commonly isolated from hospital water supplies. All *L. pneumophila* SG which were found to be implicated in nosocomial infections were also isolated from the hospital water system. That legionella pneumonias are due solely to *L. pneumophila* can be explained by the fact that in the hospital water supply only *L. pneumophila* has been found.

So far, subtyping with MAB has mainly been confined to *L. pneumophila* SG 1 isolates, the most frequent cause of legionellosis (Broome, 1984; Reingold *et al.* 1984). Clinical *L. pneumophila* SG 1 isolates of 9/12 patients were identical with at least one isolate from outlets in the patients' close environment. Where, however, in three additional patients, the isolates from the direct environment differed from the clinical ones, the same subtypes were found in other localities in the water distribution system. Our findings indicate that the hospital water has to be

considered the source of nosocomial legionella infections. This is consistent with those of other studies (Meenhorst *et al.* 1985; Neill *et al.* 1985). There is no evidence that other potential sources were implicated. Air-conditioning systems were only present in three wards and as steam was used for humidification, they can be excluded as the source of infection.

The potable water system was colonized by four different *L. pneumophila* SG 1 subtypes. Three of these were found to be involved in nosocomial *L. pneumonia*. In other studies, in which an upsurge of cases over a short period has been linked to the water supply, one responsible subtype only has been found (Neill *et al.* 1985; Hanrahan *et al.* 1987). The UKRV has a multi-branched water supply system. In recent decades, buildings and consequently parts of the water system have been reconstructed, creating multiple dead ends, a multitude of junctions with slow flow rates and reservoirs of standing water. These conditions are well known to support colonization with legionellae (Bartlett, Macrae & Macfarlane, 1986*b*; Stout *et al.* 1985) and may explain the remarkable colonization of the hospital water.

Colonization is not necessarily associated with legionella pneumonia cases (Bartlett, Macrae & Macfarlane, 1986*b*; Edelstein, 1986). However, at sites in which patients at high risk of acquiring legionellosis are being treated colonization should be prevented (Bartlett, Macrae, Macfarlane, 1986*b*; Edelstein, 1986). If an upsurge can be linked to an environmental source measures of prevention are mandatory. Part of an effective policy to control nosocomial legionella infections is administration of antibiotics effective in legionellosis in nosocomial pneumonia cases and the application of a wide spectrum of legionella diagnostic tests (Edelstein, 1986; Bartlett, Macrae & Macfarlane 1986*b*).

Heating and/or chlorination have been used for decontamination (Johnson *et al.* 1985; Shands *et al.* 1985). However, no uniform strategy has been established up to now (Bartlett, Macrae & Macfarlane, 1986*b*). The main problem of decontamination is to maintain effective free chlorine concentrations and water temperatures (Bartlett, Macrae & Macfarlane, 1986*b*). Decontamination by these methods could not have been relied upon in our hospital under the particular circumstances. Since there was no common point source, an extended reconstruction of the whole water supply system has been considered necessary.

The suggested free chlorine concentration of 8 mg/l (range 2.5–15 mg/l) necessary to maintain legionella free water (Bartlett, Macrae & Macfarlane, 1986*b*; Massanari *et al.* 1984) was considered unacceptable because of the considerable hazards to patients and staff and because of the accelerated rate of metal corrosion (Bartlett, Macrae & Macfarlane, 1986*b*). At a temperature of 58 °C legionellae are killed at a rate of 90% every 6 min (Dennis, Green & Jones, 1984). The strategy adopted therefore is to maintain the hot water supply constantly above this temperature. Since legionellae multiply rapidly at temperatures between 20 and 45 °C (Bartlett, Macrae & Macfarlane, 1986*c*) the cold water supply is to be kept constantly below this temperature range.

Reconstruction of the water supply is being undertaken in the course of an extended hospital rebuilding programme. The following system is to be implemented. Water is heated and stored in central tanks at 70 °C; the main piping for the hot water supply is being insulated so as to maintain the temperature. The distribution system of the cold water supply is also being



insulated to prevent an increase of the temperature. For departments in which immunosuppressed patients are being treated, calorifiers are being installed for heating and storage of water at 80 °C. The cold water is heated to 80 °C, cooled off again and stored at temperatures below 20 °C. The taps are fitted with forced mixing devices to avoid scalding.

Meanwhile a surveillance program has been established. Medical staff have been informed about the problem of nosocomial legionellosis and are requested to contact the Infectious Diseases Unit in all cases with nosocomial pneumonia. Antibiotics effective in legionellosis have been made part of the antibiotic regime in treating nosocomial pneumonias of unknown etiology.

Annual and seasonal variations of incidence and apparent spontaneous decreases have been observed (Muder, Yu & Woo, 1986). Further nosocomial cases are clearly not entirely prevented using heating and/or chlorination (Bartlett, Macrae & Macfarlane, 1986b). When evaluating the effectiveness of eradication programmes long-term follow up is very necessary.

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