APPLIED AND ENVIRONMENTAL MICROBIOLOGY, Apr. 1984, p. 757–762
0099-2240/84/040757-06$02.00/0

Vol. 47, No. 4

Investigations into the Survival of *Pseudomonas aeruginosa* in Poloxamer-Iodine

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Received 8 September 1983/Accepted 29 December 1983

Laboratory investigations were conducted to study potential mechanisms for prolonged survival of *Pseudomonas aeruginosa* in poloxamer-iodine (PxI). *P. aeruginosa* organisms isolated from PxI and adapted for growth in distilled water or found as part of a mixed microbial population from water in a manufacturing plant did not survive more than 15 s after challenge in stock PxI solution. Batches of PxI were compounded in the laboratory to determine the survival and growth of *P. aeruginosa* during the various stages of preparation. No *P. aeruginosa* organisms were recovered from the finished product at 1 min after the addition of iodine-iodide. However, we found *P. aeruginosa* in PxI 48 h after adding sterile PxI to the inside of a naturally contaminated polyvinyl chloride water distribution pipe. These organisms (10^4 CFU/ml) survived for as long as 98 days in contaminated stock PxI after it was removed from the polyvinyl chloride pipe. Both decreasing the free iodine level through addition of potassium iodide and increasing the free iodine level through dilution of the product resulted in an increased length of survival of *P. aeruginosa* in contaminated PxI solution. Comparative survival studies with pipes of different composition revealed that other materials may exert an effect similar to polyvinyl chloride. We concluded that polyvinyl chloride and perhaps other materials may play an important role in the survival of *P. aeruginosa* in iodophors and may be one source of resistant microbial populations when used in manufacturing plants which produce these antimicrobial solutions.

Recent investigations have documented intrinsic microbial contamination of povidone-iodine (PI) and poloxamer-iodine (PxI) solutions. Pseudobacteremia caused by *Pseudomonas cepacia* has been associated with the use of contaminated PI (4, 8, 9, 11), and *Pseudomonas aeruginosa* peritoneal infection has been attributed to the use of contaminated PxI (6, 7, 15). Confirmation of bacterial contamination of iodophors has raised questions concerning the formulation and mechanism of bactericidal activity of iodophor solutions and has left unanswered certain questions as to the reason for prolonged survival of organisms in these solutions. Some of the misconceptions concerning the chemistry and use of iodophors were recently clarified (12).

To better understand the presence of viable organisms in iodophor formulations, we developed a model to induce resistance of organisms to iodophors. We recently took the opportunity to investigate the manufacturing plant which distributed Prepodyne solution (West-Agro Chemical Co., Inc., Westwood, Kans., manufactured for AMSCO Medical Products Division, Erie, Pa.) intrinsically contaminated with *P. aeruginosa* (2). We tested, in the laboratory, hypotheses as to how these solutions became contaminated in the plant. This model for *P. aeruginosa* contamination of iodophors may explain how these solutions became contaminated during manufacture and may be used by formulary chemists as a means of improving the bactericidal quality of iodophors.

MATERIALS AND METHODS

PxI challenge experiments. Distilled water (1.0 ml) containing 1.9×10^7 CFU of *P. aeruginosa* (originally isolated from intrinsically contaminated PxI and adapted for growth in distilled water) and 1.0 ml of water (held for several days at ambient temperature) from a manufacturing plant containing a mixed microbial population (including *P. aeruginosa*) of 3.2×10^6 CFU were separately added to 20.0 ml of filter-sterilized Prepodyne (PxI). Observed initial concentrations of *P. aeruginosa* in PxI were 9.0×10^5 CFU/ml; observed initial concentrations of the mixed microbial population were 1.6×10^5 CFU/ml.

Test suspensions of PxI were thoroughly mixed, and 1.0-ml samples were removed at 15 and 30 s and at 1, 2, 4, and 10 min to determine survival rates. Quantitative assay procedures were identical to those used in previous PI challenge studies (3). For qualitative assay at each sampling time, 1.0 ml of the PxI test solution was added to 9.0 ml of brain heart infusion broth containing 0.5% sodium thiosulfate (BHIS) for iodine complex neutralization. Broths were incubated at 35 to 37°C and observed for turbidity over a 5-day period. Broths showing turbidity were streaked to Trypticase soy agar (BBL Microbiology Systems, Cockeysville, Md.) with 5% sheep erythrocytes (TSAB) and MacConkey agar plates and incubated at 35 to 37°C for 24 to 48 h.

