

Intrinsic Bacterial Contamination of a Commercial Iodophor Solution: Investigation of the Implicated Manufacturing Plant

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After an outbreak of peritoneal infections attributed to intrinsic contamination of a poloxamer-iodine solution with *Pseudomonas aeruginosa*, the manufacturer of the contaminated solution permitted investigation and sampling of materials within the plant. *Pseudomonas* spp. were recovered from two different unopened lots of solution and from numerous water samples obtained at the plant. The isolates from water identical to those of an isolate recovered from Prepodyne solution (West-Agro Chemical Co., Inc., Westwood, Kans., manufactured for AMSCO Medical Products Div., Erie, Pa.) manufactured 1 month earlier at the same plant. *P. aeruginosa* was not recovered from incoming city water. *P. aeruginosa* was recovered from sterile water and poloxamer-iodine after 48 h of incubation in a plant polyvinyl chloride pipe. Scanning electron micrographs of polyvinyl chloride pipe used in the plant showed massive concentrations of rod-shaped and coccobacillary cells apparently embedded in interior deposits of the pipe. Manufacturers of iodophors should be aware that pipes or other surfaces colonized with bacteria may be a source of contamination of their products.

Iodophors are broad-spectrum bactericidal agents which cause few side effects when used topically on skin and mucous membranes. For this reason, they are widely used for antiseptic purposes in hospitals and other health care institutions, fulfilling a useful role in current medical practice. However, reports of intrinsic bacterial contamination of two different iodophor antiseptic solutions have raised serious questions about the source of contamination during manufacture and the mechanism of prolonged survival of bacteria in these products (4, 7, 12).

After the recent outbreak of peritoneal infections attributed to the intrinsic contamination of Prepodyne solution (West-Agro Chemical Co., Inc., Westwood, Kans., manufactured for AMSCO Medical Products Division, Erie, Pa.), a poloxamer-iodine antiseptic solution, with *Pseudomonas aeruginosa*, the manufacturer of the implicated solution permitted investigation of the plant, including sampling of plant facilities, raw materials, and the finished product (12). Information regarding the manufacturing process was provided by plant officials. Generally, the findings of the investigation were that microbial contamination probably resulted from incubation of the product in pipes colonized with bacteria.

MATERIALS AND METHODS

Plant inspection and record review. The manufacturing plant was inspected, and water and product lines were traced. Records were reviewed for all batches of poloxamer-iodine made in the previous 6 months; the date of each stage in the production, storage, and bottling of each batch was recorded. City water distribution system data for the district in which the plant is located were reviewed for the previous 20-month period. The city bacteriological monitoring records for the same time period were also reviewed.

Bacteriological study of patient isolates, Prepodyne, and

raw materials. Five patient isolates, obtained from the peritoneal fluid of four patients and a wound at the dialysis catheter site of one patient, and 12 unopened 1-gal (3.785-liter) plastic containers of Prepodyne solution were obtained from the hospital at which an outbreak of *P. aeruginosa* peritoneal infections had occurred (12). In addition, four 1-pint (0.473-liter) plastic containers of Prepodyne solution of lots different from those obtained at the hospital were obtained from the manufacturing plant for culture.

To sample the containers of Prepodyne solution for microorganisms, 100-ml portions were aseptically removed from each container and were sampled through sterile sampling tubes attached to bacteriological field monitors (0.45 μ m; Millipore Corp., Bedford, Mass.). The filter was then rinsed by aspirating 75 ml of brain heart infusion broth containing 0.5% beef extract and 0.5% sodium thiosulfate (BHIES) through the filter (9). The filters were then aseptically removed and placed on Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) plates containing 5% defibrinated sheep blood (TSAB). The plates were incubated at 35 to 37°C and observed for colonies up to 7 days.

Samples of raw ingredients used in the manufacture of poloxamer-iodine (i.e., two pluronic polymers, citric acid, and sodium iodide) were obtained from the plant. For each raw ingredient, 1.0 and 5.0 ml (liquids) and 0.1 and 0.5 g (solids) were separately inoculated aseptically into 75 ml of brain heart infusion broth containing 0.5% beef extract (BHIE) and BHIES. Broths were incubated aerobically at 35 to 37°C for 7 days and were observed daily for turbidity.

