Isolation of *Legionella pneumophila* from the Cold Water of Hospital Ice Machines: Implications for Origin and Transmission of the Organism

Janet E. Stout, MS; Victor L. Yu, MD; Paul Muraca, MS

ABSTRACT

Although the mode of transmission of *L. pneumophila* is as yet unclear, the hot water distribution system has been shown to be the reservoir for Legionella within the hospital environment. In this report we identify a previously unrecognized reservoir for *L. pneumophila* within the hospital environment, ie, the cold water dispensers of hospital ice machines. The cold water dispensers of 14 ice machines were cultured monthly over a 1-year period. Positive cultures were obtained from 8 of 14 dispensers, yielding from 1 to 300 CFU/plate. We were able to link the positivity of these cold water sites to the incoming cold water supply by recovering *L. pneumophila* from the cold water storage tank, which is directly supplied by the incoming municipal water line. This was accomplished by a novel enrichment experiment designed to duplicate the conditions (temperature, sediment, stagnation, and continuous seeding) of the hot water system. Our data indicate that significant contamination of cold water outlets with *L. pneumophila* can occur. Although no epidemiologic link to disease was made, the fact that the primary source of a patient's drinking water is from the ice machines warrants further investigation of these water sources as possible reservoirs. [Infect Control 1985; 6(4):141-146.]

From the Infectious Disease Section, Veterans Administration Medical Center, Pittsburgh, Pennsylvania.

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Address reprint requests to Victor L. Yu, MD, Infectious Disease Section, VA Medical Center, University Drive C, Pittsburgh, PA 15240.

INTRODUCTION

Legionnaires' disease is now known to be a relatively common cause of nosocomial pneumonia, comprising as much as 10% to 20% of hospital-acquired pneumonias.[1-3] In a recent report we definitively established that the epidemiologic reservoir for hospital-acquired Legionnaires' disease was the hospital hot water distribution system.[4] Despite progress in the understanding of the pathogenesis of hospital-acquired Legionnaires' disease, several epidemiologic issues remain unresolved. Although it has been established that *L. pneumophila* propagates and disseminates within and throughout the hot water system,[5-8] the ultimate source of the organism is uncertain. Are plumbing systems contaminated during construction, or are they seeded with low numbers of the organism from the municipal water supply? It is also unclear how the organism is transmitted from contaminated sources to the susceptible patient. Currently, airborne transmission is the most commonly accepted theory.[9] Introduction of the organism via invasive respiratory tract procedures[10] and aspiration[11] may be alternative modes of transmission. In this report we identify a previously unrecognized reservoir for *L. pneumophila* within the hospital environment (cold water from ice machines) which may have implications regarding the aspiration hypothesis. We also provide some evidence that the incoming cold water supply is, indeed, the ultimate source for the introduction of *L. pneumophila* into hot water distribution systems.

MATERIALS AND METHODS

Specimen Collection and Processing

Samples for culture were processed as previously described.[12] Briefly, swabs were inoculated down the center of the plate and perpendicularly streaked for isolation. Water samples (0.1 ml) were inoculated by the spread plate technique. Samples were inoculated onto a selective

Figure 1. Schematic of an ice machine depicting the flow of cold water through the machine. *L. pneumophila* was isolated (+) from the waterline above the condenser/compressor, the water dispenser, and the reservoir. All other cultures were negative (−). The positivity in the line was in close proximity to the heat-generating portion of the ice machine.

Figure 2. Successful recovery of *L. pneumophila* from the incoming cold water supply was accomplished by using a novel experimental design involving 2 identical copper coils which uniquely simulated the conditions which favor the growth of *L. pneumophila* in hot water systems, ie, temperatures of 30° to 40°C, scale and sediment accumulation, presence of commensal bacteria and stagnation. Coil 1 was not heated, whereas, coil 2 was wrapped with a heat tape which heated the water within the coil to 30° to 40°C. Water from the cold water storage tank flowed through both coils at the same rate, approximately 500 ml/hour. Water from each coil was sampled from the faucet (C). Points A and B in the figure are union joints from which samples were collected by separating the joint and inserting a swab into the coil. *L. pneumophila* was isolated only from coil 2.

differential agar medium (DGVP) for the isolation of *Legionellaceae*. The medium is buffered yeast extract agar to which 0.001% bromocresol-purple, 0.001% bromothymol-blue, 0.3% glycine, 1 μg/ml vancomycin, 50 units/ml polymyxin B, and 1.5 g/l charcoal are added.[12] This medium can be obtained from Gibco (Madison, WI) or Remel (Lenexa, KS). Samples which were collected from the copper coil experiment were plated on buffered charcoal-yeast extract agar (BCYE) which has been previously described.[13] Definitive identification was performed by slide agglutination with antisera to *L. pneumophila*, serogroups 1-6.

