

***Legionella Pneumophila* in a hospital in Torino, Italy  
A retrospective one-year study.**

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SUMMARY

*Legionella pneumophila* serogroup 1 was isolated from post mortem specimens from 13 out of 58 patients with pneumonia diagnosed at autopsy. The results of a study undertaken in the hospital environment showed that the water plumbing system was colonized with *L. pneumophila* serogroup 1 which could also be isolated from respiratory devices filled with tap water. Control measures instituted are described.

INTRODUCTION

Over the last few years *Legionella pneumophila* has been found to be common in hospital water systems and has been responsible for epidemic cases of nosocomially-acquired pneumonia (Tobin *et al.* 1980; Fisher-Hoch *et al.* 1981; Stout *et al.* 1982; Meyer, 1983; Garbe *et al.* 1985; Meenhorst *et al.* 1985; Neill *et al.* 1985). Stout and colleagues (1985) report that 10–20% of nosocomial pneumonia can be ascribed to *L. pneumophila*, and Cohen *et al.* (1979) estimate that 3% of deaths due to hospital-acquired pneumonia are caused by this microorganism.

In a hospital in Torino several patients hospitalized for various diseases were found to have pneumonia or bronchopneumonia at post mortem. It was assumed, following reports in the literature, that some may have been cases of undiagnosed Legionnaires' disease. *L. pneumophila* serogroup 1 was isolated from several patients and from respiratory equipment (Moiraghi *et al.* 1987).

The investigation described was undertaken to identify the source(s) and the site(s) of the infection within the hospital environment and how the results were used to institute control measures.

## MATERIAL AND METHODS

*The hospital*

The study was carried out at the Molinette Centre of the 'Ospedale Maggiore di San Giovanni Battista e della Città di Torino', and started in March 1984. The Molinette Centre is the main part of a large hospital complex of about 2000 beds, that is both a University and Municipal General Hospital. It was built in 1934-5 and has since been extended and modified. It covers an area of 135 000 sq m, about 35 000 of which are devoted to buildings whose wings are connected through a system of underground tunnels and at the ground floor level (Fig. 1). The wings are 4 or 5 storeys in height. In every wing there are two sections with 35 to 50 beds in rooms with 3 or 6 beds.

Potable water is supplied from nine different points of the municipal system, each supplying one or more buildings, and in some cases part of a building. The hot water distribution system consists of a central calorifier (Fig. 1) and a main pumped recirculating pipe from which branches supply the different wings.

Each room has a single sink with a mixer tap supplying cold and hot water. Water for a separate heating system is provided via a water softener.

Self-contained air-cooling systems serve the Cardiac Surgery and the Neurosurgery wards. There are no cooling towers or humidifiers in the hospital.

*Case findings*

All autopsies performed at the hospital from March 1984 to April 1985 were studied and lung tissue from 58 patients showing signs of pneumonia was examined. Histopathological and bacteriological examinations, including direct immunofluorescence (DFA) for *L. pneumophila* serogroups 1 to 6, were performed in order to detect *L. pneumophila*. Respiratory secretion was examined from one patient who underwent heart-surgery and died from a complicating bronchopneumonia and on whom an autopsy was not performed. When the results were positive for legionella the hospital records of these patients were examined.

*Case definition*

Cases were defined as legionella pneumonia according to the following criteria based on the hospital records: macroscopic and histologic evidence of pneumonia at post mortem; isolation of *L. pneumophila* from autopsy specimens or bronchial aspirate; medical records showing evidence of symptoms of pneumonia, cough, high temperature, leukocytosis and chest radiographic changes.

Cases were considered as nosocomially acquired if disease developed after more than 4 days of hospitalization.

A positive DFA for *L. pneumophila* was not accepted as definitive evidence of Legionnaires' disease.

