Effect of Disinfectants on Pseudomonads Colonized on the Interior Surface of PVC Pipes

ROGER L. ANDERSON, PHD, BETTY W. HOLLAND, JANICE K. CARR, BS, WALTER W. BOND, MS, AND MARTIN S. FAVERO, PHD

Abstract: We investigated the effect of disinfectants on microbial contamination present on the interior surface of polyvinyl chloride (PVC) pipes filled with 600 ml of water contaminated with *Pseudomonas picketii* and *P. aeruginosa*. After eight weeks, water was removed, and the test pipes exposed to various types of aqueous disinfectants. Disinfectant samples were removed, neutralized, and examined for recovery of microorganisms by membrane filtration. After seven-days exposure, disinfectant solutions were removed and pipes filled with sterile distilled water. Water was examined by membrane filtration at seven-day intervals to determine whether the organisms had survived in the pipes. Colonization of PVC surfaces were examined during each study phase by scanning electron-microscopy (SEM). *P. aeruginosa* was isolated directly from iodophor disinfectant, phenolic germicide, and iodophor antiseptic solutions. After addition of sterile water, *P. aeruginosa* was recovered from PVC pipes previously exposed to chlorine, phenolic, quaternary-ammonium, and iodophor disinfectants; *P. pickettii* was recovered from water in pipes treated with iodophor disinfectant, chlorine, and ethanol. The existence of glycocalyx-like cellular masses on the interior wall of PVC pipes most likely protected embedded organisms from the microbicidal action of some of the disinfectants tested and served as the reservoir for continuous contamination. (*Am J Public Health* 1990; 80:17–21.)

Introduction

The resistance of microorganisms to antimicrobial agents in general and chemical germicides in particular is controlled by a number of factors including the culture history and strain of the microorganism, the nature of the suspending menstruum as well as a variety of physical factors such as temperature, pH, and hardness. Further, microorganisms in their naturally occurring state have been shown to be significantly more resistant to chemical germicides than microorganisms that have been subcultured on artificial culture media.[1,2]

In the last several years, however, there have been reports of bacteria surviving in concentrations of chemical germicides that go significantly beyond the perceived limits of resistance. For example, recent epidemiologic and microbiologic investigations have documented intrinsic microbial contamination of iodophor antiseptic solutions.[3–6] Pseudobacteremia caused by *Pseudomonas cepacia* has been associated with the use of contaminated povidone-iodine, and *Pseudomonas aeruginosa* peritoneal infections has been attributed to the use of contaminated poloxamer-iodine. Presently, epidemiologic and laboratory investigations involving a contaminated povidone-iodine solution are in progress. Peritoneal infections in infants and false positive blood cultures from intensive-care unit patients with *P. cepacia* have been associated with the use of this intrinsically contaminated povidone-iodine antiseptic solution.[7] In addition, the prolonged survival of *P. aeruginosa* in iodophor solutions has been demonstrated after the iodophor was exposed to the inside surfaces of naturally contaminated PVC water distribution pipes.[8,9] When these organisms were isolated from iodine solutions and then tested, high numbers ($>10^6$ per ml) of *P. aeruginosa* were inactivated by iodophor solutions in minutes. More recently, studies have been completed which show recovery of *P. aeruginosa* from iodophors after unused or new PVC, stainless steel, copper, and glass pipes were artificially contaminated with water containing these organisms and subsequently exposed to iodine antiseptic solutions.[8]

The isolation and extended survival of microorganisms in these iodine-containing solutions may be due to extracellular glycocalyx-like material that microorganisms form and deposit on a variety of different types of surfaces.[10–12] Microbial colonization on the interior surfaces of PVC pipes and subsequent protection of organisms from the microbicidal action of antiseptic solutions may be due to the formation and adherence of extracellular matrixes to PVC surfaces. The end result is the production of thick masses of cells and extracellular material (biofilms). Once these masses are formed, antibacterial agents such as disinfectants or antiseptics must saturate the matrix before they can kill bacteria within adhesive biofilms.

Because PVC pipes are commonly used to distribute water in many pharmaceutical and manufacturing plants, in hospital dialysis units, and in various laboratory facilities, we initiated laboratory studies to determine: 1) the effects of various chemical germicidal disinfectants on *Pseudomonas* spp. colonizing the interior wall surfaces of new PVC pipe; 2) the observation and importance of thick cellular masses on PVC surfaces by SEM; and 3) if bacteria survive in and recolonize test pipes after exposure to chemical disinfectants. The purpose of these studies was to formulate a model system to determine the reason for the extraordinary ability of *P. cepacia* and *P. aeruginosa* to survive for extended periods of time after exposure to chemical germicides.

