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LEGIONELLACEAE IN THE HOSPITAL WATER-SUPPLY

Epidemiological Link with Disease and Evaluation of a Method for Control of Nosocomial Legionnaires' Disease and Pittsburgh Pneumonia

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Summary An epidemiological link was found between contamination of a hospital water-supply by *Legionella pneumophila* and by Pittsburgh pneumonia agent (PPA) and subsequent cases of nosocomial legionnaires' disease and Pittsburgh pneumonia. The extent of *L pneumophila* isolation from the water-supply paralleled the occurrence of disease. Whenever *L pneumophila* was isolated from more than 30% of ten selected water sites, nosocomial legionellosis occurred. The temperature of the hot water tanks was raised to 60–77°C for 72 h, and water outlets were flushed for 30 min with hot water. A decline in numbers of *L pneumophila* and PPA in the water-supply was followed by a fall in the incidence of legionnaires' disease and Pittsburgh pneumonia. In addition, intermittent raising of the temperature in the hot water system decreased both the number of months in which disease occurred and the proportion of nosocomial pneumonias caused by these organisms.

Introduction

WE have established that *Legionella pneumophila* is ubiquitous in the potable water distribution system in a hospital with nosocomial legionnaires' disease.[1] Pittsburgh pneumonia agent (PPA), was also widely distributed within the hospital's water-supply.[2] Other workers[3–5] have likewise reported isolation of *L pneumophila* from potable water supplies but no epidemiological link has been established between organisms from this source and hospital-acquired legionnaires' disease and Pittsburgh pneumonia.

Several eradication measures have been instituted in an effort to eliminate *L pneumophila* from the water-supply,[3,4,6] but there has been no long-term study of effectiveness in

suppressing or eradicating *L pneumophila,* and, more importantly, no long-term study of efficacy in decreasing the incidence of legionnaires' disease. In this report, we present evidence of an epidemiological link between isolation of *L pneumophila* from potable water and nosocomial legionellosis. In addition, we describe an eradication method directed at *L pneumophila* and PPA in the water-supply, and assess its efficacy not only in decreasing the rate of isolation of these organisms from the hospital water-supply, but also in preventing legionnaires' disease and Pittsburgh pneumonia.

Materials and Methods

Sampling of Hospital Surveillance Sites

Ten hospital water sites, representing patient and non-patient areas as well as east and west hospital wings, were monitored monthly from April, 1981, to determine the extent, duration, and degree of Legionellaceae distribution. Twenty other sites were monitored eight times during this investigational period to determine the validity and reproducibility of the results obtained from the monthly survey sites. Cultures were taken from two cooling towers within 300 m of the hospital using guineapig inoculation[7] and selective media.

Specimen Collection and Preparation

After swabbing the water outlet with a sterile 'Dacron' swab, 100–200 ml water samples were collected and both were inoculated onto a selective differential dye-containing medium for isolation of Legionellaceae.[2] Water from one of two cold water storage tanks and one of four hot water storage tanks was also cultured monthly. One-litre specimens were taken from the bottom of each tank via drain pipes. These samples were plated directly onto the media (0·1 ml per plate by the spread plate technique). *L pneumophila* and PPA (*Tatlockia micdadei*[8] or *Legionella micdadei*[9]) identification was confirmed by slide agglutination[10] and direct fluorescent antibody testing.[11]

Intermittent Temperature Elevation Methods

Beginning in July, 1981, the temperature of the hot water storage tanks was raised on eight occasions to 60–77°C in an attempt to eradicate *L pneumophila* and PPA (table). The hot water was then allowed to return to the usual 45°C. We had previously established that *L pneumophila* and PPA were present most abundantly in sediment (scale).[12] Therefore, in conjunction with seven of the eight heating procedures, taps (faucets) and showers were flushed with a constant flow of hot water for 20–30 min (table). Patients and personnel were alerted by notices in the hospital daily bulletin and by signs at every water outlet: "Danger! Use with caution. Very hot water".

