Editorial

Iodophor Antiseptics: Intrinsic Microbial Contamination with Resistant Bacteria

Roger L. Anderson, PhD

The development of hospital infections following use of an iodophor antiseptic solution has raised its ugly head again. The article "*Pseudomonus* aeruginosa Infections Associated with Use of Povidone-Iodine in Patients Receiving Continuous Ambulatory Peritoneal Dialysis" by Goetz and Muder *(Infect Control Hosp Epidemiol* 1989; 10:447-450) describes an epidemiologic investigation showing that use of povidone-iodine solutions to cleanse the catheter site of patients receiving continuous ambulatory peritoneal dialysis was significantly associated with *P aeruginosa* infections (i.e., peritonitis and catheter site infections). Unfortunately, *P* aeruginosa organisms were not recovered from any of the sampled povidone-iodine products used in the hospital, nor were appropriate typing or marker systems (e.g., serotyping, plasmid profiles, pyocin typing or ribotyping) used to denote whether the clinical strains of *P aeruginosa* were similar or dissimilar. This added microbiologic information would certainly have enhanced the epidemiologic findings described in this article. This editorial reviews several instances in which viable bacterial pathogens (pseudomonads) have been isolated from commercially available iodophor antiseptic solutions and points out the emerging hypotheses concerning the mechanism of extreme iodine resistance.

The resistance of microorganisms to antimicrobial agents in general and chemical germicides in particular is controlled by the culture history and strain of the microorganism and the nature of the suspending menstruum, as well as by 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 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.[3-7] Pseudobacteremia caused by *Pseudomonas cepacia* has been associated with the use of contaminated povidone-iodine, and peritoneal infections caused by *P* aeruginosa have been attributed to the use of contaminated polox-amer-iodine.

Presently, the Centers for Disease Control (CDC) is conducting epidemiologic and laboratory investigations involving a contaminated povidone-iodine antiseptic solution. 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-

From the Nosocomial *Infections Laboratory Branch, Hospital Infections Program, Centers for Disease Control, Atlanta, Georgia.*

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Address reprint requests to Roger L. Anderson, PhD, Hospital Infections Program, CVC, 1600 Clifton Road, 1-B341, Atlanta, GA 30333.

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iodine antiseptic solution.[8] These contaminated iodophor antiseptics were being used in various ways to prepare the skin before venipuncture and to disinfect the tops of blood culture bottles and multidose vials of dialysis fluid additives, peritoneal fluid administration set connectors, catheter and machine tubing, and ports of peritoneal dialysis systems.

Since the initial recognition of intrinsic microbial contamination of iodophor antiseptics, many laboratory investigations have been performed to determine the mechanism by which pseudomonads could survive in iodophor antiseptic solutions for prolonged periods of time.[9,10] Until that time, microbiologists who were knowledgeable about the microbicidal activity associated with iodine solutions did not believe that *P cepacia* and *P aeruginosa* could survive for long periods in povidone-iodine or poloxamer-iodine containing 1% available iodine (i.e., titratable iodine). In addition, studies have clarified iodine and povidone-iodine chemistry and the subsequent effect of dilution on the bactericidal activity of iodophor antiseptics,[11,12] which has led to a greater understanding of the resistance mechanisms involved and of the dilution phenomenon associated with increased microbicidal efficiency.

The chemistry and subsequent bactericidal activity associated with iodophor antiseptics are complex and often misunderstood. Iodine in the elemental form (1,) can be present in two chemical disinfectant formulations:

- as solutions of iodine and iodide (aqueous or alcoholic tincture), and
- as iodophors—chemical complexes that contain a mixture of elemental iodine, iodide and a solubilizing agent or carrier. Common carriers of iodine used in commercial iodophors include polyvinylpyrrolidone (povidone) and ethoxylated nonionic detergents (poloxamers). The complexing of iodine with a carrier not only increases the solubility of elemental iodine, but it also provides for a sustained-release reservoir of free iodine.

