Bacterial Fouling of a Hospital Closed-Loop Cooling System by *Pseudomonas* sp.

GARY C. DU MOULIN,* GREGORY O. DOYLE, JOHN MacKAY, AND JOHN HEDLEY-WHYTE

Department of Anaesthesia, Harvard Medical School, Charles A. Dana Research Foundation, Boston, Massachusetts 02215

Received 3 February 1981/Accepted 16 March 1981

During the summer of 1979 the air-conditioning system at a hospital in Boston deteriorated, and this led to total failure of some chilling units. Patient care and operating-room areas were affected. Investigation of the problem ruled out mechanical and electrical causes, but revealed a strain of *Pseudomonas* sp. biofouling heat transfer tubes of the closed chilled water system. The pseudomonads apparently were stimulated to grow by low concentrations of ethylene glycol antifreeze. The proximate source of these organisms was an expansion tank located in a 33°C environment. The organisms probably originated from the potable water supply of the hospital. Fouling was eventually cleaned by prolonged and expensive treatments of the closed chilled water system. *Pseudomonas* sp. is frequently isolated from hospital-acquired infections at our institution (Beth Israel Hospital, Boston, Mass.); however, our studies with fluorescent dye tracers indicated that organisms were prohibited from entering patient areas via contaminated water from the chillers. Microbiologists must become cognizant of seemingly unimportant microbial environments within hospitals that may indirectly contribute to hospital-acquired infections.

The piping configurations of central air-conditioning systems usually are either "open" or "closed." Open cooling systems require evaporative condensers and cooling towers which are necessarily exposed to both air and aquatic environments. Open systems are more susceptible to microbial contamination and have been implicated in recent outbreaks of *Legionella pneumophila* disease (3, 11, 12). In closed systems the only environmental input is the domestic water that supplies a system of piping interposed with Freon-charged chillers. Closed piping system contamination is rare. This report concerns an occurrence of closed-circulation bacterial fouling in a hospital air-conditioning system by a waterborne strain of *Pseudomonas* sp.

(This study was presented in part at the 80th Annual Meeting of the American Society for Microbiology, Miami Beach, Fla. [G. C. du Moulin, Abstr. Annu. Meet. Am. Soc. Microbiol. 1980, N94, p. 179].)

MATERIALS AND METHODS

Events leading to chiller failure. The closed chilled-water system of our 450-bed hospital (Beth Israel Hospital, Boston, Mass.) is a two-pipe system. Chilled-water supply and chilled-water return of approximately 15,000 gallons (ca. 57 kl) form a continuous loop through approximately 80% of the hospital (Fig. 1). The remaining areas are cooled by smaller,

localized units. Water within the system passes through cooling coils in room and central air-conditioning units. Cooling needs are determined by thermostats that monitor the supply air discharge temperatures. The heart of the system is the chiller room, which contains all the mechanical equipment needed to produce and pump the chilled water. Three new electrically driven centrifugal chillers (Carrier, Inc., Syracuse, N.Y.) and one older steam absorption unit (Trane, Inc., LaCrosse, Wis.) are in the system. When activated, each chiller takes water by a primary pump from the common return line, cools the water by vaporization of dichlorofluoromethane (Freon 12; Du Pont Co., Wilmington, Del.), and returns the water to a common supply line. A secondary pump distributes chilled water through piping to the hospital. Each chiller receives water at 16 to 24°C (60 to 75°F), extracts heat, and discharges cooled water at 6 to 8°C (43 to 46°F).

The three new centrifugal chillers were routinely checked and tested in April before use in May. During the nonoperational and secured winter months, water in the system was drained and replaced with aqueous ethylene glycol (1:1 [vol/vol], Zerex; Du Pont), followed by draining again. Residual water in the coils was thus protected from freezing. One 600-ton chiller (1 ton = 12,000 British thermal units) was activated in May. In June the cooling efficiency of the unit progressively decreased. Typical mechanical and electrical sources were ruled out. The unit stopped cooling, and at this time we investigated the evaporator tubes.