The free chlorine level of the city-supplied water is 0·1–0·5 parts

INTERMITTENT TEMPERATURE ELEVATION METHODS FROM JULY 1981 TO DECEMBER 1982

Dates	Hot water tank temperature (°C)	Duration of temperature elevation (h)	Environmental site positivity (%) for *L pneumophila*		
			Pre-heat	1 wk post-heat	1 mo post-heat
July 4–6, 1981	60	72	60	20	30
Nov 14–15, 1981*	60	48	30	10	40
Jan 22–24, 1982	70	72	40	0	10
March 28–30, 1982	77	72	50	0	20
May 8–10, 1982†	77	72	30	10	30
June 11–14, 1982	77	72	30	0	10
Sept 23–26, 1982	77	72	30	0	20
Dec 16–19, 1982	77	72	30	0	

*Temperature elevation maintained for 48 h only.
†Distal water sites not flushed.

per million. No further chlorination of the cold water system was instituted. The cooling towers were never treated.

Case Detection and Definition

The study period for case detection began in March, 1979, when specialised laboratory tests for isolation of *L pneumophila* were introduced into our diagnostic microbiology laboratory; all nosocomial pneumonias were evaluated for the possibility of legionnaires' disease, as previously described.[13] Special culture media and direct fluorescent antibody reagents used for isolation of PPA were not introduced until April, 1981; consequently, there is an underestimate of cases of PPA in our institution from March, 1979, to April, 1981. Cases of legionnaires' disease and Pittsburgh pneumonia were identified by standard criteria[13] including: (1) undiagnosed pneumonia with an antibody titre to *L pneumophila* of $\geqslant$1:128 (17 cases); (2) fourfold rise in *L pneumophila* (serogroups 1–6) and PPA antibody titre to $\geqslant$1:160 (enzyme-linked immunosorbent assay)[14] during the course of the pneumonia (38 cases); (3) greater than 25 organisms in respiratory secretions staining typically with fluorescent antibody (46 cases); or (4) isolation of the organism by culture (32 cases). All *L pneumophila* isolated were serogroup 1. (The total of cases is more than 100 because 33 patients fulfilled more than one of the above criteria.)

Statistical Analysis

Environmental and patient data were stored and analysed in a computer data bank (Prophet System, Division of Research Resources, National Institutes of Health). Probability values were

calculated by the Fisher exact test. Sensitivity, specificity, and predictive values were calculated as previously described.[15]

Results

During this eighteen month study period, the number of positive surveillance sites varied from 0 to 10 (100%). The concentration of *L pneumophila* cultured from the monthly surveillance sites ranged from 20 to 300 colony-forming units per plate. We were unable to establish a correlation between quantitative counts of *L pneumophila* and the frequency of isolation (as measured by site positivity) and number of Legionellaceae cases. This was not surprising since we had previously shown that the quantity of organisms was dependent upon the amount of sediment (scale) in the water fixture; the amount of sediment varies with use of the water fixture as well as the amount of sediment dislodged during the long-term surveillance process.

85% of the isolates were serogroup 1, and the remaining 15% were serogroup 5. PPA was isolated from six surveillance sites. Twenty other environmental sites were monitored on eight separate occasions to validate the percentage positivity obtained from the ten monthly surveillance sites. The percentage positivity in these additional sites paralleled that in the monthly sites. There was no isolation of *L pneumophila* or PPA from multiple samples of the two adjacent cooling towers.

All cases of nosocomial legionellosis from March, 1979, to December, 1982, inclusive, are detailed in the accompanying figure. There were 76 cases of legionnaires' disease (all serogroup 1), 17 cases of Pittsburgh pneumonia, and 7 cases of "dual infection" caused by both *L pneumophila* and PPA.[16] In the figure, the line graph depicts monthly surveillance site positivity and each testing period is depicted by the dark arrow. The eradication methodology is detailed in the table.

A substantial reduction in surveillance site positivity (as represented by the line graph) followed all but two of the heating procedures (November, 1981, and May, 1982). The two unsuccessful heatings differed from the six successful heatings (table): in November, 1981, duration was 48 h instead of 72 h; and in May, 1982, distal water sites were not flushed with hot water.