Survival of organisms during batch preparation of PxI. Batches of PxI were compounded in the laboratory to determine the survival and growth of *P. aeruginosa* during the various stages of preparation. Naturally contaminated plant water stored in 1-gal (3.785-liter) plastic containers and contaminated water that had been stored in a section of polyvinyl chloride (PVC) pipe obtained from the manufacturing plant were used to prepare two batches of PxI. Both samples of contaminated water contained *P. aeruginosa*. Other ingredients used in the production of PxI included citric acid, sodium hydroxide, two surfactants, and a solution of iodine-iodide. These ingredients were obtained from the manufacturing plant and when previously sampled were shown to be free of *P. aeruginosa* (2). Ingredients were added to 1,500-ml sterile screw cap flasks in concentrations and sequences which simulated manufacturing procedures; 1,000-ml quantities of each batch were made.

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Microbial plate counts were completed for both batches after each specific ingredient was added during the batch processing. After the iodine-iodide suspension was added, 1.0-ml samples were removed at 1, 4, 10, and 60 min and at 4 and 24 h and were separately added to 9.0 ml of BHIS. In addition, at 10 and 60 min and at 4 and 24 h after the addition of iodine-iodide, duplicate 25-ml samples of the finished product were passed through sterile sampling tubes attached to bacteriological membrane field monitors (0.45 μm; Millipore Corp., Bedford, Mass.): one filter was rinsed with 75 ml of BHIS and aseptically placed on the surface of TSAB plates for a quantitative count; the other filter was removed and placed in 75 ml of BHIS for qualitative assay. All broths and plates were incubated at 35 to 37°C for 24 to 48 h and observed for microbial growth.

At 51 h, both batch formulations were tested for free and available iodine by using the *n*-heptane extraction (17; A. Cantor and M. W. Winicov, U.S. patent 3,028,300, April 1962; M. W. Winicov and W. Schmidt, U.S. patent 3,028,299, April 1962) and thiosulfate titration (18) procedures, respectively.

Recovery of *P. aeruginosa* from PxI after exposure to PVC pipe. PVC (schedule 80) pipe (50.8 cm long, 3.8 cm in diameter) with a coupling close to one end was obtained from the water distribution system of the manufacturing plant proximal to the vat where the contaminated lots of PxI had been mixed. At the time of removal, the pipe was filled with plant water and stoppered at both ends. The water in the pipe was removed and quantitatively cultured. Two colonies of each morphological type were picked to triple sugar iron agar and then identified by standard biochemical and serological procedures (5, 19).

Antimicrobial susceptibility testing was done on selected *P. aeruginosa* isolates recovered from water originally stored in the pipe and iodophor found subsequently to be contaminated (14).

Approximately 250 ml of membrane (0.45 μm) filter-sterilized PxI was deposited into the PVC pipe, and the ends were plugged with sterile rubber stoppers. PVC pipe containing PxI was left at room temperature (25 ± 2°C) in a horizontal position. Samples (50 ml) of PxI were separately filtered through sterile bacteriological membrane field monitors (0.45 μm) at 10 min, 1 h, and 1, 2, and 3 days. After sampling on day 3, the pipe was refilled with additional (250 ml) sterile PxI and subsequently sampled at 1, 2, 3, and 4 days. After filtration, the filters were rinsed with 75 ml of BHIS, aseptically removed, and placed on TSAB plates for enumeration of colonies after incubation. Plates containing filters were incubated for 24 to 48 h at 35 to 37°C.

One 17-ml sample of contaminated PxI was removed at 72 h and transferred to a sterile glass beaker. At 1, 4, and 10 min and at 3 h, samples (5, 5, 5, and 2 ml, respectively) of PxI were removed from the glass beaker, placed separately into 75 ml of broth containing neutralizer, and mixed, and the entire contents were sampled by membrane filtration. The filters were placed onto TSAB plates for incubation and subsequent enumeration of colonies.