*Environmental study*

When the first cases were detected in March 1984 and for the next year, cold and warm water from sink and bath taps or from showers, and the water from respiratory equipment (oxygen bubble humidifiers and an underwater chest drain) in the wards where the cases had been admitted, were examined for the presence

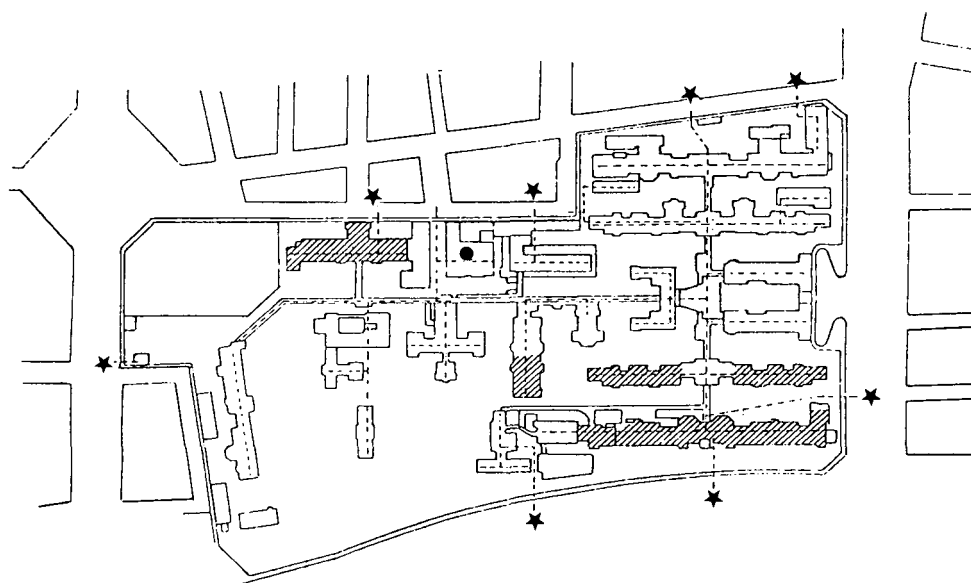


Fig. 1. San Giovanni Battista Hospital 'Molinette Centre'. ▨, Wards where legionellosis cases occurred; ●, central water heater; ★, the city net-work takeoffs; ----, drinking water main supply.

of *L. pneumophila*. Moreover sampling was carried out from the following: 1) the water supplied by the city system at the nine takeoff points; 2) the hospital hot water system; 3) the air-conditioning systems in Cardiac Surgery and Neurosurgery; 4) the demineralized water system. Temperature and free chlorine concentration were measured at the time of sampling.

#### *Bacteriological procedures*

Frozen ( $-70^{\circ}\text{C}$ ) or fresh lung tissue specimens from 58 patients were examined by direct immunofluorescence (DFA) test and tissue suspensions were cultured. Bronchial aspirate was examined from the patient on whom an autopsy was not performed.

Water samples (5 litres from taps and showers, and from blow-back water of the demineralizing system; 200 ml from oxygen humidifiers; 500 ml from the underwater chest drain) were concentrated at least 50-fold by filtration through millipore filters at  $0.45$  or  $0.22\ \mu\text{m}$  pore size. Deposits from taps and showers, and fragments of filters and the condensation water of air-conditioning systems were inoculated directly on agar. Heat and/or acid pretreatments (Bopp *et al.* 1981; Edelstein, Snitzer & Bridge, 1982) were performed if necessary on contaminated samples.

The medium used was BCYE agar (Oxoid) with and without selective supplements, MWY (Edelstein, 1982) or GVCP (Dennis, Bartlett & Wright, 1984). Suspect colonies were identified by DFA and by gas-liquid chromatography of bacterial fatty acids. DFA staining was performed with antisera to *L. pneumophila* serogroups 1 to 6 supplied by the Biological Products Division, Centers for Disease

Control, Atlanta, GA. Strains isolated from patients and some strains from the hospital environment were studied for plasmid pattern and by monoclonal antibody subtyping. Plasmid analysis of cultures grown on BCYE agar was performed by the rapid method of Kado & Liu (1981) using horizontal agarose gel electrophoresis, and the molecular weight of the crude plasmid DNA was determined by comparison with standard plasmids: R27, 112 megadaltons (Mdal); R16, 69 Mdal; R471, 52 Mdal; R94, 36 Mdal. Restriction endonuclease digestion was performed on purified plasmid DNA with Bam HI and Hind III (Boehringer Mannheim) following the manufacturer's instruction. The digestion products were then analysed by electrophoresis as above. Monoclonal antibody subtyping was performed as described by Watkins *et al.* (1985).

