Prolonged Survival of *Pseudomonas cepacia* in Commercially Manufactured Povidone-Iodine

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*Pseudomonas cepacia* organisms were recently recovered from a povidone-iodine antiseptic solution. During the subsequent investigation, laboratory studies were initiated to determine the survival time of these organisms in the iodophor solution, which contains 1% titratable iodine. The solution was sampled weekly upon receipt in our laboratory, and *P. cepacia* was subsequently recovered through 29 weeks of sampling. Current laboratory data and lot production date information from the manufacturer indicate that *P. cepacia* survived for up to 68 weeks from the time of manufacture. Scanning electron microscopic examination of contaminated solution demonstrated bacterial cells embedded in extracellular material.

Epidemiologic investigations have been completed involving a contaminated povidone-iodine (PI) antiseptic solution (Clinidine, lot 823529; expiration date, September 1991; manufactured by the Clinipad Corp., Guilford, Conn.). Peritoneal infections in infants and false-positive results have been associated with the use of an intrinsically contaminated iodophor antiseptic solution (7). This contaminated solution was being used for skin antisepsis before venipuncture and to disinfect the tops of blood culture bottles and multidose vials of dialysis fluid additives, peritoneal fluid administration set connectors, and ports of peritoneal dialysis systems. In this report, we describe the 68-week survival of *P. cepacia* in a 1-gal (ca. 4-liter) container of commercially prepared PI antiseptic germicidal solution containing 1% available iodine.

The following general procedures were used to sample the PI for bacterial contamination. For weekly sampling, the container of PI was thoroughly mixed and a 1.0-ml sample was removed and placed in a 9.0-ml sterile deionized water dilution blank containing 0.5% sodium thiosulfate for iodine complex neutralization. In addition, 10-fold dilutions (10^{-1} to 10^{-7}) of an undiluted test portion were made for quantitative assay. Samples were mixed, and the contents were membrane filtered through bacteriologic 0.45-μm field monitors (Millipore Corp., Bedford, Mass.). As microbial counts decreased, larger aliquots (60 to 100 ml) of PI were removed and sampled by membrane filtration, and the filters were rinsed with 75 ml of brain heart infusion broth containing 0.5% sodium thiosulfate. All filters were aseptically removed and placed on the surface of Trypticase soy agar plates (BBL Microbiology Systems, Cockeysville, Md.) with 5% sheep erythrocytes and incubated for 96 h at 35 to 37°C; colonies were counted and recorded per milliliter of PI.

The available or titratable iodine level present in the contaminated bottle of PI was determined by sodium thiosulfate titration (15), and free iodine concentrations were performed colorimetrically from the amount of iodine (I\textsubscript{2}) extracted into n-heptane, an immiscible organic solvent (14).

For scanning electron microscopy (SEM), 10 ml of contaminated PI was mixed with an equal volume of a fixative solution consisting of 5% glutaraldehyde in cacodylate buffer (0.067 M; pH 6.2) with 0.30% ruthenium red. This preparation stood for 24 h at room temperature and was filtered through a 0.4-μm Nuclepore filter (Nuclepore Corp., Pleasanton, Calif.). Filters were rinsed in Sorenson buffer of pH 5.0, placed in 1% osmium tetroxide for 1 h, dehydrated in a graded ethanol series, exposed to hexamethyldisilazane (Polysciences, Inc., Warrington, Pa.) for 1 h, and held in a desiccator overnight. Filters were mounted on aluminum stubs, sputter coated with gold, and examined in a Philips SEM 515.

The microbial counts and survival times for *P. cepacia* in the contaminated PI are shown through 30 weeks of sampling (Fig. 1). The counts of *P. cepacia* per milliliter varied during the 29 weeks of survival in PI. Initial sampling of PI in June 1989 demonstrated counts of 14/ml. These counts increased to a high of 2.8 × 10^7/ml at 10 weeks, which was followed by fluctuating microbial counts during 11 to 29 weeks and complete die off of *P. cepacia* at 30 weeks. This lot of PI was manufactured in September 1988, thus indicating a prolonged survival period of 68 weeks from the date of manufacture. The titratable iodine present in the contaminated bottle of PI was 1.0%, and free iodine levels ranged from 0.23 to 0.32 ppm (0.23 to 0.32 μg/ml) during three sampling periods. SEM examination of contaminated PI demonstrated bacterial cells embedded in extracellular material and strands of glycocalyx between cells (Fig. 2).