Water samples, obtained at different locations throughout the plant, were shipped by air freight to the Centers for Disease Control for processing. Samples of influent lake water to the city treatment plant, treated water from the city treatment plant, and water from the city water main at the site of entry into the plant were also obtained. At the time of sampling, sodium thiosulfate was added to neutralize residual chlorine.

Water was assayed for microbial content. Portions (100 ml) of each sample were cultured by using the membrane

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filter technique, and the filter was then aseptically placed on the surface of TSAB plates (4). Samples (1 and 0.1 ml) were examined by using the standard pour plate method with Standard Methods agar (1). In addition, 5.0-ml samples were added to 75 ml of BHIE broth. Plates and broths were incubated at 35 to 37°C and observed daily for visible colonies or turbidity for 7 days.

Stimulation of plant preparation and storage of poloxamer-iodine. Naturally contaminated plant water was combined with other raw materials to produce a poloxamer-iodine solution containing 1% available iodine, according to the methods of the manufacturer. At each stage of the batch process, the mixture was sampled for aerobic bacterial growth (2). Sampling was performed at 1, 4, 10 and 60 min and at 4 and 24 h.

Several sections of polyvinyl chloride (PVC) pipe proximal to the mixing tank in which the contaminated solution had been made were removed and examined. One pipe section (3.8-cm diameter) was filled with 55 ml of sterile deionized water and was incubated at 25°C for 24 h; the water was then cultured by the method described above for water assay. Similarly, 250 ml of sterile membrane-filtered Prepoddyne solution was placed in another section of PVC pipe (3.8-cm diameter) and was incubated in the pipe at 25°C (2). Portions (50 ml) were sampled at 10 min and at 1, 24, 48, and 72 h. Samples were cultured by using the technique for Prepoddyne assay described above.

Typing systems used with *P. aeruginosa* isolates. All gram-negative nonfermenting isolates were identified by standard biochemical testing (16). Many of the confirmed *P. aeruginosa* isolates were also tested by using several epidemiological marker systems. Serological identifications were performed by slide agglutination with the *P. aeruginosa* International Antigenic Typing Schema and commercially prepared antisera (Difco Laboratories, Detroit, Mich.) (8, 10); analysis of plasmid DNA extracted from *P. aeruginosa* strains was performed by agarose gel electrophoresis (5, 11); and antimicrobial susceptibility testing was performed by the Bauer-Kirby method (3).

Chemical analyses of Prepoddyne solution and plant water. The pH of all lots of contaminated Prepoddyne solutions and of several noncontaminated lots of Prepoddyne solutions was determined. Free and available iodine determinations were done by the *n*-heptane extraction method and sodium thio-sulfate titration, respectively (15; M. W. Winicov and W. Schmidt, U.S. patent 3,028,299, April 1962). Total and free chlorine levels were determined on selected water samples at the time of collection by the diethyl-*p*-phenylenediamine colorimetric method (1).

Electron microscopy of PVC pipe. PVC pipe from the manufacturing plant was sectioned (ca. 10 by 10 mm), and the sections were critical-point dried, sputter coated with gold-palladium, and examined with an AMR-1000 (AMRAY Inc., Bedford, Mass.) scanning electron microscope at various magnifications.

RESULTS

Plant inspection and record review. Water lines and product lines at the plant were made of a variety of materials, including cast iron, galvanized steel, copper, and PVC pipes ranging in diameter from 1.2 to 6.3 cm. The water used to manufacture the product passed through a heat exchanger and was sometimes heated before a particular product was mixed; however, the water was not filtered or otherwise treated and was not held in storage tanks. The water was combined with the other raw materials in a fiber glass mixing

tank, and the product was then pumped to another fiber glass tank for storage. The product pipes were not flushed with water until a batch was completely bottled, and records showed that batches subsequently found to be contaminated were bottled 5 to 18 days after production. Thus, some product may have stood within pipes between the mixing tank and storage tank and between the storage tank and bottling area for this period of time. Pipes were not otherwise cleaned. Review of city water distribution records did not reveal any significant pressure drop problems, nor did the city bacteriological records reveal any significant water quality problems.