#### *Serological procedures*

Single serum samples from 23 members of the hospital staff working in the cardiology ward, where the first two patients with legionella infection were detected, were examined by indirect immunofluorescence (IFA) test against *L. pneumophila* serogroup 1 to 6 antigens.

## RESULTS

#### *Cases*

*L. pneumophila* serogroup 1 was isolated from 13 of the 58 post mortem lung tissue specimens examined (Table 1) and from the respiratory secretion of the patient on whom autopsy was not performed (patient no. 4). In addition, in seven patients *L. pneumophila* serogroup 1 was seen in lung tissue only by direct immunofluorescence. In the present work, however, patients with these findings were not considered as infected as legionellae were identified only microscopically and were therefore not consistent with the case definition.

The histological findings in the cases from which *L. pneumophila* was isolated were consistent with the post mortem finding of pneumonia or bronchopneumonia. In 13 of the 14 patients from whom *L. pneumophila* was isolated, the infection was acquired nosocomially (HAI) following the case definition. One patient's (Table 1, patient no. 14) infection was community acquired (CAI). The 13 subjects with HAI had been admitted to the following wards: Cardiac Surgery, Nephrology, Cardiology, Medicine E, Medicine B, Geriatrics, Neurosurgery. Several cases were clustered in Cardiac Surgery (4 patients) and Nephrology (3 patients).

Twelve patients (6 males and 6 females) were aged 55–74 years. One male was aged 47. All suffered from chronic and debilitating diseases, and 5 had undergone surgery (4 cardiac surgery). Three patients had used the bath or shower, 6 received oxygen, and 4 had an underwater chest drain. Six subjects occupied beds less than 2.5 metres from the sink. Two of them had occupied the same bed in the Nephrology ward (patients no. 6 and 7).

#### *Bacteriological and serological results*

*L. pneumophila* serogroup 1 was isolated from patients and from water. *L. pneumophila* serogroup 3, *L. erythra*, *L. jordanis* and *L. rubrilucens* were also isolated from water. The plasmid pattern and monoclonal subtype of some of the

Table 1. Hospitalized patients from whom *Legionella pneumophila* was isolated

Patient	Age/Sex	Ward	Admission diagnosis	Autopsy findings in lungs
1	57/F	Cardiac surgery	Tricuspid valve regurgitation in previous mitral replacement	Bronchopneumonia, fibrothorax
2	67/M	Cardiac surgery	Coronary arteriosclerosis	Pneumonia
3	47/M	Cardiac surgery	Combined aortic and mitral valve disease	Pneumonia, pleural effusion
4	58/M	Cardiac surgery	Ischaemic cardiopathy	Autopsy not performed†
5	61/M	Nephrology	Chronic renal failure	Pneumonia, emphysema
6	62/M	Nephrology	Chronic glomerulonephritis	Bronchopneumonia, fibrothorax, emphysema
7	65/F	Nephrology	Cirrhosis with chronic renal failure	Pneumonia, abscess, fibrothorax
8	58/F	Cardiology	Combined mitral stenosis and regurgitation	Bronchopneumonia, chronic stasis, pleuritis
9	55/F	Cardiology	Combined mitral stenosis and regurgitation	Bronchopneumonia
10	72/M	Medicine E	Agranulocytosis with nephroangiosclerosis	Bronchopneumonia, pleuritis, emphysema
11	62/F	Medicine B	Dolicho-megacolon	Bronchopneumonia
12	77/M	Geriatrics	Hodgkin's disease	Bronchopneumonia
13	55/F	Neurosurgery	Acoustic neuroma	Bronchopneumonia, abscesses, pleuritis
14*	47/F	Emergency	Bronchopneumonia	Bronchopneumonia