Recovery of *P. aeruginosa* from PI after exposure to PVC pipe. A section of schedule 80 PVC pipe (24.1 cm long, 3.8 cm in diameter with a coupling at one end) located proximal to the mixing vats was used in this study. Upon removal, the pipe was filled with plant water, and both ends were stoppered.

The plant water was removed from the pipe in the laboratory, and the pipe was filled with ca. 175 ml of membrane (0.45 μm) filter-sterilized Betadine (Purdue Frederick Co., Norwalk, Conn.). Then 1.0-ml samples of PI were removed at 1, 2, 3, 4, and 7 days from the PVC pipe and separately placed in 9.0 ml of sterile deionized water dilution blanks containing 0.5% sodium thiosulfate (SDWS). In addition, 10-fold dilutions (10^{-1} to 10^{-4}) of a undiluted test portion were made for quantitative assay. All samples were mixed, and the contents were membrane filtered through bacteriological field monitors (0.45 μm). Filters were placed on the surface of TSAB plates and incubated for 24 to 48 h at 35 to 37°C; colonies were counted and recorded per milliliter of test sample.

Resistance of *P. aeruginosa* to varying levels of free iodine. A piece of plant PVC pipe (schedule 80) with a dead end (27.9 cm long, 3.8 cm in diameter) located proximal to mixing tanks was filled with filter-sterilized PxI, and the solution was sampled at various times to determine the level of microbial contamination. After 9 days, samples of this iodophor contained 10^4 CFU of *P. aeruginosa* per ml. This contaminated PxI was then used to make five formulations with various free and total iodine concentrations: stock test solution, 1:2 and 1:4 dilutions of the test solution, and two 50-ml quantities of stock test solution to which 312 and 624 mg of KI had been added. The test solutions were held in 50- and 125-ml Erlenmeyer flasks enveloped in aluminum foil to prevent exposure to light.

At various sampling times, 1.0-ml portions of test solution were removed and separately placed in 9.0 ml of SDWS and assayed by using methods previously described.

Free iodine determinations by the *n*-heptane extraction method (17; A. Cantor and M. W. Winicov, U.S. patent 3,028,300, April 1962; M. W. Winicov and W. Schmidt, U.S. patent 3,028,299, April 1962) were performed at 0 h and at various other sampling times during the study.

Contamination of unused PVC pipe. Two sections of unused schedule 80 PVC pipe (25.4 cm long, 3.8 cm in diameter) were used in these studies. One section of pipe was filled with ca. 180 ml of membrane-filtered PxI, the ends were plugged with sterile stoppers, and the pipe was allowed to stand at ambient laboratory temperature for 1 week (PxI-treated pipe). The second pipe was left empty and stoppered (untreated pipe).

After 7 days, the PxI in the PxI-treated pipe was discarded, and the pipe interior was rinsed twice with sterile deionized water. Both PxI-treated and untreated pipes were then filled with plant water contaminated with *P. aeruginosa* and held at ambient temperature for 1 week. The contaminated water (containing 4.0×10^4 CFU/ml) was then discarded, and both sections were filled with filter-sterilized PxI solution.

Samples (25 ml) were removed from both pipes at 10 min, 1 h, and 1, 2, 6, 7, and 9 days and were membrane filtered for quantitative assay by using the methods previously described. In addition, 1-ml undiluted samples and 1 ml from 10-fold dilutions (10^{-1} to 10^{-3}) were neutralized in 9.0 ml of SDWS and separately membrane filtered.

Contamination of unused pipes of different manufacture and composition. Six different unused pipes were employed for these investigations: white (schedule 40) PVC, stainless steel, and galvanized steel (all 30.4 cm long, 3.8 cm in diameter); gray (schedule 40) PVC and copper (both 30.4 cm long, 3.1 cm in diameter); and a piece of glass tubing (38.1 cm long, 2.5 cm in diameter). All pipes were sterilized with ethylene oxide gas. The six pipes were filled with contaminated plant water containing *P. aeruginosa* and held at room temperature for 1 week; pipe ends were fitted with sterile rubber stoppers. After 7 days, the water was removed from

each pipe, and all were filled with membrane-filtered PxI. Microbial counts of water removed from the test pipes ranged from 3.4×10^4 to 9.9×10^4 CFU/ml at 7 days.