Water samples were bled from a single point, col-

FIG. 1. Schematic of closed-circulation chilled-water system. Quantitative cultures were obtained from a single bleed-off point in the chilled-water supply line.

lected daily into sterile flasks, and processed immediately. During heavy contamination, 1-ml samples were diluted 1:100 and 1:1,000 in sterile water and filtered through 0.45- μ m cellulose ester filters (Sybron Corp., Rochester, N.Y.). During periods of little or no contamination, water was filtered directly, without dilution, in 100-ml portions. In all cases filtration was followed by sterile water rinses of 100 ml to prevent water treatment chemicals from contaminating the agar media. Filters were aseptically removed and placed on plates containing tryptic soy agar with 5% sheep blood cells (TSAB) (Scott Laboratories, Fiskeville, R.I.). Sterile cotton-tipped swabs were applied to areas for qualitative culture, and organisms were rolled onto a TSAB plate and streaked in a standard way. All plates were incubated at 35°C for 18 h and subsequently held at 22°C for 72 h with daily examination for growth. Plates containing between 30 and 300 organisms on filter membranes were enumerated and converted to log₁₀ colony-forming units (CFU) per 100 ml of water.

Representative colonies were isolated in pure culture and identified as to species with the aid of the API 20E system (19) (Analytab Products, Plainview, N.Y.) and other appropriate biochemical tests (see Table 1). Antibiotic susceptibility tests were carried out by the U.S. Food and Drug Administration modification of the Kirby-Bauer disk diffusion assay (2).

In vitro studies. Susceptibilities of the bacterial strain to the commercial biocides applied to the chilled-water system were tested, as was the effect of ethylene glycol in promoting growth. One-milliliter amounts of log-phase organisms grown overnight in dextrose-phosphate broth (approximately 10⁷ organisms per ml) were added to a series of tubes containing either *n*-alkyldimethylbenzylammonium chloride or *n*-alkyl-1,3-propanediamine biocides diluted from 10 to 100 μ g/ml in 10- μ g/ml gradations in sterile distilled water. Organisms were incubated in contact with the biocides for 24 h at ambient temperature (25°C). After incubation, 1-ml portions were passed through 0.45- μ m cellulose ester filters and rinsed with 100 ml of sterile distilled water. Filters were applied to TSAB and incubated overnight at 35°C. CFU were counted to determine minimum killing concentrations of the biocides.

Growth encouraged by ethylene glycol was tested in the laboratory by simulating those conditions existing in the closed system during the period of fouling and incorporating ethylene glycol as a sole carbon source. Concentrated ethylene glycol was diluted in sterile tap water to 1, 5, 10, 25, 50, and 75% (vol/vol). Undiluted ethylene glycol and sterile tap water were also included in the growth-supporting experiments. At time zero, 8.7×10^5 CFU of the fouling organism per ml was seeded into flasks containing each concen-

tration and incubated at 33°C (temperature of the expansion tank) with vigorous aeration in a shaking water bath. 0.1-ml samples were withdrawn periodically up to 170 h, grown on TSAB, and enumerated.

Backflow preventer studies. To test the integrity of the backflow prevention system, a modification of a fluorescein tracer technique was used (13). Fluorescein disodium salt (Eastman Kodak Co., Rochester, N.Y.) was added on two occasions to the expansion tank, in concentrations of 0.0015 and 0.010 mg/liter, respectively. Samples of water were taken from the expansion tank and the upstream side of the backflow preventer, a device designed to prevent the contamination of potable water sources (1). Samples were filtered to remove extraneous sources of fluorescence (0.45- μ m; Sybron Corp., Rochester, N.Y.). Fluorescence was determined by spectrophotofluorometry (Aminco-Bowman spectrofluorometer, American Instrument Co., Inc., Silver Spring, Md.) at an excitation wavelength of 484 nm and an emission wavelength of 518 nm.

RESULTS

A brown, odorous sludge coated the inside surfaces of the evaporator tubing. This material was removed from each evaporator tube with a scrub brush device, and the machine was reassembled and started. Efficiencies improved to near-normal ranges. However, during the next 2 weeks, cooling efficiency decreased again, and the circulating water from the chilled system was brown, odorous, and turbid. Wet preparations of sediment from centrifuged water were observed by microscopy and revealed large numbers of motile bacteria. No fungi or algae were seen. The bacterium was identified and chemical

treatment of the water was instituted.

Figure 2 depicts the percent loss of cooling efficiency with the quantitation in $\log_{10}$ CFU per 100 ml of water from the closed-loop system during the period of fouling. Initially, a single bacterial species was isolated from circulating chilled water and was identified as a pseudomonad by colony morphology and biochemical reactions (Table 1). Although some biochemical assays deviated from the established schema, this species most closely resembled *Pseudomonas fluorescens* (7, 20). Reactions which were found to deviate from classical descriptions of *P. fluorescens* included an ability to grow at 42°C and an inability to grow on cetrimide agar. The API 20E system, by its nine-digit code, classified the strain as "fluorescent *Pseudomonas* group" (API no. 2202004-43).