\* Community-acquired infection

† Isolation from respiratory secretions

isolates are shown in Table 2. Some 58.3% of the human strains and 50% of strains isolated from the plumbing system had plasmids. Unexpectedly the isolates from the devices filled with tap water were all found to contain plasmids. Plasmids were found to be of 70 and 90 megadaltons. All the plasmids of the *L. pneumophila* serogroup 1 strains tested had the same enzyme restriction pattern (Castellani Pastoris, Mingrone & Passi, 1987). All but five of the strains subtyped by monoclonal antibody belonged to the Pontiac 1a subtype (Watkins *et al.* 1985). None of the sera taken from the staff of the Cardiology ward reacted at a dilution 1/16 against *L. pneumophila* serogroup 1 antigens.

#### Environmental results

*L. pneumophila* serogroup 1 was isolated from water taken from the plumbing system of all but one of the wards where cases with culture proven infections or DFA positive patients were located. The hot water temperature was about 35 °C at the tap and cold water about 14 °C. The free chlorine level was 0.1 mg/l.

*L. pneumophila* serogroup 1 was also isolated from water taken from the oxygen bubble humidifiers which had been filled with tap water, and from the underwater chest drain (Moiraghi *et al.* 1987).

The same microorganism was found in samples taken from cold blowback water

Table 2. *L. pneumophila* serogroup 1 tested for plasmid content and monoclonal subtype, by their isolation source

	Number of strains	Plasmids	Monoclonal subtype
Patients	7	+	Pontiac 1a
	5	-	Pontiac 1a
Devices	1 (Chest drain)	+	Pontiac 1a
	2 (Oxygen humidifier)	+	Pontiac 1a
	1 (Oxygen humidifier)	+	Pontiac 4a
	1 (Oxygen humidifier)	+	Pontiac 1c
Plumbing systems	5	+	Pontiac 1a
	2	-	Pontiac 1a
	3	-	Olda/Pontiac
	1	+	not done
	1	-	not done

+ , plasmids of 70 and 90 Mdal present.

in the demineralizing system. Culture of filter fragments and condensation water of air-conditioners in the Cardiac Surgery and the Neurosurgery wards did not yield legionella.

Legionellae were not isolated from the municipal water sampled at the nine entry points.

#### *Preliminary intervention measures*

The following prevention and control measures were adopted. In all wards, 1: cleaning, descaling and disinfection of oxygen bubble humidifiers, and replacement of many of them with new ones; 2: preparation of single-dose containers of sterile distilled water to fill the oxygen humidifiers; 3: adopting underwater chest drains that can be disassembled and sterilized.

Owing to technical difficulties, it was impossible to add chlorine to the hospital cold water system, so control measures were confined to raising the hot water temperature to 60 °C at the taps for 24 h each week.

*L. pneumophila* serogroup 1 was seldom isolated from tap water samples taken at weekly intervals the day before the temperature was raised. During 17 months of surveillance, 94 pneumonia cases came to autopsy. Three which occurred within the first 10 months of control measures were culture positive for *L. pneumophila*. No other cases have been diagnosed since.

#### DISCUSSION

This study shows that there were undiagnosed cases of legionellosis within the hospital, and enabled us to verify that *L. pneumophila* serogroup 1 was the cause of pneumonia in 24% of the 58 patients in whom pneumonia was detected post mortem. The patients had serious diseases and in some cases required a long period in hospital. Many had individual risk factors commonly associated with pneumonia caused by *L. pneumophila*.