There was a direct correlation between site positivity for *L pneumophila* and incidence of nosocomial legionellosis. Whenever monthly site positivity exceeded 30%, cases of legionnaires' disease or Pittsburgh pneumonia invariably appeared in those months. Similarly, when positivity fell to

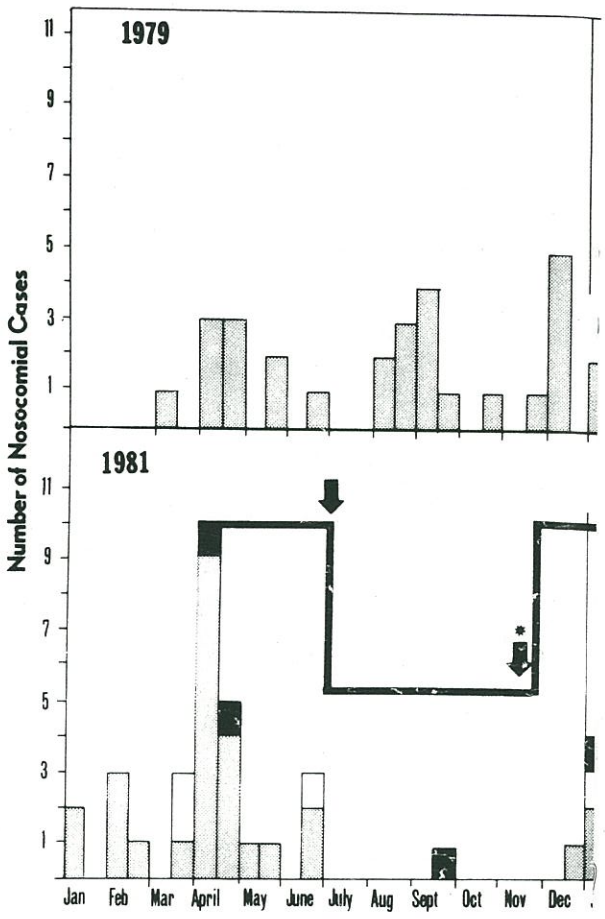
20% or less, no case of disease was observed. In our institution, site positivity of 30% seemed to be a "grey" area with respect to occurrence of disease; no cases were observed in July, August, October, and November of 1981, or in May and December, 1982, while there was one in September, 1981 and one in June, 1982. Cases also occurred during the months following the two unsuccessful heatings.

This correlation can be further analysed by determining the efficacy of using the monthly surveillance site positivity as a predictor for occurrence of nosocomial legionellosis. If ⩾30% isolation of *L pneumophila* from the monthly surveillance sites is designated as a "positive" test, then "true positive" is defined as a month with cases of legionellosis and "false positive" is a month with no cases of legionellosis when the site positivity for that month is ⩾30%. Correspondingly, when site positivity is <30%, "false negative" is defined as a month with cases and "true negative" represents a month without cases when the site positivity for that month is <30%.

According to these criteria, the sensitivity of ⩾30% positivity as a predictive test was 100% and the predictive value of a negative result (ie, site positivity <30%) was also 100%. The specificity is 54% (7 true negatives/[7 true negatives + 6 false positives]); if the months within the 30% grey area are discarded in this analysis, the specificity increases to 100%.

Cases of nosocomial legionellosis decreased overall once heating was initiated in July, 1981. This can be shown by comparison of the eighteen-month period when heating procedures were instituted (July, 1981, to December, 1982) with an equal period before initiation of heating procedures (January, 1980, to June, 1981). If a disease-free month is defined as a month in which no cases of legionellosis are observed, then it can be shown that the number of disease-free months in the eighteen-month preheat period (0 of 18) was significantly less than the number of disease-free months after initiation of heating procedures (12 of 18) ($p<0\cdot01$, Fisher's exact test). Furthermore, in the eighteen-month preheat period, the proportion of nosocomial pneumonias attributed to legionellosis was 31% (61 of 198 nosocomial pneumonias) *vs* 7% (12 of 178 nosocomial pneumonias) in the eighteen-month period after initiation of heating; this difference is also statistically significant ($p<0\cdot01$, Fisher's exact test).