Samples of PxI were removed from each test pipe at 10 min, 1 h, and 2, 5, 7, and 20 days for quantitative microbial assay as described above.

RESULTS

PxI challenge and batch experiments. Challenge organisms survived for only a short period of time when added to stock PxI. *P. aeruginosa* from the mixed microbial population in plant water survived for 15 s by both quantitative and qualitative methods but not for 30 s. Gram-positive rods (morphologically observed as raised yellow and white colonies) in the mixed population survived for 15, 30, and 60 s but not for 2 min. *P. aeruginosa* organisms adapted to distilled water and challenged with PxI were not recovered at 15 s.

Microbial levels of 10^6 CFU/ml (2-log increase) were observed in both laboratory prepared batches of PxI before iodine-iodide was added. When iodine-iodide solution was added 51 h later, no *P. aeruginosa* organisms were isolated from 1.0-ml samples of PxI at 1 min; the 25-ml membrane-filtered samples tested were also negative. The batch of PxI made with contaminated plant water contained 1.07% available iodine and 1.52 ppm (μg/ml) free iodine; the batch made with contaminated water from PVC had 1.02% available iodine and 0.94 ppm free iodine.

Recovery of *P. aeruginosa* from PVC pipe. *P. aeruginosa* was recovered from PxI 2 days after the PxI was added to the inside of contaminated PVC pipe (Table 1). The microbial counts in PxI generally increased and reached a level of more than 100 CFU/ml at 4 days. *P. aeruginosa* organisms recovered from water initially present in the PVC pipe and from the subsequently contaminated PxI were serogroup O:3 and had identical antimicrobial susceptibility patterns. Isolates were resistant to ampicillin, cefamandole, cefoxitin, cephalothin, chloramphenicol, sulfamethoxazole-trimethoprim, and tetracycline and sensitive to amikacin, carbenicillin, gentamicin, and tobramycin. Organisms isolated from these two environmental sources had the classic biochemical reactions of *P. aeruginosa*.

P. aeruginosa was recovered from PxI after removal of PxI from PVC pipe. At 3 h, 100 CFU/ml were recovered.

P. aeruginosa was also recovered from PI after exposure to the inner surface of contaminated PVC pipe. CFU observed per milliliter were as follows: 1 at 2 days, 1.6×10^3 at 3 days, 2.0×10^3 at 4 days, and 6.7×10^4 at 7 days. The free iodine content of the test PI was 1.07 ppm at 1 day.

Effect of various levels of free iodine on organisms present in pipe-contaminated PxI. Prolonged survival of *P. aeruginosa* was observed with all PxI solutions studied (Table 2). Organisms were recovered from the stock test solution at 98 days but not at 133 days, from the 1:2 dilution at 133 days but not at 168 days, from the 1:4 dilution at 168 days but not at 192 days, and from the stock test solutions with added KI at 192 days.

TABLE 1. Recovery of *P. aeruginosa* from PxI during exposure to the inside of a contaminated PVC water distribution pipe

Sampling time	Quantitative count (CFU/ml)
10 min	0
1 h	0
1 day	0
2 days	0.5
3 days[a]	2
1 day	1.4
2 days	5.4
3 days	48
4 days	>100

[a] At 3 days, the PVC pipe was refilled with 250 ml of filtered PxI.

Over a 168-day period, free iodine levels (ppm) associated with these test PxI solutions ranged from 0.52 to 0.67 for stock solution, 1.03 to 1.67 for the 1:2 dilution, 2.01 to 3.26 for the 1:4 dilution, and 0.20 to 0.32 and 0.10 to 0.17 for stock PxI containing 312 and 624 mg of KI, respectively (Table 3). Free iodine levels of each test solution gradually decreased during the 168 days.