Methods

Preparation of PVC Test Pipes and Sample Remnants

Schedule 80 PVC pipe (4.1 cm in diameter) was used in these investigations. Test pipes consisted of two 30.4 cm PVC sections attached to a 90° PVC elbow. Straight pieces of PVC were separately attached to the elbow by pipe putty (Rectorseal, The Rectorseal Corp, Houston, TX), teflon tape (Teflon Thread Seal, DuPont, Wilmington, DE), organic solvents (Oatey PVC Cement, Oatey Co, Cleveland, OH), or dresser couplings (Flo-Control, Burbank, CA). Thus, four

From the Hospital Infections Program, Center for Infectious Diseases, Centers for Disease Control, Atlanta. Address reprint requests to Roger L. Anderson, PhD, Hospital Infections Program, Centers for Disease Control, 1600 Clifton Road, 1-B341, Atlanta, GA 30333. This paper, submitted to the Journal February 6, 1989, was revised and accepted for publication July 17, 1989.

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sections of PVC (distinguished by the type of attachment) were included in each test group. All PVC test pipes were thoroughly rinsed with tap water, dried, and separately packaged before ethylene oxide sterilization; rubber stoppers used to plug the pipe ends were steam sterilized.

Remnant samples for SEM were prepared by cutting small (1.5 by 1.5 cm) squares of PVC pipe. A small hole was bored at one end and a single 30.5 cm piece of monofilament fishing line tied to each PVC square. Remnant samples were packaged and sterilized with ethylene oxide before suspending them in PVC test pipes.

Microbiologic Conditioning of Test PVC Pipes and Remnant Samples

PVC test pipes (two test groups evaluated each week for four weeks) were filled with 600 ml of water containing a mixed, naturally occurring bacterial population which included only *P. aeruginosa* and *P. pickettii*. These organisms were originally present in naturally contaminated manufacturing plant water and served as the inocula for maintaining a source of contaminated water for these investigations. A reservoir of contaminated water was maintained by inoculating an 18-liter plastic carboy of sterile deionized water with 500 ml of contaminated plant water. Water in the carboy was held at ambient temperature (25°C), and contained a total microbial level of 10^4 colony-forming units (CFU) per ml at the beginning of this study. Initially, contaminated water present in the PVC test pipes contained 10^2 *P. aeruginosa* and 10^2 *P. pickettii* per ml of water.

Five remnant samples were suspended in rubber-plugged pipes filled with contaminated water. Pipes were then stored for eight weeks at 25°C to effect microbial colonization of the interior PVC surfaces. Microbial populations in the pipe waters were sampled weekly using membrane filtration procedures as described. Ten-fold water dilutions were made and passed through sterile sampling tubes attached to bacteriological membrane field monitors (0.45 micron; Millipore Corp, Bedford, MA). PVC remnants were removed at various time intervals for SEM observation of microbial colonization (i.e., formation of cellular and extracellular material accumulations).

Scanning Electron Microscopic Examination of PVC Remnants

For SEM studies, the PVC remnants were fixed in 1 percent glutaraldehyde in Sorenson's PO_4 buffer (pH 4.9) for 16 hours at room temperature, then rinsed three times in PO_4 buffer (pH 7.0). Postfixation was in osmium tetroxide (1 percent in PO_4 buffer, pH 7.0) for four hours at room temperature. The remnants were dehydrated in a graded ethanol series and then placed in a critical-point drying apparatus. Critical-point drying was carried out with CO_2 as the transitional fluid-drying gas. Specimens were loaded into the unit while immersed in 100 percent ethanol, and drying was accomplished by successive changes of liquid Co_2 while the apparatus was chilled by an ice/water bath. The CO_2 was removed by the temperature and pressure being raised to the critical point (32°C and 1100 psi) and slowly exhausted. Specimens were sputter-coated with gold palladium and examined in a Philips SEM 515.