Sampling of Ice Machines

Monthly surveillance: the cold water dispensers of 14 hospital ice machines were cultured monthly between January 1982 and January 1983 (excluding February, March, and April). A rayon swab was inserted into the opening of each water dispenser and rotated several turns. Ice machine manufacturers included Market Forge (Ferno-Forge, Wilmington, MA), Crystal Tips (Crystal Tips—McQuay, Inc., Minneapolis, MN), York (B.W. Central Systems, York, PA), and DSI (DSI, Easton, PA).

Culture of the Internal Parts of Contaminated Ice Machines

Two machines (Market Forge, 6 West, and Crystal Tips, 8 West) were selected for intensive culture of internal parts based on positive culture results obtained from both water dispensers. Swabs and water samples were obtained from the incoming water line, the piping above the compressor, the water valve, the water reservoir, the pump, cooling unit, and ice bin (Figure 1).

Dye-Tracer Study of the Hospital Water Supply

A non-toxic, biodegradable fluorescent dye (PYLATEL fluorescent yellow, PYLAM Products Co., Garden City, NY) was used to examine the possibility of cross connections between the hot and cold water distribution systems. The dye is not visibly detectable at concentrations below 5 ppm. However, the dye fluoresces yellow when solutions of water containing concentrations of 0.5 to 5 ppm are exposed to ultraviolet light. The amount of the dye to be added to the hot water storage tank was calculated to achieve approximately 2.5 to 5-0 ppm in the circulating hot water. The dye was allowed to circulate within the system for 3 hours before hot and cold water samples were collected from 5 ice machines and 30 other selected sites. Water was allowed to flow from the cold water inlet of the machines for 10 minutes. This would allow standing water within the pipes leading to the machine to be cleared. Water samples were collected, and the presence of yellow fluorescence was recorded. The dye which remained in the recirculating hot water system was removed 24 hours later.

Recovery of *L. pneumophila* from the Incoming Water Supply

Over a 3-year period, we had failed to recover *L. pneumophila* from the cold water storage tank by direct culture,

TABLE 1
MONTHLY SURVEILLANCE OF ICE MACHINE WATER DISPENSERS* FOR *L. PNEUMOPHILA*

	Recovery of *L. pneumophila* (CFU/plate)/Month									
Location	**J†**	**M**	**J**	**J**	**A**	**S**	**O**	**N**	**D**	**J**
11 North	0	10	1	7	28	>300	>300	>300	12	4
9 East‡	>300	150	3	0	>300	185	0	95	>300	22
8 West	0	>300	0	0	0	0	0	0	0	200
6 North	0	>300	12	31	0	3	33	4	27	287
6 East	0	>300	122	0	0	0	0	0	0	70
6 West	0	10	0	0	0	0	0	0	0	1
4 North	0	1	0	21	0	0	0	0	0	0
4 West‡	>300	0	0	0	0	0	0	0	0	0

**Cultures obtained in January, May through December 1982, and January 1983. Cultures were not obtained for February, March, and April 1982.*
†Fourteen ice machine water dispensers were cultured the first week of each month using a sterile swab. The water dispensers of 6 machines were consistently culture-negative and were therefore omitted from the Table.
‡Decontamination procedures were instituted in March, May, June, and September 1982, for ice machines on 9 East and 4 West.

continuous centrifugation, and concentration by filtration. A new experimental design was implemented based on an understanding of those factors which favor the growth of *L. pneumophila*, ie, optimal growth temperature (35° to 37° C), stagnant water, accumulated sediment deposits, and a continuing source of the organism itself.[14]

A 1/2 inch copper pipe was connected to the drain pipe of a 20,000-gallon cold water storage tank. Water for this tank is supplied directly from the incoming city water main. Two 6-foot lengths of ⅝ inch flexible copper pipe were used to form a series of 5 coils, approximately 6 inches in diameter (Figure 2). The coils served to increase the surface area and maximize sediment (scale) accumulation. One coil was wrapped with electric heat tape (Thermwell Products Co., Inc., Patterson, NJ) for thermal enrichment, and the other coil was left unwrapped as a non-heated control. The heat tape was connected to a variable autotransformer which maintained the water within the coil at a temperature of 30° to 40°C (the optimal growth temperature for *L. pneumophila* is 35° to 37°C). The temperature of the water from the non-heated coil was 10° to 15°C. Water was allowed to drip from the brass faucets at a rate of 500 ml/hour; this allowed for continual seeding of both coils by microorganisms from the incoming water supply (presumably with *L. pneumophila* as well). Water was allowed to flow through the coils at a very slow rate for 2 weeks at a time. The coils were then allowed to fill with water and remain stagnant for an additional 10 days. Samples were obtained from both coils by swabbing the faucet, the mid-coil union, and the union connecting the coil to the ½ inch copper pipe. Water samples taken from each coil were plated onto buffered charcoal yeast extract agar and DGVP, both directly and after concentration by centrifugation at 5000 rpm.