The strain was found to be susceptible to carbenicillin (100 μ g), gentamicin (10 μ g), polymyxin (300 U), tobramycin (10 μ g), and amikacin (30 μ g) and resistant to ampicillin (10 μ g), chloramphenicol (30 μ g), kanamycin (30 μ g), and penicillin (10 μ g). This pattern of susceptibility was commonly found among clinical isolates. Two other species in smaller numbers were transiently isolated from water during the period of investigation, *Pseudomonas aeruginosa* and *Achromobacter xyloxidans*. CFU decreased as a result of periodic applications of microbicidal chemicals, principally *n*-alkyldimethylbenzylammonium chloride, a quaternary ammonium compound (approximate concentration, 100 μ g/ml), and *n*-alkyl-1,3-propanediamine (approximate

FIG. 2. Percent loss of cooling plotted against the recovery of $\log_{10}$ CFU per 100 ml of water from the closed-loop system (1 ton of air conditioning = 12,000 British thermal units). Symbols: ●, percent loss of cooling; ○, $\log_{10}$ CFU per 100 ml of water; ■, *n*-alkyl-1,3-propanediamine (~100 μ g/ml) added; ▲, *n*-alkyldimethylbenzylammonium chloride (~100 μ g/ml) added; *, maximum air-conditioning load attempted.

TABLE 1. *Biochemical reactions of Pseudomonas sp.*

Test	Reaction
Triple sugar iron	K/K ^a
Glucose oxidation	+
Glucose fermentation	—
Growth on cetrimide agar	—
Growth at 42°C	+
Motility	+
Pyocyanin	—
Fluorescent pigments	+
Nitrate reduction	—
N ₂ production	—
MacConkey agar	+
Oxidase	+
Inositol oxidation	—
Melibiose oxidation	—
L-Arabinose oxidation	—
β -D-Galactosidase	+
Arginine dihydrolase	+
Lysine decarboxylase	—
Ornithine decarboxylase	—
Citrate utilization	+
H ₂ S production	—
Urease production	—
Tryptophan deaminase	—
Indole	—
Voges-Proskauer	—
Gelatin liquefaction	—
Mannitol oxidation	—
Sorbitol oxidation	—
Amygdalin oxidation	—

^a K/K, Alkaline/alkaline.

mate concentration, 100 μ g/ml) (Fig. 2). Gradual purging of the contaminated chilled water and replacement with untreated fresh water from an expansion tank appeared to improve the efficiency of the air-conditioning system. However, samples later taken from the expansion tank showed contamination with *Pseudomonas* sp. The temperature of the water in this tank was consistently 33°C. The sources of heat were the large motors in the small area where the expansion tank was located. After this tank was subjected to chemical decontamination, the number of circulating organisms quickly dropped. The efficiency of the air-conditioning system began to improve with the physical removal of the bacterial slime mass from the evaporator tubes, a gradual purging of the contaminated water, and chemical decontamination.

Water specimens later obtained in a hospital water main as well as a bubbler in a remote area of the hospital grew *Pseudomonas* sp. with biochemical characteristics identical to those of the fouling strain. It was present in tap water at concentrations of 7,200 CFU/100 ml in the water main and 200 CFU/100 ml in the specimen drawn from the bubbler.

In vitro studies: susceptibility to biocides. The *Pseudomonas* sp. was tested for susceptibility to various concentrations of *n*-alkyldimethylbenzylammonium chloride and *n*-alkyl-1,3-propanediamine in the laboratory after a 24-h contact time. *n*-Alkyl-1,3-propanediamine was effective in concentrations greater than 10 μ g/ml. The quaternary ammonium biocide inhibited growth in concentrations greater than 20 μ g/ml, possibly explaining the inability of the strain to grow on cetrimide agar containing another quaternary ammonium compound, hexadecyltrimethylammonium bromide.

In vitro studies: utilization of ethylene glycol. Figure 3 depicts growth of the *Pseudomonas* sp. in ethylene glycol as a sole carbon source. The strain failed to grow in concentrations of ethylene glycol greater than 10% and failed to grow in tap water alone. In a 1% concentration of ethylene glycol, organisms were recovered on one occasion in low numbers after 3 h of incubation. In 5 and 10% concentrations, the strain flourished. By 50 h, approximately 10⁶ CFU/ml was recoverable. In the last sample taken (at 170 h), 10⁵ CFU/ml was recovered from these concentrations.