Disinfection of Contaminated PVC Test Pipes

After eight weeks of incubation, contaminated water in the PVC test pipes was removed and cultured quantitatively using ten-fold dilutions and membrane filtration procedures as previously described. Two disinfectants were evaluated each week in two groups of PVC test pipes. Four contaminated PVC test sections (representing the four types of pipe attachment), or one PVC test group, were totally filled (*ca.* 600 ml) with each of the following disinfectants: 1) a 1:213 aqueous dilution (pH 2.9) of an iodophor detergent-germicide (Wescodyne, West Chemical Co, Lynbrook, NY); 2) a 1:128 aqueous dilution (pH 9.2) of a phenolic germicidal detergent (Vesphene II, Vestal Laboratories, St. Louis, MO); 3) a 1:256 aqueous dilution (pH 8.9) of a quaternary-ammonium germicidal detergent (Hi-Tor, Huntington Laboratories Inc, Huntington, IN); 4) a stock solution (pH 5.0) of an iodophor antiseptic (Prepodyne, West-Agro Chemical Co, Inc, Westwood, KS, manufactured for AMSCO Medical Products Division, Erie, PA); 5) 2 percent glutaraldehyde (Cidex, Surgikos, Inc, Arlington, TX) of pH 8.0; 6) a aqueous dilution (pH 7.5) of purified-grade sodium hypochlorite (Fisher Scientific Co, Fair Lawn, NJ) having 10–50 mg of free available chlorine per liter, as determined by the DPD colorimetric test method (Hack Company, Loveland, CO); 7) a 2 percent aqueous dilution (pH 3.4) of formaldehyde (Whiteworth Inc, Gardena, CA); and 8) 70 percent (vol/vol) aqueous ethyl alcohol (Warner-Graham Co, Cockeysville, MD) of pH 5.4. Two control PVC pipes were evaluated with each disinfectant test group: one noncontaminated PVC pipe with disinfectant and one water-contaminated PVC pipe with sterile distilled water.

Aliquots (10 to 50 ml) of the disinfectant were removed and sampled using membrane filtration procedures at 0, 2, 4, and 6 hours and 1, 2, 3, 5, and 7 days. After sampling, each pipe was refilled with an equal amount of the appropriate disinfectant. Filters were rinsed with 250 ml of brain heart infusion broth with 0.5 percent beef extract and the appropriate neutralizer. The filters were placed on blood agar plates. The following chemicals (i.e., in final percent concentration) were added to the enriched broth to neutralize any residual disinfectant: 0.5 percent sodium thiosulfate for iodophors and chlorine solutions; 0.5 percent Tween 80 for phenolics; 0.7 percent lecithin (soybean) for quaternary ammonium compounds; and 0.25 percent sodium bisulfite for aldehydes. Blood agar plates with filters were incubated for 24–48 hours at 35–37°C. The microbial count was recorded per aliquot sampled and subsequently expressed as CFU per ml. To insure that *P. aeruginosa* and *P. pickettii* were the only organisms in the mixed microbial population that was used to condition the PVC pipes, two representative colonies of each morphologic type were picked from the filters and inoculated to triple sugar iron agar slants. Organisms showing nonfermentative reactions on these slants were identified by standard biochemical tests.[13]

Recolonization of PVC Pipes Previously Exposed to Disinfectants

After the contaminated pipes were exposed to disinfectants for seven days, the pipes were emptied and each was refilled with 600 ml of sterile distilled water. Pipes were resealed with fresh sterile rubber stoppers. Water was removed at seven-day intervals and various ten-fold dilutions were examined by membrane filtration procedures as previously described to determine reestablishment of microbial contamination in PVC test pipes previously exposed to chemical disinfectants. After dilutions were filtered, the membrane filters were placed directly on blood agar plates (no neutralizer rinse) and incubated.

Results

Microbiologic Conditioning of PVC Test Pipes

Over the eight-week conditioning period, water counts

for *P. pickettii* (biovar Va-1) were higher (10^5 to 10^6 CFU per ml) than counts observed for *P. aeruginosa* (10^3 to 10^5 CFU per ml). The microbial count for each organism increased each week during the eight-week sampling time. Total viable counts were approximately ten-fold higher in PVC test pipes that used pipe putty (a pipe-threading compound) to attach individual straight pieces to the 90° PVC elbow than pipes that did not have putty.

SEM Examination of PVC Remnants Exposed to Contaminated Water

The presence of bacterial colonization (i.e., formation of cells embedded in extracellular material) on the interior surface of PVC sample remnants was observed by SEM after remnant exposure to contaminated water (Figure 1). Microorganisms were not observed on control PVC remnants (samples not exposed or conditioned with contaminated water).