RESULTS

Sampling of Ice Machines

Monthly surveillance: *L. pneumophila* serogroup 1, was isolated from 8 of 14 ice machine water dispensers during the study period (Table 1). The concentration of *L. pneumophila* ranged from 1 to >300 CFU/plate. The cold water dispensers of ice machines on 11 North, 9 East, and 6 North were consistently culture-positive, while other water dispensers demonstrated sporadic positivity. Comparison of the number of positive cultures from a given water dispenser with the characteristics of the ice machine did not demonstrate any obvious correlation (Table 2).

Culture of the Internal Parts of Contaminated Ice Machines

L. pneumophila was not recovered from the internal parts of the ice machine on 8 West (Crystal Tips). However, the cold water line located above the compressor and the cold water reservoir of the Market Forge on 6 West were culture-positive for *L. pneumophila*, serogroups 1, 10 and 8 CFU/plate, respectively (Figure 1).

Dye-Tracer Study of the Hospital Water Supply

Water obtained from the hot water tank and other hot water sites demonstrated bright yellow fluorescence under ultraviolet light. The water collected from the cold water lines leading to the 5 ice machines and all other cold water samples were negative for fluorescence.

Decontamination of Ice Machine Water Dispensers

The January 1982, culture results of ice machine water dispensers identified machines on 9 East and 4 West to be positive for *L. pneumophila* (Table 1). In conjunction with a hospital-wide eradication protocol, these dispensers were flushed with 170°F water for 30 minutes.[15] Since ice

TABLE 2
ICE MACHINE CHARACTERISTICS SHOW NO CORRELATION WITH ISOLATION OF *L. PNEUMOPHILA*

Location	Model	No. of Positive Cultures*	Position of Water Line	Pressure Regulator†	Insulated Water Lines	Type of Water Dispenser
11 North	Crystal Tips	9	floor	+	+	lever
9 East	Market Forge	8	wall	–	–	lever
6 North	York	8	floor	+	+	lever
6 East	Market Forge	3	wall	–	–	faucet
6 West	Market Forge	2	wall	–	–	faucet
8 West	Crystal Tips	2	wall	+	+	button
4 North	York	2	floor	+	+	lever
4 West	Market Forge	1	wall	–	–	lever
8 East	Crystal Tips	0	wall	+	+	button
7 East	Crystal Tips	0	wall	+	+	button
7 West	Crystal Tips	0	wall	+	+	button
5 East	Market Forge	0	wall	–	–	faucet
3 North	DSI	0	wall	+	–	lever
2 North	Crystal Tips	0	floor	+	–	lever

**The water dispenser of each machine was cultured monthly for 10 months. Numbers represent total months for which cultures were positive for* L. pneumophila.
†(+, –) Indicates presence or absence of a pressure regulator or insulated water lines.

machines have no hot water line, we attached a hose from the machine water line to an adjacent sink. This procedure was performed on March 3, 1982; May 8, 1982; June 14, 1982; and September 24, 1982. The dispenser on 4 West became culture-negative in March and subsequently remained negative for the remainder of the study. The dispenser on 9 East, however, repeatedly recolonized with *L. pneumophila* (Table 1). Subsequently, the cold water dispensers were removed from all hospital ice machines.

Recovery of *L. pneumophila* from the Incoming Cold Water Supply

Samples from the copper coils were taken from January 1982 to March 1984. After 1 month of operation, all water obtained from the heated coil demonstrated a greater concentration of bacterial flora than the non-heated coil, often >300 CFU/0.1 ml compared to <10 CFU/0.1 ml. The water obtained from the heated coil also became visibly turbid with a rusty appearance after 1 month. This was in contrast to the non-heated coil in which the water remained generally clear during the entire 15-month study period. The bacteria recovered from the heated coil were identical to those previously recovered from the hot water storage tanks.[14] All cultures were negative for *L. pneumophila* up to January 1984. Two months later, *L. pneumophila,* serogroup 1, was isolated from the water collected from the heated coil, at a concentration of 10 CFU/ml on direct plating to buffered charcoal yeast extract agar. *L. pneumophila* was never isolated from the non-heated coil.

DISCUSSION

The currently accepted theory regarding the mode of transmission of *L. pneumophila* from contaminated water sites is aerosolization, although we have presented circumstantial evidence suggesting that aspiration may be a

which favored the establishment of a commensal bacterial population. We ultimately isolated *L. pneumophila*, serogroup 1, from the heated copper coil, the first isolation of *L. pneumophila* from the cold water storage tank in 3 years! No *L. pneumophila* was isolated from the unheated control coil. The positivity of the main cold water supply now rendered the positivity of the ice machine water dispensers interpretable. Cold water sites could be continually seeded by low numbers of the organism via the incoming cold water supply.