Results of the fluorescence studies are depicted in Fig. 4. Fluorescence measured in the upstream side of the backflow preventer was at all times negligible. Integrity of the valves and proper operation of the backflow preventer isolated even high concentrations of dye from potable water sources.

DISCUSSION

Biofouling of this magnitude within a closed-loop system has not been previously reported (4, 15). Ethylene glycol treatment of the chilled-

FIG. 3. Growth of *Pseudomonas* sp. in 1, 5, and 10% concentrations of ethylene glycol in tap water at 33°C in a shaking water bath. Bacteria failed to grow in tap water and in 25, 50, 75, and 100% concentrations of ethylene glycol.

FIG. 4. Effectiveness of valves and backflow preventer in protecting upstream potable water.

water coils followed by winter shutdown is a procedure used as an energy-efficient method of maintaining a closed system over the winter months. The availability of ethylene glycol in utilizable concentrations in addition to suitable temperatures, water, oxygen, and turbulence created an excellent environment for growth when the system became operational in the spring. Biocidal treatment to remove contamination was complicated by simultaneous bleeding of the system and replacement of the loss with water from a contaminated expansion tank serving as a 33°C incubator. This, in effect, reintroduced large numbers of organisms into the chilled water while decreasing the effective concentration of biocide.

These studies suggest that 5 and 10% concentrations of ethylene glycol could easily stimulate growth of the strain under the physical conditions that were present during the period of fouling. The concentration of ethylene glycol initially added to the coils was 50%, a concentration found to inhibit growth. However, during the spring maintenance of the system, this concentration was washed out, and lower concentrations sufficient for growth could have conceivably been present to serve as a bacterial substrate.

Water specimens taken when the chilled-water system first became operational in April were clear, colorless, and low in bacterial numbers. Therefore, bacterial growth and subsequent fouling encompassed a period of approximately 6 weeks before mechanical difficulties were first observed.

Exocellular slime formation is a common characteristic of the *Pseudomonadales* (5, 6) and can be readily observed by electron microscopy

after critical-point drying and freeze-etching (5). Slime formation is difficult to demonstrate in the laboratory, where the protected environment of pure cultures on rich growth media discourages this phenomenon. Formation of a glycocalyx provides bacteria with adherence characteristics in hostile environments and easier access to nutrients (5, 6). In addition, the use of quantitation of CFU to measure success of decontamination can be misleading because, the greater mass of organisms are firmly affixed by the bacterial glycocalyx to the lining of the pipes. Only those organisms that are viable and free-floating in the circulation can be quantitatively measured. Presumably, these are the organisms having greater effective contact with the biocides.

Water used in filling closed cooling systems is drawn from the domestic water supplies of the local community. The source of the water and its prior treatment determine the microbial load that will ultimately enter the closed system. In most cases, the lack of utilizable nutrients and competition among bacteria and other organisms is sufficient to limit waterborne levels of bacteria. Nevertheless, potentially pathogenic bacteria do enter the hospital environment (8, 9, 18) and may be responsible for hospital-acquired morbidity and mortality among susceptible patients (18). Spread of these organisms is further encouraged by the crowding of patients and by breaks in aseptic techniques by hospital personnel (10, 14, 17). *Pseudomonas* sp. with biochemical characteristics and antibiotic susceptibility patterns identical to those of the fouling strain became a common isolate from clinical specimens in our institution, particularly on surgical services, such as the respiratory-surgical intensive care unit. During an 8-month period in 1979, 17 of 371 patients admitted to the respiratory-surgical intensive care unit developed infections due to this organism. We felt that the fouling episode might be related to the increased incidence of the organism in the hospital. This led to the fluorescent dye experiments, which showed that contaminated chilled water was prevented from passing upstream of the backflow preventer. The backflow preventer is inspected periodically in our institution. However, in some other hospital facilities, little or no attention may be given to the maintenance of this and other such complex devices. This negligence may seed pathogenic bacteria into potable water supplies (16).

The antibiotic susceptibility patterns of organisms isolated from the chilled-water system and patient care areas were identical. *Pseudomonas* sp. was in the potable water supply of the hos-

pital, and we believe this water infected our patients and fouled the cooling system of the hospital. We are looking into additional environmental areas where these pseudomonads may propagate.