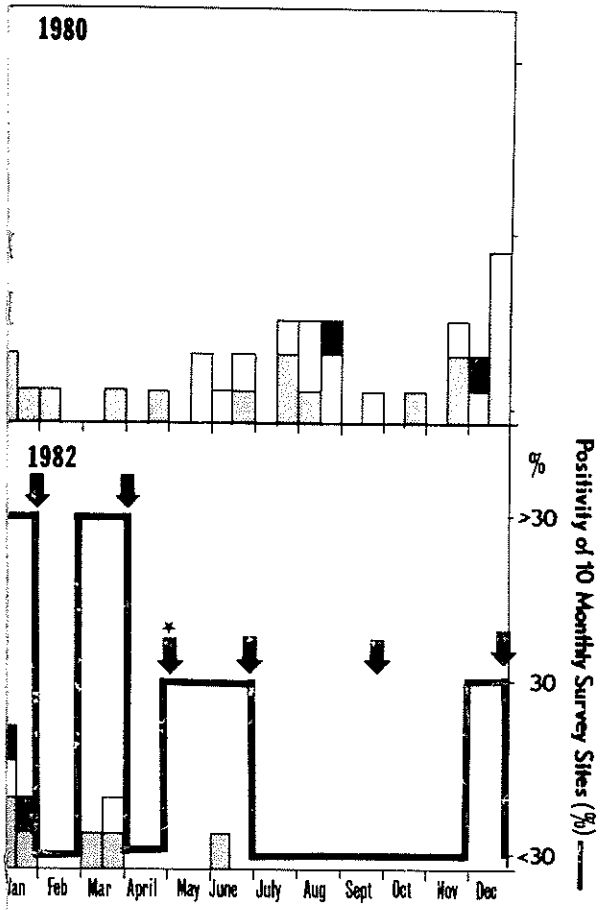
With the initiation of eradication measures in July, 1981, it seemed not only that cases of legionellosis had become infrequent, but also that the few patients who did become infected tended to have been in hospital for longer before onset of pneumonia, and these same patients tended to have



Correlation between number of nosocomial cases of legionellosis (hist measured by percentage of 10 monthly survey sites (line graph), a

For July, 1981, we have shown the percentage positivity as 30% (July 10) inst figure more accurately reflects the status of the water system for that month. □ =1 to heat treatments. Heating measures in November, 1981 (*) and May, 1982 (

more serious underlying disease than those who had been infected before July, 1981. This impression was confirmed in the following analysis. Cases of legionellosis arising before the eradication measures (88 cases) differed significantly ($p<0\cdot01$, Fisher's exact test) from cases arising later (12 cases) in number of days in hospital (mean 16 days *vs* 30 days), and



gram), contamination of the potable water supply by *L pneumophila* as d heating measures for eradication from 1979 to 1982 (arrows).
ead of 60% (July 1) because heating of the water-supply began on July 3. the 30% egionnaires' disease; [ ] = Pittsburgh pneumonia; ■ = dual infection. Arrows point) were considered suboptimal.

in the percentage deemed at risk because of renal failure, diabetes, collagen-vascular disease, or malignant disease (53% *vs* 100%). When the same analysis was restricted to cases occurring in equal 18-month periods before and after heating (January, 1980, to June, 1981 *vs* July, 1981, to December, 1982), the number of days in the hospital before

onset of pneumonia remained significantly greater in the post-heating period ($p>0\cdot05$, Mann-Whitney U test). No significant differences were found between the two groups for age, sex, surgical procedure, and laboratory abnormalities.

Discussion

Since March, 1979, we have diagnosed 100 cases of nosocomial legionnaires' disease and Pittsburgh pneumonia in our institution. We hypothesised that the potable water supply was the source of the organisms, particularly because clinical cases of legionnaires' disease were caused by the serogroup of *L pneumophila* predominant in the water supply. Thus, in July, 1981, we began efforts to eradicate these organisms from the supply. Since we had previously shown in vitro that Legionellaceae could easily be killed at temperatures greater than 60°C,[17] and since *L pneumophila* and PPA were concentrated in the hot water tanks,[12] we increased the temperature of the hot water tanks above 60°C. Because we had also found that sediment (scale) within the plumbing system and water fixtures harboured the organisms, we flushed all water outlets with the hot water.