Bacteriological study of patient isolates, Prepoddyne, and raw materials. The five patient isolates were confirmed as *P. aeruginosa*. *P. aeruginosa* or *Pseudomonas* spp. were recovered from 1 of 12 1-gal containers and all 4 1-pint containers of Prepoddyne solution. No organisms were recovered from the other gallon containers of Prepoddyne solution or from any of the raw materials tested. *P. aeruginosa* was recovered from 33 of 45 water samples taken from within the plant, including the potable water supplying the fiber glass tank in which the contaminated batches were made. A polymicrobial population was observed in the raw and treated city water off plant premises, but *P. aeruginosa* was not recovered from these sources.

P. aeruginosa recovered from the patients and from a 1-gal container of Prepoddyne solution demonstrated identical markers (i.e., serotype, plasmid profile, and antimicrobial susceptibility pattern) (Table 1).

The *P. aeruginosa* isolates from the pint bottles of Prepoddyne solution taken from the plant possessed different epidemiological markers from those of the *P. aeruginosa* isolates obtained from the patients and the 1-gal containers. In addition, pint bottles of a lot different from those containing *P. aeruginosa* were found to be contaminated with *Pseudomonas* spp. which could not be identified. No contaminated lot of Prepoddyne solution demonstrated polymicrobial content. All of these lots had been manufactured during different months of the previous year.

Water isolates of *P. aeruginosa* demonstrated a wide

TABLE 1. Epidemiological markers used for *P. aeruginosa* isolates

Source of isolate	Serotype	Plasmid profile (approx mol wt in megadaltons)	Antibiogram ^a
Patients (peritoneal fluid and wound) in hospital A	9,10	75, 5	a
Prepoddyne solution used at hospital A	9,10	75, 5	a
Prepoddyne solution at plant	3	60-65 ^b	b
Water entering tank in which contaminated lots were made	9,10	60-65	b
	3	60-65	b
	9,10	No plasmid DNA band	b
	Other		

^a a, Sensitive to amikacin, carbenicillin, colistin, gentamicin, sulfamethoxazole-trimethoprim, tetracycline, tobramycin, cefoperazone, piperacillin, mezlocillin, and moxalactam; resistant to ampicillin, cefamandole, cefoxitin, and cephalothin. b, Similar to (a) except resistant to sulfamethoxazole-trimethoprim and tetracycline.

^b One weak band.

range of serotypes; other epidemiological markers were determined only for isolates with serotype 3 or 9, 10 which were recovered from the tap supplying the tank in which the contaminated solutions were made (Table 1). Water isolates of *P. aeruginosa* had a serotype, plasmid DNA profile, and antimicrobial sensitivity pattern identical to those of isolates recovered from samples of one of the lots of Prepodyne.

Simulation of plant preparation of poloxamer-iodine. No bacteria were isolated from the batches of final product made in the laboratory with contaminated water. *P. aeruginosa* was recovered from sterile water incubated in a section of pipe for 24 h. No bacteria were recovered from the Prepodyne solution incubated in PVC pipe for 10 min or 0, 1, or 24 h (2). However, 27 viable colonies per 50 ml were recovered from Prepodyne solution incubated in the pipe for 48 h, and at 72 h the counts increased to ca. 100 CFU/50 ml (2). These organisms were identified as *P. aeruginosa*, serotype 3. Survival of *P. aeruginosa* was also demonstrated at 3 h after the Prepodyne was removed from the pipe.

Chemical analyses of Prepodyne and plant water. The pH and available and free iodine contents of the contaminated Prepodyne solutions were similar to those of Prepodyne in which *Pseudomonas* contamination was not demonstrated. The pH ranged from 4.7 to 5.0, the free iodine ranged from 0.65 to 0.70 ppm ($\mu\text{g/ml}$), and the available iodine ranged from 1.01 to 1.14%. Chlorine analysis of the plant water

taken during inspection of the plant at mid-week demonstrated a free chlorine residual of 0.7 mg/liter in the water coming into the plant and free chlorine levels ranging from 0.0 to 0.7 mg/liter in water samples taken at various points in the plant. Of the 34 samples, 18 (53%) contained no measurable free or combined chlorine residual.

Scanning electron micrographs of the interior pipe surface. Scanning electron microscopy of the interior PVC pipe surface showed massive concentrations of rod-shaped and coccobacillary cells on the surface of and apparently imbedded in craggy mineral-like deposits composed of a rough base overlaid with a granular structure (Fig. 1).