Thus, *L. pneumophila* from the incoming cold water supply could contaminate hospital ice machines should a favorable environment for growth be established. The heat generated by the condenser/compressor housed in the ice machine could provide favorable growth temperatures for *L. pneumophila*. In fact, the culture of one contaminated ice machine demonstrated that the cold water line just above the compressor, as well as the water reservoir could be sources for the organism (Figure 1).

In summary, this study sheds light on the source of *L. pneumophila* contamination in water distribution systems and has implications for its mode of transmission. We have established that cold water sites as well as hot water sites yield high concentrations of *L. pneumophila*. (We have also isolated *L. pneumophila* from drinking fountains in our hospital and from ice machines of 3 other hospitals.) The ice machine site may be relevant because it is the primary source of drinking water for patients. The ultimate source of *L. pneumophila* within the hospital water system is the incoming cold water from the municipal water supply. Cold water transports low numbers of the organism to the hot water recirculating system (or other niches favorable to growth) where they proliferate.

The above information will be pertinent to the design of eradication measures for decontamination of the water distribution systems as well as infection control measures used to protect susceptible patients from water sources contaminated with *L. pneumophila*. Eradication measures must take into account the continuous re-seeding of the water system from the incoming cold water supply, as well as the concentration of the organism at distal hot *and* cold water fixtures.

REFERENCES

1. Muder RR, Yu VL, McClure J, et al: Nosocomial Legionnaires' disease uncovered in a prospective pneumonia study: Implications for underdiagnosis. *JAMA* 1983; 249:3184-3192.
2. Yu VL, Kroboth FJ, Shonnard J, et al: Legionnaires' disease: New clinical perspective from a prospective pneumonia study. *Am J Med* 1982; 73:357-361.
3. Johnson J, Yu VL, Best M, et al: Nosocomial Legionella pneumonia in a population of head and neck cancer patients. *Laryngoscope* 1985, to be published.
4. Best M, Yu VL, Stout J, et al: Legionellaceae in the hospital water supply — Epidemiologic link with disease and evaluation of a method for control of nosocomial Legionnaires' disease and Pittsburgh pneumonia. *Lancet* 1983; 2:307-310.
5. Wadowsky R, Yee R, Mezmar L, et al: Hot water systems as sources of *Legionella pneumophila* in hospital and non-hospital plumbing fixtures. *Appl Environ Microbiol* 1982; 43:1104-1110.
6. Stout J, Yu VL, Vickers R, et al: The ubiquitousness of *L. pneumophila* in the water supply of a hospital with endemic Legionnaires' disease. *N Engl J Med* 1982; 306:466-468.
7. Plouffe JF, Webster LR, Hackman B: Relationship between colonization of hospital buildings with *Legionella pneumophila* and hot water temperatures. *Appl Environ Microbiol* 1983; 46:769-770.
8. Dennis PJ, Taylor JA, Fitzgeorge R, et al: *Legionella pneumophila* in water plumbing systems. *Lancet* 1982; 1:949-951.
9. Dondero TM, Rendtorff RC, Mallison GF, et al: An outbreak of Legionnaires' disease associated with a contaminated air conditioning cooling tower. *N Engl J Med* 1980; 302:365-370.
10. Arnow P, Chou T, Weil D, et al: Nosocomial Legionnaires' disease caused by aerosolized tap water from respiratory devices. *J Infect Dis* 1982; 146:460-467.
11. Yu VL, Stout J, Zuravleff JJ: Aspiration of contaminated water may be a mode of transmission for *Legionella pneumophila*. Interscience Conference on Antimicrobial Agents and Chemotherapy 1981; Abstract 297, Chicago.
12. Stout J, Yu VL, Vickers RM, et al: Potable water supply as the hospital reservoir for Pittsburgh pneumonia agent. *Lancet* 1982; 1:471-472.
13. Edelstein PH: Improved semiselective medium for the isolation of *Legionella pneumophila* from contaminated clinical and environmental specimens. *J Clin Microbiol* 1981; 14(3):298-303.
14. Stout J, Yu VL, Best M: The ecology of *L. pneumophila* in water distribution systems. *Appl Environ Microbiol* 1985; 49:221-228.
15. Best MG, Goetz A, Yu VL: Heat eradication measures for control of nosocomial Legionnaires' disease: Implementation, education, and cost analysis. *Am J Infect Control* 1984; 12:26-30.

Note: Hsu and colleagues isolated Legionella *organisms from 5 of 856 water samples taken from municipal water supplies. Hsu S, Martin R, Wentworth B: Isolation of* Legionella *species from drinking water.* Appl Environ Microbiol *1984; 48:830-832.*