Much confusion has existed in the scientific community concerning the terms "free iodine" and "available iodine." Free iodine (I_2) is the chemical and microbicidal species, whereas available iodine refers to the total amount of iodine that can be titrated with sodium thiosulfate. Concentrations of free and available iodine can vary depending on the formulation. For example, a typical 10% povidone-iodine solution containing 1% available iodine (10,000 ppm) will continually release free iodine to provide an equilibrium value of approximately 1 ppm. Thus, the complexed iodine (i.e., most of the available iodine) is held as a reservoir and released as free iodine to maintain the 1 ppm equilibrium level. Iodophor formulations diminish the unpleasant odor, irritation, tissue staining and corrosion of some metal surfaces that occur with pure aqueous and alcoholic iodine disinfectant solutions. They are marketed as skin antiseptics and chemical disinfectants.

Recent investigations have demonstrated that increased concentrations of free iodine will occur when an iodophor antiseptic is diluted with water. These elevated levels of free iodine directly correlate with increased microbicidal activity.[11,12] Most likely, the aqueous dilution of povidone-iodine and poloxamer-iodine causes a weakening of the iodine linkage to the carrier polymer with a concomitant increase in the amount of free iodine in solution. The following factors favor increased complexation of iodine to the carrier and thereby lower the free iodine concentration in solution:

- higher carrier content;
- higher carrier molecular weight (as in the case of povidone);
- higher iodide concentration; and
- lower temperatures.

Therefore, the specific formulation, the type and concentration of the carrier molecule and the presence of water contribute to the amount of free iodine, and thus to the bactericidal effectiveness of the iodophor.

To date, prolonged survival of *P aeruginosa* could be shown in the laboratory only by exposing iodophor antiseptic solutions (both povidone-iodine and poloxamer-iodine) to the inside surfaces of naturally contaminated polyvinyl chloride (PVC) water distribution pipe segments obtained from a manufacturing plant. In one study, we isolated *P aeruginosa* from a poloxamer-iodine solution 48 hours after membrane-filter sterilized poloxamer-iodine was added to the interior surface of a naturally contaminated PVC water pipe.[10] Continuous exposure of poloxamer-iodine to this pipe resulted in a level of 10^4 colony forming units (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 used as the reservoir for prolonged microbial survival studies. Using this contaminated stock solution, we found that *P aeruginosa* (10^4 CFU per ml) survived for as long as 98 days after its removal from the PVC pipe. Based on this investigation, we developed a suitable laboratory model for induction of survival of *Pseudomonas* species and for studying their mechanism of resistance to iodophors. We were unable to demonstrate the survival of *P aeruginosa* (10^6 CFU per ml) after direct iodophor challenge experiments.

We also prepared poloxamer-iodine by using new ingredients including contaminated water obtained from the plant. Large concentrations of *P aeruginosa* (10^6 CFU per ml) 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 poloxamer-iodine after the addition of iodine-iodide to the batch, the final production step. Thus, we were unsuccessful in inducing resistance by simulating poloxamer-iodine manufacture in the laboratory. The population of *P aeruginosa* in poloxamer-iodine after its removal from the PVC pipe was extraordinarily resistant to iodine, and the D value (i.e., the time required to reduce the population by 1 log) was shown to be approximately 1.7 hour at 25°C.[13] By comparison, the D value of *P aeruginosa* in

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culture, when inoculated into poloxamer-iodine in large numbers, cannot be calculated because the rate of kill is almost instantaneous.

How do we account for three documented instances of intrinsically contaminated iodophor antiseptic solutions? The laboratory studies completed and discussed above give some indication why these solutions were found contaminated with pseudomonads. All intrinsically contaminated iodophor antiseptic products described occurred because of the lack of good manufacturing practices used in their preparation. For example, one instance of *P cepacia* contamination was believed to be the result of heavily contaminated deionizing resin beds that subsequently contaminated the process water and distribution system during the manufacture of povidone-iodine. 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 bottlmg area) prior to bottling. 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 povidone-iodine contamination.