During the time the electric chillers were non-operational, the steam heat exchanger unit showed little effect from the fouling. Possible explanations include the fact that the absorption machine is used as standby equipment and the chilled water does not normally circulate through it unless the primary pump is operating. At the time of the problem the machine had not run on a regular basis. Also, the heat transfer area on the tubes within the absorption unit is far greater, per ton, than that of the electric chillers. Finally, the water flow through the tubes is much slower, and heat transfer does not have to be as efficient.

ACKNOWLEDGMENT

This study was supported in part by Public Health Service grant GM15904 from the National Institute of General Medical Sciences.

LITERATURE CITED

1. Anderson, G. D. 1979. Backflow prevention. Why and How. Heat. Piping Air Cond. 51:61-67.
2. Bauer, A. W., W. M. M. Kirby, S. Sherris, and M. Turck. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45:493-496.
3. Bauer, R. O. 1979. Industrial cooling water systems. Potential breeding grounds for Legionnaires' disease. Plant Eng. 6:137-138.
4. Boehler, R. A. 1969. Role of microorganisms in industrial corrosion. Heat. Piping Air Cond. 41:62-67.
5. Cagle, G. C. 1975. Fine structure and distribution of extracellular polymer surrounding selected aerobic bacteria. Can. J. Microbiol. 21:395-408.
6. Costerton, J. W., G. G. Gelsey, and K. J. Cheng. 1978. How bacteria stick. Sci. Am. 238:86-95.
7. Doudoroff, M., and N.J. Palleroni. 1974. Genus *Pseudomonas* Miguela 1894, 237 nom. cons. Opin. 5, Jud. Comm. 1952, 121, p. 217-243. In Bergey's manual of determinative microbiology, 8th ed. The Williams & Wilkins Co., Baltimore.
8. du Moulin, G. C. 1979. Airway colonization by *Flavobacterium* in an intensive care unit. J. Clin. Microbiol. 10: 155-160.
9. Eichhorn, J. H., M. L. Bancroft, L. H. Laasberg, G. C. du Moulin, and A. J. Saubermann. 1977. Contamination of medical gas and water pipelines in a new hospital building. Anesthesiology 46:296-298.
10. Johanson, W. G., A. K. Pierce, J. P. Sanford, and G. D. Thomas. 1972. Nosocomial respiratory infections with gram-negative bacilli. Ann. Intern. Med. 77:701-706.
11. Miller, R. P. 1979. Cooling towers and evaporative condensers. Ann. Intern. Med. 90:667-670.
12. Morris, G. K., C. M. Patton, J. C. Feeley, S. E. Johnson, G. Gorman, W. T. Martin, P. Skaliy, G. F. Mallison, B. D. Politi, and D. C. Mackel. 1979. Isolation of the Legionnaire's disease bacteria from environmental samples. Ann. Intern. Med. 90:664-666.
13. Reznick, S., W. Hayden, and M. Lee. 1979. Analytical note—fluorescein tracer technique for detection of groundwater contamination. Am. Water Works Assoc. J. 71:586-587.
14. Salzman, T. C., J. J. Clark, and L. Klemm. 1968. Hand contamination of personnel as a mechanism of cross-infection in nosocomial infections with antibiotic-resistant *Escherichia coli* and *Klebsiella-Aerobacter*, p. 97-100. Antimicrob. Agents Chemother. 1967.
15. Smith, A. L., and R. A. Muia. 1973. Identify and control microbiological organisms in cooling water systems. Power 117:16-19, 91-95.
16. Springer, E. K. 1979. Manual of cross-connection control. Foundation for Cross-Connection Control and Hydraulic Research, University of Southern California, Los Angeles.
17. Steere, A. C., and G. F. Mallison. 1975. Handwashing practices for the prevention of nosocomial infection. Ann. Intern. Med. 83:683-690.
18. Teres, D., P. Schweers, L. S. Bushnell, J. Hedley-Whyte, and D. S. Feingold. 1973. Sources of *Pseudomonas aeruginosa* infection in a respiratory/surgical intensive-therapy unit. Lancet i:415-417.
19. Washington, J. A., II, P. K. W. Yu, and W. J. Martin. 1971. Evaluation of accuracy of multitest micromethod system for the identification of *Enterobacteriaceae*. Appl. Microbiol. 22:267-269.
20. Weaver, R. E., H. W. Tatum, and D. G. Hollis. 1972. The identification of unusual pathogenic gram-negative bacteria. Center for Disease Control, U.S. Public Health Service, Atlanta.