Recovery of Organisms from Test Disinfectants

P. aeruginosa was recovered directly from an iodophor disinfectant and a phenolic germicidal detergent in 48 hours and from an iodophor antiseptic solution in five days (Table 1). *P. pickettii* was not isolated from any of the test chemicals through seven days of sampling. *P. aeruginosa* and *P. pickettii* organisms were recovered from each of the 32 sterile water controls (i.e., four controls for each test disinfectant). The method of attaching PVC sections to the 90° PVC elbow did not correlate with recovery of organisms from test disinfectants.

Reestablishment of Organisms in Sterile Water

P. aeruginosa was recovered from water placed in PVC pipes previously exposed for seven days to use dilutions (i.e., diluted product as recommended by the manufacturer) of either an iodophor disinfectant, a phenolic germicidal detergent, a quaternary-ammonium compound, an iodophor antiseptic, or a dilution of sodium hypochlorite; *P. pickettii* was recovered from the pipes treated with an iodophor disinfectant, a chlorine solution, or 70 percent ethanol (Table 2). There was no evidence of microbial contamination during 42 days of sampling from contaminated PVC pipes exposed to 2 percent glutaraldehyde or 2 percent formaldehyde and refilled with sterile water. In some instances, organisms were found in water present in the PVC test pipes but were not recovered from the corresponding disinfectant initially used to disinfect that group of pipes.

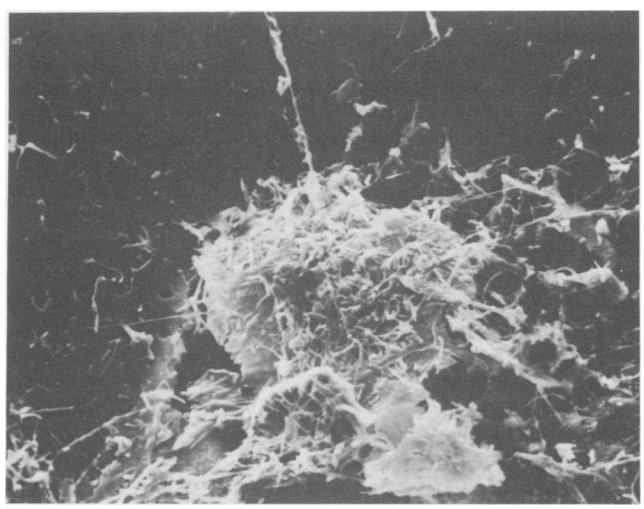

FIGURE 1—Scanning Electron Micrograph of the Interior Surface of a PVC Remnant Sample after Eight Weeks of Exposure to Contaminated Water, × 3,800

Discussion

Previous investigations have demonstrated the prolonged survival of *P. aeruginosa* in naturally contaminated PVC water distribution pipe segments after they were exposed to iodophor antiseptic solutions.[8,9] In one study, *P. aeruginosa* was isolated from a poloxamer-iodine solution 48 hours after membrane-filter sterilized poloxamer-iodine was added to the interior surface of naturally-contaminated PVC water pipe. Continuous exposure of poloxamer-iodine to this pipe resulted in a level of 10^4 CFU per ml of *P. aeruginosa* at nine days. This contaminated solution of poloxamer-iodine was removed from the pipe, stored at 25°C, and served as the reservoir for prolonged microbial survival studies. Using this contaminated stock solution of poloxamer-iodine, we found that *P. aeruginosa* (10^4 CFU per ml) survived for as long as 98 days in stock solution after removal from the PVC pipe.[8] Based on this investigational approach, a suitable laboratory model for induction of survival of these organisms and for studying the mechanism of *Pseudomonas* resistance to iodophors was developed.

In addition, studies have been done in which unused pipes of different composition were artificially contaminated by exposure to contaminated water containing *P. aeruginosa*.[8] After seven days, the water was removed from each pipe, and all were filled with membrane-filtered poloxamer-iodine. *P. aeruginosa* was recovered from poloxamer-iodine after exposure to artificially-contaminated PVC, stainless steel, copper, and glass pipes, but not galvanized steel pipe.

Thus, the newly developed model of using both naturally and artificially contaminated PVC pipes proved useful in demonstrating iodophor-resistance of *P. aeruginosa*. To date, the addition of iodophor antiseptic solutions to contaminated PVC pipe is the only laboratory method now available to show recovery and survival of *P. aeruginosa* from iodophor antiseptics.