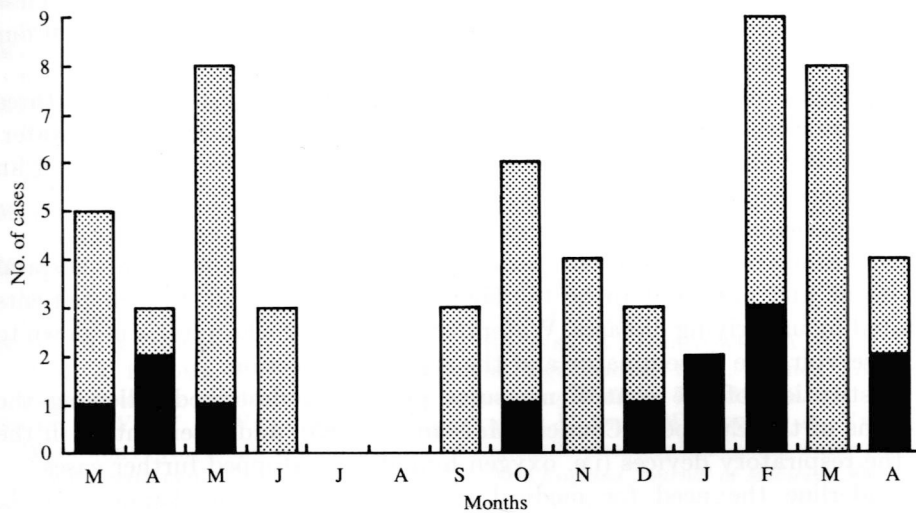


Fig. 2. Temporal distribution of the pneumonia cases occurred from March 1984 to April 1985. ▨, Negatives; ■, positives.

The cases detected probably represent the 'tip of the iceberg' of legionella infection in our hospital since a study on possible non-fatal cases was not performed.

All but one of the cases were located on one side of the hospital (Fig 1). The temporal distribution of the 58 cases is shown in Figure 2.

The organism appeared to be transmitted through the hospital environment by the hot and cold water, these being the only source of infection. *L. pneumophila* serogroup 1 was isolated from all but one of the water samples taken from wards where the cases occurred. Air conditioning did not seem to be responsible for the spread of infection; legionellae were not isolated from the two air-conditioning systems (Cardiac Surgery and Neurosurgery).

It is possible that legionellae were present in the city piped water system. Although none were isolated from the water samples taken at the nine take off points, the isolation of *L. pneumophila* from blowback water of the hospital demineralizing system suggests their presence in the municipal water at very low concentration. This hypothesis however is not completely confirmed by the results of subtyping by monoclonal antibodies (Table 2).

There are three possible ways in which patients could be infected: 1) by inhaling contaminated water droplets while having a bath or showering; 2) by inhaling aerosols created by water jets in hand basins; 3) by using other colonized devices. In the first instance the patients most likely to be exposed would be those in Cardiac Surgery, who had a bath or shower before surgery, and also the patient admitted in the Nephrology ward for a long period of time (Table 1, patients no. 1, 3, 5). However the health status of the other patients together with information from the hospital staff enabled us to rule out the possibility that patients might have had a shower or bath while in hospital.

The second possibility is linked to the position of the beds next to the sink. Microorganisms may spread in the environment through aerosols generated by

running water (Dandalides, Rutala & Sarubbi, 1984). Therefore, beds situated less than 2-5 metres from the sink may be reached by aerosols. In fact some of our patients were less than 25 m from the sink.

With reference to the third mode of infection it seems likely that at least three patients were infected by oxygen humidifiers filled with contaminated tap water. In one case the only possible source of infection appeared to be the use of an underwater chest drain (Moiraghi *et al.* 1987).

For two patients no possible source of infection was found.

In conclusion, in our study *L. pneumophila* was isolated from the hospital environment and appeared to be the significant cause of infection in patients debilitated by underlying diseases. Water distributed in the hospital was shown to be the reservoir, the disseminator and the source of infection.

The institution of the control measures previously described following the suggestions of the European Council (Maisonnet, 1985), and intervention in the use of the respiratory devices (i.e. oxygen humidifiers) stopped further cases.

We underline the need for medical staff to consider the diagnosis of *L. pneumophila* in cases of nosocomial pneumonia and to observe health regulations in the use and maintenance of hospital devices.

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