The extended survival of, and the ability to isolate microorganisms in these iodine-containing solutions may be caused by the extracellular glycocalyx-like material that microorganisms form and deposit on a variety of different types of surfaces.[14-16] 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 (biolilms). Once these masses are formed, antibacterial agents such as disinfectants or antiseptics must saturate the matrix before they can kill bacteria .

The prolonged survival of *Serratia marcescens* in 2% chlorhexidine was most likely due to the embedding of these microorganisms in a thick matrix that adhered to the walls of storage jugs containing this antiseptic solution.[17] Viable S *marcescens* was recovered from 2% 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.[9] Scanning electron-microscopy (SEM) micrographs of these pipes demonstrated large concentrations of rod shaped and coccobacillary cells embedded in interior deposits of the pipe.

In addition, we have completed studies using artificially contaminated PVC pipes to demonstrate the resistance to *P aeruginosa* in iodophors.[18] 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 a&ion of iodophor and phenolic germicides.[18] SEM examination of the most recent intrinsically contaminated povidone-iodine solution has demonstrated large numbers of bacterial cells embedded in extracellular material (Figure). These physically protected *P cepacia* cells have survived in povidone-iodine solution for 41 weeks.

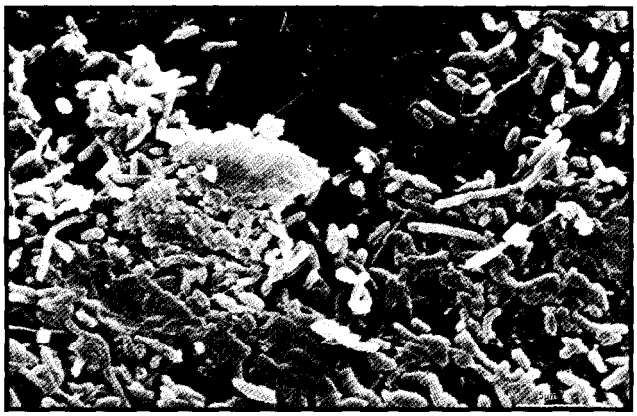

Figure. SEM of *P cepacia* protected cells in intrinsically contaminated povidone-iodine, ×52,800.

Other investigators have hypothesized the importance of cell masses such as the glycocalyx as a potential reservoir of microbial contamination.[19,20] 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.

Iodophors still fill a unique and useful role in current medical practice. Extrinsic microbial contamination of these products has not been demonstrated because of the bactericidal nature of these antiseptic solutions on contaminating organisms or challenge inocula. On the other hand, microbial contamination of iodophors from unopened bottles has occurred in three different instances. This contamination was intrinsic in nature and resulted from production practices used in the manufacturing plants.

Process water used to formulate iodophor products can become contaminated with pseudomonads or other naturally occurring gram-negative water bacteria. This contamination, if left uncontrolled or sporadically treated, can colonize water and product dis-

tribution lines and effect the manufacture and quality of formulated iodophors. The physical thickness of cellular and extracellular material that forms as a result. of bacterial attachment on PVC pipe surfaces could protect organisms from the action of germicidal chemicals and serve as a continuous reservoir for microbial contamination in distribution pipes and of water flowing through pipes.

It has been reported that it is common and good practice to perform bacteriologic quality control on such waters.[20,21] Scheduled bacteriologic quality control checks of process water and finished product, maintenance of resin beds and filters, and sanitization of water and product distribution pipes (e.g., 60°C hot water for one hour) would all seem prudent as remedial measures. Manufacturers of iodophors and other healthcare professionals should be aware that pipes or other surfaces colonized with bacteria may be a source of contamination. These adherent bacteria could contaminate commercial products produced within manufacturing or pharmaceutical plants and other solutions prepared in the hospital or laboratory setting.

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