We report experiments designed to determine: 1) the effect of various germicides on *Pseudomonas* spp. colonizing PVC pipe; 2) the existence of glycocalyx-like structures as a potential survival mechanism for these organisms; and 3) the reestablishment of *Pseudomonas* spp. in sterile distilled water added to contaminated and disinfectant-treated PVC pipes. *P. aeruginosa* was recovered from three test germicides during a seven-day exposure period: an iodophor disinfectant and phenolic germicide in 48 hours and an iodophor antiseptic in five days. *P. aeruginosa* was not recovered from either a quaternary-ammonium germicidal detergent, 2 percent glutaraldehyde, 10–50 ppm chlorine, 2 percent formaldehyde, or 70 percent ethyl alcohol; *P. pickettii* was not isolated from any of the eight disinfectants tested. The variable microbicidal capabilities of the germicides tested could be due to the type and amount of bacterial colonization present in the PVC pipes, the presence of morphologically abnormal bacterial cytoplasm in cells, or the specific mode and site of action of test disinfectants on bacterial cells (such as microorganisms embedded in a matrix of extracellular material).

Recent review articles have discussed contaminated antiseptics and disinfectants and the various types of orga-

TABLE 1—Recovery of Organisms from Chemical Disinfectants Exposed to Contaminated PVC Test Pipes

Test Pipe Group and Disinfectant Used	Recovery of Organisms per Aliquot of Chemical Sampled:		Recovery of Pseudomonads from Sterile Water Controls:	
	P. aeruginosa	*P. pickettii*	*P. aeruginosa*	*P. pickettii*
A[a]-Iodophor Disinfectant	48h, 2/50 ml	–[b]	+[c]	+
B-Phenolic Germicide	48h, 4/50 ml	–	+	+
C-Quaternary Ammonium Comp.	–	–	+	+
D-Iodophor Antiseptic	5d, 39/10 ml	–	+	+
E-2% Glutaraldehyde	–	–	+	+
F-Chlorine (10-50 ppm)	–	–	+	+
G-2% Formaldehyde	–	–	+	+
H-70% Ethanol	–	–	+	+

[a]Each group consisted of four test PVC pipes.
[b]No recovery after seven days of chemical exposure.
[c]Recovery of organisms from sterile water controls of each disinfectant test group (a total of 32 positive controls).

TABLE 2—Reestablishment of Organisms in Sterile Distilled Water after Exposure of Contaminated PVC Pipes to Disinfectants

Test Pipe Group and Disinfectant Used	Recovery of Organisms from Water after Pipes Were Exposed to Chemicals	
	P. aeruginosa	*P. pickettii*
A[a]-Iodophor Disinfectant	7d, 3.1×10^5/ml	7d, 7.5×10^4/ml
B-Phenolic Germicide	7d, 7.5×10^5/ml	–[b]
C-Quaternary Ammonium Comp.	7d, 25/50 ml	–
D-Iodophor Antiseptic	7d, 2.8×10^2/ml	–
E-2% Glutaraldehyde	–	–
F-Chlorine (10–50ppm)	7d, 2.1×10^3/ml	7d, 3.5×10^5/ml
G-2% Formaldehyde	–	–
H-70% Ethanol	–	21d, 1.4×10^6/ml

[a]Each group consisted of four test PVC pipes.
[b]No recovery after 42 days of water sampling.

nisms associated with these contaminated chemical solutions[14] as well as the resistance of bacteria to antiseptics and disinfectants.[15] A more definitive answer as to why *Pseudomonas* may survive or even grow in germicidal solutions will most likely not be available until laboratory studies are completed which clarify the mechanism of germicidal activity and determine the biologic and ecologic characteristics of *Pseudomonas* spp. that permit their tolerance to or utilization of various chemical compounds.

Massive concentrations of bacterial cells were observed by SEM on the surface of PVC remnant samples after incubation in contaminated water. These cellular masses (or glycocalyx-like structures) appeared to develop in greater numbers as the length of exposure time to contaminated water increased. As suggested in this investigation, the existence of glycocalyx-like structures and the shedding of these structures from interior PVC wall surfaces most likely served to shield *P. aeruginosa* from the microbicidal action of iodophor and phenolic germicides (Table 1).