DISCUSSION

Intrinsic contamination with viable vegetative bacteria of both povidone-iodine and poloxamer-iodine antiseptic solutions containing 1% available iodine has been reported (4, 7, 12). Since the initial report of iodophor contamination, however, the source of bacteria and the mechanism of resistance have remained unclear. The manufacturer of a contaminated povidone-iodine antiseptic solution reported that a deionizing resin bed was causing contamination of the water supply used to manufacture the iodophor (4). Therefore, contaminated water was believed to be the source of the viable bacteria in the iodophor, and many manufacturers

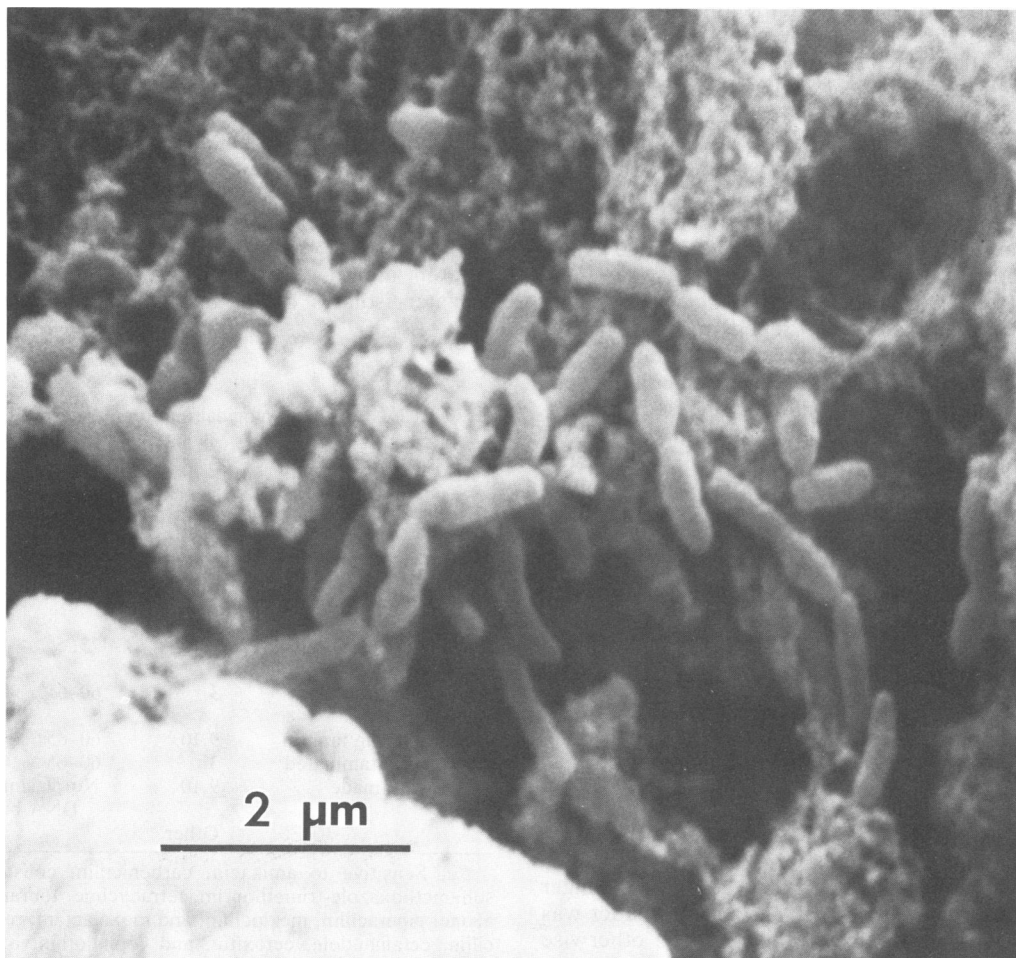


FIG. 1. Scanning electron micrograph of the interior surface of a plant PVC pipe.

of iodophors concentrated on manufacturing a sterile product by bacteriological monitoring of the water supply.

After the finding of contaminated Prepodyne antiseptic solution, water used in the manufacture of the iodophor was again suspected to be the source of contamination. Of the raw ingredients and water specimens sampled, only plant water was found to be contaminated with *P. aeruginosa*; some water isolates possessed an identical serotype and plasmid profile to those recovered from Prepodyne solution manufactured the previous month. However, use of contaminated plant water to mix poloxamer-iodine solution in a laboratory setting did not result in recovery of viable bacteria from the laboratory-produced product.