Contamination of unused PVC pipes and of unused pipes of different composition. *P. aeruginosa* was recovered from PxI after the inside of unused PVC pipe was exposed to contaminated water. Higher microbial counts were obtained from the pipe not pretreated with PxI than from the PxI-pretreated PVC pipe (Table 4).

Contamination of other types of pipe with *P. aeruginosa* is shown in Table 4. These organisms were isolated from PxI after exposure to artifically contaminated PVC, stainless steel, copper, and glass pipes, but not galvanized steel pipe.

DISCUSSION

In this investigation, experiments were designed to look at potential models for the development of *P. aeruginosa* resistance to iodophors. Experiments were designed to simulate, as closely as possible, conditions which could have occurred in the plant which distributed intrinsically contaminated Prepodyne solution. Details of the plant investigation which gave us some clues for the design of these experiments are reported in the accompanying paper (2).

First, we challenged plant water which contained *P. aeruginosa* in a mixed microbial population and *P. aerugiosa* which had been adapted to grow in distilled water with PxI to determine whether this organism was inherently resistant to iodophors. We found no evidence for resistance under the laboratory conditions described; *P. aeruginosa* from the mixed microbial population was recovered after 15 but not 30 s, and *P. aeruginosa* which had been adapted for growth in distilled water was not recovered after 15 s of exposure to PxI.

We then considered that under conditions of PxI manufacture, where hours or days might have elapsed between the addition of various components, growth of bacteria in preliminary stages might account for a resistant population in the final product. We prepared PxI by using raw ingredients, including contaminated water obtained from the plant. Large concentrations of a mixed microbial population (10^6 CFU/ml), including *P. aeruginosa*, were observed during the various stages of simulated batch production in the laboratory. However, survival of *P. aeruginosa* could not be shown from samples of PxI after the addition of iodine-iodide to the batch, the final production step. Thus, we were unsuccessful in inducing resistance by simulating PxI manufacture in the laboratory.

We next examined the role of PVC pipe in the protection of *P. aeruginosa* from the microbiocidal action of PxI in a more direct fashion. PVC pipe was chosen for study for the following reasons. (i) PVC pipe was found throughout the manufacturing plant and was used to distribute water into mixing tanks and to distribute finished product from the mixing tank into the final product containers. (ii) Previous studies have shown that the interior surface of PVC pipe

TABLE 2. Survival of *P. aeruginosa* in five test formulations of contaminated PxI after removal from PVC pipe

Sampling time	CFU/ml in the following formulation:				
	Stock[a]	1:2 dilution	1:4 dilution	Stock[b] with 312 mg of KI	Stock[b] with 624 mg of KI
1 min	8.4×10^4	4.5×10^4	2.0×10^4	7.2×10^4	9.1×10^4
60 min	5.9×10^4	4.3×10^4	1.7×10^4	8.3×10^4	8.7×10^4
4 h	12×10^4	4.4×10^4	0.8×10^4	8.4×10^4	5.3×10^4
1 day	62×10^4	37×10^4	7.0×10^4	45×10^4	47×10^4
3 days	16×10^4	15×10^4	7.3×10^4	15×10^4	47×10^4
7 days	11×10^4	13×10^4	0.8×10^4	10×10^4	8.0×10^4
15 days	8.0×10^4	7.0×10^4	1.2×10^4	11×10^4	21×10^4
63 days	6.7×10^4	6.5×10^4	4.2×10^4	6.0×10^4	10×10^4
98 days	1.0×10^4	8.7×10^4	3.1×10^4	7.3×10^4	10×10^4
133 days	—[c]	4.1×10^4	2.0×10^4	2.5×10^4	3.3×10^4
168 days	—	—	0.1×10^4	0.2×10^4	2.2×10^4
192 days	NS[d]	NS	—	0.8×10^4	5.9×10^4

[a] There was 1.9×10^4 CFU of *P. aeruginosa* per ml of PxI at the beginning of the study.
[b] Fifty milliliters of stock PxI.
[c] —, No recovery.
[d] NS, Not sufficient quantity to sample.

used for water distribution may become heavily colonized with *Pseudomonas* spp. (Informal Quarterly Report, April to June 1975, Phoenix Laboratories Division, Centers for Disease Control). (iii) Scanning electron micrographs of the interior of PVC pipes obtained from the implicated plant proximal to the mixing tank showed heavy colonization with bacilli imbedded in an amorphorus matrix (13). These organisms may have been *P. aeruginosa* because water sampled within the plant revealed heavy growth of *P. aeruginosa*. (iv) Product distribution lines were frequently flushed with water which was allowed to remain within these lines until new batches of product were formulated.