The protective effect of the bacterial glycocalyx has been suggested by several investigators.[8–12,16] But it should be noted that our preparative techniques for SEM observations were not sufficient to preserve the entire glycocalyx matrix as described by Coseterton, *et al.*[10] Although we observed a considerable degree of microbial adhesion accompanied by notable accumulations of extracellular material on the PVC surfaces, our visual observations are likely an underestimation of the actual amount of physical mass. The prolonged survival of *Serratia marcescens* in 2 percent chlorhexidine was most likely due to these microorganisms being embedded in a thick matrix which adhered to the walls of storage jugs containing this antiseptic solution.[17] Viable *S. marcescens* was recovered from 2 percent chlorhexidine during a storage period of 27 months. The recovery of *P. aeruginosa* from unopened bottles of poloxamer-iodine could be the result of contaminated water and PVC product distribution pipes from a manufacturing plant.[16] SEM micrographs of these pipes demonstrated large concentrations of rod-shaped and coccobacillary cells embedded in interior deposits of the pipe. Also, large numbers of *P. aeruginosa* (10^4 CFU per ml) survived for as long as 98 days in a stock solution of poloxamer-iodine after exposure and removal from naturally contaminated PVC water distribution pipe.[8]

In the present study, microorganisms were observed to survive and reestablish in sterile water placed in PVC pipes previously exposed to chemical germicides. Although *Pseudomonas* spp. were not recovered from some of the test disinfectants, they were isolated from water that was placed in the corresponding chemically-treated test pipes. This reestablishment of microbial contamination suggests a continuous reservoir of organisms adhering to and shedding from the interior PVC wall surface. Organisms showing survival in five PVC test pipes were *P. aeruginosa* (after exposure to chlorine or a quaternary ammonium compound) and *P. pickettii* (after exposure to a iodophor disinfectant, chlorine, or 70 percent ethanol). This is strong evidence that these organisms survived within the colonized PVC pipes after exposure for seven days to a variety of chemical treatments. The physical thickness of cellular and extracellular material that form on PVC pipe surfaces could protect organisms from the action of germicidal chemicals and serve as a continuous reservoir for microbial contamination in test pipes and of water flowing through pipes.

Other investigators have hypothesized the importance of cell masses such as the glycocalyx as a potential reservoir of microbial contamination.[18,19] Several recent investigations have implicated *P. aeruginosa* as the etiologic agent of whirlpool-associated infections (i.e., folliculitis). These organisms are capable of growing in water and colonizing various whirlpool surfaces including water pipes. Such cellular and extracellular material accumulations adhering to whirlpool surfaces could protect bacteria from the action of chlorine and provide a constant microbial reservoir resulting in the contamination of water after a chemical treatment.

The public health significance of this investigation and its findings are widespread. PVC pipes are commonly used in

laboratory facilities, pharmaceutical and manufacturing plants, hospitals, therapeutic and public whirlpool-type hot tubs, and homes to distribute water for various usages. In 1984, during an investigation of a manufacturing plant which produced iodophor antiseptics, *P. aeruginosa* contamination was recovered throughout the PVC water distribution system.[16] Subsequently it was found that intrinsic contamination of a poloxamer-iodine antiseptic solution resulted when the formulated iodophor was allowed to stand in contaminated PVC pipes (pipes between the mixing tank and storage tank and between the storage tank and the bottling area) prior to bottling. As remedial measures, the company removed all PVC pipes, installed new stainless pipe, and initiated a hot water sanitizing procedure (60°C water for one hour) of all process water and product distribution pipes. In addition, better and more frequent microbiological quality control procedures on municipal and process water were instituted.

These types of laboratory studies are important in identifying possible mechanisms by which microorganisms could survive in disinfectant solutions for extended periods of time and to determine how bacterial organisms could continuously contaminate the various parts within a water distribution system. The best way to maintain a water distribution system in a safe condition is to limit the number of microorganisms by proper and scheduled maintenance and to routinely sanitize pipes or tubing that transport water. The laboratory findings presented here add to development of effective strategies for disinfecting water or product distribution lines and holding tanks in manufacturing plants and in laboratory, medical, and pharmaceutical facilities where microbial contamination by pseudomonads or other naturally-occurring gram-negative water bacteria is a problem.

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Public Health Workforce Consortium Recommended

Development of a Public Health Workforce Consortium with public and private representatives—to study health personnel implications of strategic public health issues and improve data collection efforts—has been recommended by a work group established by the Health Resources and Services Administration (HRSA).

The HRSA-supported group recommended the study of selected issues such as those delineated by the Year 2000 Health Objectives for the Nation. It also recommended an incremental approach to the eventual creation of an analytical system capable of assessing the public health work force.

Information on the Public Health Workforce Consortium project and its recommendations is available from B. Jerald McClendon, Division of Associated and Dental Health Professions, Bureau of Health Professions, Room 8C-09, DHHS/PHS, 5600 Fishers Lane, Rockville, MD 20857. Telephone: (301) 443-6757.