The major question to be answered from this investigation is by what mechanism did *P. cepacia* organisms survive in a germicidal antiseptic solution such as PI. To date, the only laboratory model available to demonstrate the prolonged survival of microorganisms in iodophor antiseptic solutions involves exposing these solutions to the inside surfaces of naturally contaminated polyvinyl chloride (PVC) water distribution pipe sections obtained from a manufacturing plant (2, 4). In one study, continuous exposure of poloxamer-iodine to naturally contaminated pipe resulted in a level of 10^6 CFU of *P. aeruginosa* per ml of poloxamer-iodine at 9 days (2). Using this contaminated stock solution, we found that *P. aeruginosa* survived for as long as 98 days after its removal from the PVC pipe.

How do we account, then, for three documented instances of intrinsically contaminated iodophor antiseptic solutions? All intrinsically contaminated iodophor antiseptic solutions...
FIG. 1. Survival of *P. cepacia* in an intrinsically contaminated PI antiseptic solution. Symbol: *, CFU per ml = 0 and CFU per 100 ml = 0.

occurred because of the lack of proper manufacturing practices used in their preparation. For example, one instance of *P. cepacia* contamination resulted from heavily contaminated deionizing resin beds that subsequently contaminated the process water and distribution system during the manufacture of PI (5, 11). In another, poloxamer-iodine solution was contaminated with *P. aeruginosa* 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 (1, 3, 6, 13). Finally, contaminated process water and an antiquated PVC water distribution system were the most likely sources of *P. cepacia* in the most recent episode of PI contamination (1). We believe the extended survival of *P. cepacia* for 68 weeks in PI as occurred in this investigation is due to the extracellular glycocalyx-like material that microorganisms form and deposit on various types of surfaces (8–10). The shedding of this material from interior PVC wall surfaces most probably was the origin of intrinsic microbial contamination. The SEM of contaminated PI certainly show embedded cells in a potentially protected state (Fig. 2).

The fluctuating microbial counts shown in Fig. 1 are of great interest. These results could be interpreted as growth of *P. cepacia* over time or the gradual breakdown of extracellular material containing embedded *P. cepacia* cells. The dissolution of extracellular mass could yield smaller matrices with more numerous CFU and a resultant increase in colony count. If growth is occurring, then the cells must be multiplying within the extracellular matrix and not as free-floating cells in the PI solution. Samples removed from

FIG. 2. SEM of *P. cepacia* cells in intrinsically contaminated PI. Note strands of glycocalyx (G) between cells and cells embedded (E) in extracellular material. Bar, 5 μm.
nonmixed and subsequently mixed PI demonstrated similar logarithmic survival counts at each sampling. This finding suggests the homogeneous dispersal of cells from the extracellular matrix. Previous studies have shown that *P. aeruginosa* organisms isolated from an iodophor antiseptic and adapted to water were not recoverable at 15 s when challenged to stock poloxamer-iodine at $9.0 \times 10^2$ CFU/ml (2). If organisms are multiplying within the matrix, then the free iodine must affect the protective matrix before microbicidal action and cellular death are noted. The 68-week survival period for *P. cepacia* we describe supports these hypotheses.

Low, free iodine levels as measured in this contaminated lot of PI might affect the overall quality of an iodophor antiseptic. A typical 10% PI solution containing 1% available iodine (10,000 ppm) will continually release free iodine to provide an equilibrium value of approximately 1 ppm (1, 12). The low I$_2$ values found in the contaminated bottle of PI and the higher values associated with other Clinidine lots suggest that the product was not being formulated uniformly. One percent available iodine was present in the contaminated bottle under test and suspect lot of PI as specified on the product label. Since free iodine is the major microbicidal species in iodophor solutions, the presence of low levels might affect the bactericidal effectiveness of formulated PI antiseptic solutions.

Of 19 1-gal bottles of the implicated lot sampled, 6 (31.5%) were positive for *P. cepacia*, with counts ranging from $10^5$ to $10^9$ per ml of PI. This finding suggests that only part of the PI lot was exposed to contaminated surfaces within the manufacturing plant. A plant investigation was initiated to determine the source of *P. cepacia* contamination. Upon arrival at the plant, the process water and product distribution system had been revamped and no PVC piping was available for examination. Extrinsic contamination of this PI product during packaging would be remote because of the high microbial counts observed in the contaminated bottles and the inability of challenge studies to demonstrate microbial recovery after short exposure times.

Manufacturing plant water used to compound iodophor products can become contaminated with a variety of gram-negative water bacteria. This contamination, if left uncontrolled, can colonize water and product distribution lines and affect the manufacture and quality of formulated iodophors. It is common and good practice to perform bacteriologic quality control on such water (12). Scheduled bacteriologic quality control checks of process water and finished product, maintenance of resin beds and filters, and sanitation of water and product distribution pipes (e.g., $60^\circ$C hot water for 1 h) would all seem prudent as remedial measures. Manufacturers of iodophors and other health care professionals should be aware that pipes or other surfaces colonized with bacteria may be a source of contamination.

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**LITERATURE CITED**