Concentrations of *P. aeruginosa* up to 10^4 CFU/ml were recovered from stock PxI held in PVC pipe previously contaminated with plant water. Although this plant water initially contained a mixed microbial population, only *P. aeruginosa* was recovered from the PxI. Additionally, *P. aeruginosa* was recovered from PxI for up to 3 months after the contaminated solution was removed from the pipe and placed in glass beakers.

With the discovery of a mechanism for producing extraordinarily iodine-resistant *P. aeruginosa*, we were able to perform several survival studies, using various formulations of *P. aeruginosa*-contaminated PxI designed to alter the free and total iodine content of the solution.

Dilutions of PxI resulted in an expected increase of the free iodine concentration from 0.66 ppm for stock solution to 3.26 ppm for the 1:4 dilution. Increased free iodine concentrations with PI dilutions have been previously observed (3). Survival studies of *P. aeruginosa* in these solutions were both surprising and paradoxical; the duration of *P. aeruginosa* survival progressively increased from 98 to 133 days to 133 to 168 days as the free iodine concentration increased. These results are not what one would anticipate, based on previous reports (3). On the other hand, prolonged survival of *P. aeruginosa* in PxI to which potassium iodide—known to lower the free iodine concentration of iodophor preparations—had been added was in keeping with expected results (1).

Since the manufacturing plant also contained pipes composed of material other than PVC, we extended our model to determine whether resistance of *P. aeruginosa* to PxI could be induced with galvanized steel pipe obtained from the water distribution system of the implicated plant and fresh pipes of different manufacture and composition. Fresh pipes of different composition varied in their qualitative and quantitative ability to induce PxI resistance after these pipes were colonized with *P. aeruginosa* in the laboratory.

Contaminated white PVC pipe demonstrated higher microbial counts after exposure to PxI than did gray and untreated PVC (Table 4). Additionally, low numbers of *P. aeruginosa* were recovered from contaminated stainless steel and glass pipes after exposure to PxI; organisms were not recovered from contaminated galvanized steel pipe. Differences in microbial recovery from PxI may be due to the time allowed for conditioning of the pipes. In these studies, contaminated water was added to the interior of pipes and left to incubate for 7 days. Perhaps a longer

TABLE 3. Free iodine concentrations of five test formulations of PxI used in resistance studies

Sampling time	Free iodine level (ppm)[a] in the following formulation:				
	Stock	1:2 dilution	1:4 dilution	Stock[b] with 312 mg of KI	Stock[b] with 624 mg of KI
0 h	0.66	1.67	3.26	0.32	0.17
12 days	ND[c]	1.45	3.10	ND	ND
14 days	0.64	1.51	2.94	0.30	0.13
21 days	0.67	1.44	2.80	0.25	0.12
85 days	0.52	1.13	2.39	0.23	0.10
168 days	ND	1.03	2.01	0.20	0.11

[a] Determined by the *n*-heptane extraction method.
[b] Fifty milliliters of stock PxI.
[c] ND, Not done.

TABLE 4. *P. aeruginosa* contamination of unused pipes of different composition

Pipe composition[a]	CFU/ml after a contact time of:								
	10 min	1 h	1 day	2 days	5 days	6 days	7 days	9 days	20 days
PVC untreated	—[b]	—	1	8	ND[c]	4.0	6.0	2.0	ND
PVC pretreated with PxI	<1	—	—	<1	ND	—	—	—	ND
Gray PVC	—	—	ND	—	2	ND	2.0	ND	—
White PVC	—	—	ND	11	100	ND	180	ND	390
Stainless steel	—	—	ND	<1	<1	ND	2.0	ND	3.0
Galvanized steel	—	—	ND	—	—	ND	—	ND	—
Copper	—	—	ND	96	TNTC[d]	ND	1.1×10^4	ND	—
Glass	—	—	ND	—	<1	ND	—	ND	2.0