To substantiate our contention that the hospital potable water system was, indeed, the source of legionnaires' disease in our institution, we invoked criteria defined in Koch's postulates and modified by Evans, and applied them to this epidemiological study. We believe that fulfilment of these criteria provides satisfactory confirmation of our hypothesis. The criteria listed by Evans[18] are as follows:

1. *The incidence of disease should increase in relation to the duration and intensity (dose) of the suspected factor.*—The figure shows that extent of *L pneumophila* isolation from the water-supply correlates with the incidence of disease. Specifically, whenever the site positivity rate exceeded 30%, cases of legionellosis always appeared. In April, 1981, when site positivity reached 100%, there was an outbreak of nosocomial legionnaires' disease. In two months (November, 1981, and May, 1982) when eradication measures were suboptimal, site positivity persisted or increased, and cases of legionnaires' disease subsequently occurred.

2. *The distribution of the suspected factor should parallel that of the disease in all relevant aspects.*—We previously demonstrated the ubiquity of *L pneumophila* in our hospital[1] by isolating the organism from water sites on every nursing unit and every floor in our hospital. Correspondingly, cases of legionellosis showed no space clustering and had been identified on every nursing unit in the hospital.

3. *A spectrum of illness should be related to exposure to the suspected factor.*—With the institution of heating in July, 1981, it is clear from the figure that overall patient exposure to *L pneumophila* in the

water-supply decreased, as survey site positivity decreased. It is thus noteworthy that the clinical characteristics of patients who acquired legionellosis after July, 1981, when exposure was curtailed, differed considerably from those of patients who acquired the disease before the eradication measures. It appears that, once the epidemiological reservoir is suppressed, the disease is likely to infect only patients with prolonged stays in hospital and severe underlying disease.

4. *Reduction or removal of the factor should reduce or stop the disease.*—As the figure shows, eradication measures effective in curtailing Legionellaceae in the hospital water supply also reduced the incidence of nosocomial disease due to those organisms. The number of months when cases of legionellosis were observed and the proportion of nosocomial pneumonias caused by *L pneumophila* and PPA decreased significantly with reduction of *L pneumophila* and PPA from the water supply.

5. *Human populations exposed to the factor in controlled studies should acquire the disease more commonly than those not exposed.*—While we have confirmed this phenomenon within our hospital, fulfilment of this postulate in the general population is beyond the scope of this investigation. In addition, we have yet to identify a hospital whose hot water system is not contaminated with *L pneumophila*. We have conducted a prospective pneumonia study at another hospital with *L pneumophila* in its water-supply. Hitherto, this hospital had never diagnosed a case of legionnaires' disease. When specialised laboratory methods were introduced for isolation of *L pneumophila* 14% of the nosocomial pneumonias seen in the course of three months proved to be caused by *L pneumophila* of the serogroup predominant in the water-supply.[19]

Our schedule for control of *L pneumophila* and PPA entails intermittent boosting of the hot water tank temperature to 60–77°C (140–171°F) with two 30 min flushes (24 h apart) in patient areas. After 72 hours, the water returns to less than 54°C—the maximum specified by the Joint Commission on Accreditation of Hospitals. The frequency of heating can be adjusted according to results of surveillance cultures.

Advantages of our method include the fact that no chemicals or equipment are necessary and that it can be implemented immediately. The posting of signs and alerting of personnel and patients seem to be sufficient to prevent accidental scalding or burns; during our 18-month evaluation no such incidents have occurred.

This is the first report in which measures to reduce *L pneumophila* and PPA have been linked statistically with a reduction in the incidence of clinical legionnaires' disease and Pittsburgh pneumonia. In view of the frequency of nosocomial legionellosis[20,21] as well as a flurry of recent reports which imply an association between *L pneumophila* isolation in the water-supply and legionnaires' disease,[19,22,23] our results should be applicable well beyond this institution.

Before this report, there was uncertainty about the wisdom of performing environmental cultures for *L pneumophila* and the efficacy of eradication measures if the organism was isolated from the water supply. This report addresses both of these issues. Since the link between isolation of the organisms and occurrence of disease has finally been established, we conclude that, in any hospital with cases of legionnaires' disease or Pittsburgh pneumonia, cultures must be taken from the water distribution system. Our eradication method, which has proven efficacious and safe, now needs to be compared with other potentially useful methods including chlorination, ozonation, and ultraviolet irradiation.

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