It had been previously documented that PVC pipe readily supports the growth of bacteria (13), and, indeed, we demonstrated that incubation of sterile deionized water or sterile poloxamer-iodine in a section of plant PVC pipe for 24 and 48 h, respectively, resulted in the subsequent recovery of viable bacteria from both the water and iodophor. Furthermore, an increased number of bacteria were found with time, and survival of bacteria for more than 3 months in an iodophor solution after incubation in contaminated pipe has been demonstrated (2).

These investigations support the hypothesis that the product was contaminated after formulation. Bacteria in the water are known to be able to establish themselves on surfaces under flowing conditions. Incubation of the product for several days in pipes whose surfaces were contaminated with gram-negative bacteria probably resulted in contamination of the iodophor. Bacterial resistance to iodophors after incubation in pipe sections may be a result of glycocalyx formation, a coating of bacterial origin possibly induced by interaction between bacteria and the pipe surface and which may create a mechanical protection for the bacteria (6). Scanning electron micrographs of the interior surface of PVC pipes demonstrated numerous bacteria imbedded in an amorphous matrix which might represent glycocalyx formation.

The manufacturing plant had pipes made of a variety of material. PVC pipe was used in sections throughout the plant in no systematic fashion, but its use included parts of the water line entering the tank in which the contaminated Prepodyne solution was made and the end product line between the storage tank and the bottling area. Whether other materials such as the fiber glass tanks in which the product was mixed and stored can also support growth in iodophors is being studied (2).

The manufacturers of Prepodyne solution first attempted eradication of *Pseudomonas* spp. from the pipes through hyperchlorination (5 to 10 ppm [$\mu\text{g}/\text{ml}$]) of plant water. However, this attempt was reportedly unsuccessful; the mechanism allowing survival of bacteria in iodophors may also successfully operate to protect bacteria from other halogens, or dead end pipes not under circulation may have provided a reservoir of unexposed organisms available to recontaminate the plumbing system. The manufacturer subsequently moved to a building in which the pipes could be steam sterilized. Steam sterilization may be a more effective means of preventing contamination of iodophor products than chemical treatment of water; however, this remains to be shown.

Other methods of manufacture may also reduce the chances of product contamination. The time the iodophor is allowed to stand in storage tanks or pipes, or both, before bottling should be kept to a minimum, since this may be an important determinant of whether a product becomes con-

taminated. The type of container in which the iodophor is bottled may also be important to prolonged survival of bacteria in contaminated iodophors; preliminary studies in our laboratory have indicated that some plastics (e.g., polyethylene) may support bacterial growth in contaminated iodophors better than glass or other plastics (i.e., polypropylene). However, further studies are necessary to confirm these results, and no formal recommendations can be made at this time.

Pseudomonas spp. have been found frequently in chlorinated water supplies throughout the country at different times (14), and the original source for contamination of the pipes in the plant with *Pseudomonas* spp. was probably the city water. An increased number of samples on different days may have been necessary to document the intermittent presence of *P. aeruginosa* in the water supply of this city. In any case, control of *Pseudomonas* spp. in the water supply would most likely not be an effective means of eliminating bacterial contamination of iodophors.

This investigation helps clarify the probable source of the bacteria which contaminated the Prepodyne solution, and it provides insight into the means of long-term survival of bacteria in iodophors. The exact mechanism of bacterial interaction with the pipe surface to allow for prolonged survival remains unclear. Further insight, however, may be gained through more in-depth studies related to the interaction of pipes and other plant surfaces with iodophors of different composition and different iodine levels. Many of these studies have already been accomplished, and the results are reported and discussed in the accompanying paper (2).

Iodophor antiseptic solutions are currently used in health care for many purposes, including skin antiseptics before surgery, irrigation of wounds and ulcers, and in many instances, incorrectly for disinfection of patient-care items. Clinically adverse effects from use of contaminated antiseptic iodophor solutions in patient care have been documented. In each instance, contamination of the implicated iodophor occurred at the site of manufacture; extrinsic contamination by patient-care providers has not been reported. It is important that manufacturers of iodophors take measures to prevent microbial contamination of their products.

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