[a] Results shown in this table represent two different experiments: artificial contamination of unused PVC untreated and pretreated pipe and artificial contamination of six unused pipes of different composition (two PVC and one each of stainless steel, galvanized steel, copper, and glass).
[b] —, No recovery.
[c] ND, Not done.
[d] TNTC, Too numerous to count.

contact time of contaminated water with the interior pipe surface would enhance better conditioning of the pipes and result in greater recovery of organisms from PxI. The interior surface of copper pipe was colonized quite easily after exposure to contaminated water. High numbers of *P. aeruginosa* were recovered from PxI at 7 days (1.1×10^4 CFU/ml), but no organisms were isolated from this solution at 20 days. A color change was observed with the PxI solution after exposure to copper pipe; at 48 h the PxI was bright yellow, whereas at 20 days the iodophor solution had turned light blue. These color changes indicate a chemical reaction between copper pipe and PxI and suggest a toxic effect on organisms, as observed by the dramatic drop in recoverable organisms at 20 days. The results of this comparative study should be viewed as preliminary; recommendations to manufacturers regarding construction or modification of facilities or procedures based on these data are thus unwarranted at this time.

We extended our investigations into the survival of *P. aeruginosa* by adding a different iodophor, a PI, to contaminated PVC water distribution pipe. Although studies with PI were not as extensive as those with PxI, *P. aeruginosa* at 6.7×10^4 CFU/ml was recovered from PI at 7-days exposure to the PVC pipe. Most certainly these organisms were associated with a resistant population.

The ability of *P. cepacia* and *P. aeruginosa* organisms to survive in iodophor solutions for long periods of time has mystified many scientists. Indeed, this mystery has been compounded by the absence of a suitable model for induction of survival of these organisms in iodophors under laboratory conditions. We believe this experimental model may be useful in studying further the mechanism of *Pseudomonas* resistance to iodophors. Although it is generally accepted that the free iodine content of iodophor solutions is an active microbiocidal component of these solutions, additional work needs to be done to verify this assumption. Some cationic and anionic surfactants are known to interact with the outer structure of bacteria and to exert a bacteriostatic or bactericidal effect. Inherent polymer properties have been suggested to exert a complementary effect to the bactericidal action of free iodine (16). Other physical or chemical mechanisms associated with the iodine complex, such as the total iodine present, the ability to penetrate, and the amount of surface tension, may also play an important role in the susceptibility of organisms present in a resistant or protected microbial population.

Other questions remain. Do *P. aeruginosa* organisms multiply during their exposure to PxI while the contaminated solution is in the pipe, or are they shed (without significant multiplication) from the pipe walls into the solution? When contaminated solutions are removed from the pipe, is there a balance between the multiplication and death of *P. aeruginosa* organisms (i.e., are they in a stationary growth phase?), or are vegetative organisms merely persisting without multiplication until the time of sampling? What accounts for their rather abrupt, unanticipated die-off after removal of contaminated solutions from the pipe? Do *P. aeruginosa* organisms growing on surfaces, particularly PVC, acquire intrinsic resistance to iodine? Does the glycocalyx-like structure decribed by Costerton et al. (10), which was observed on electron micrographs of the interior of the pipe surfaces, shield microorganisms from the microbiocidal action of the iodophor? These and other questions all need further laboratory study; many of these questions may soon be answered, as a model is now available to show prolonged survival of organisms in iodophors.

ACKNOWLEDGMENTS

We thank Anita K. Highsmith, Nosocomial Infections Laboratory Branch, and Bertha C. Hill, Antimicrobics and Infection Mechanisms Branch, Hospital Infections Program, Center for Infectious Disease, Centers for Disease Control, for completion of serological typing and antimicrobial susceptibility testing of *P. aeruginosa*, respectively. In addition, the assistance provided by West-Agro Chemical Co., Inc., in acquiring PVC pipe for laboratory testing was most appreciated.

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