Resistance of antimicrobial skin preparations to saline rinse using a seeded bacteria model

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Objective: We describe a randomized blinded study to evaluate the antimicrobial persistence following saline exposure of 2 commercially available skin antiseptic agents. One agent contained iodine povidone in alcohol and the second contained chlorhexidine gluconate in alcohol.

Method: Both agents were applied to the forearms of 36 healthy subjects according to manufacturers' instructions and allowed to dry. The sites were then exposed to either a saline rinse or to a saline-saturated gauze, similar to the challenges that preps would face during most surgical procedures. Two analyses were performed: (1) An indicator organism was seeded onto the treated sites. After 30 minutes, samples were collected from the treated sites and surviving bacterial colonies were enumerated and log reductions calculated. (2) The saline-saturated gauze was analyzed chemically for presence of chlorhexidine or iodine.

Results: The baseline densities (stated as logarithms of colony forming units "log CFU") of the sites to which the agents were applied had statistically equivalent microbial densities. Both agents reduced the density of organisms in a statistically significant manner. Chemical analysis of the gauze samples indicated that 35 of 36 samples had detectable chlorhexidine while no samples had detectable iodine (P < .0001).

Conclusion: The results indicate that chlorhexidine is removed by saline-soaked gauze while the iodine povidone in water-insoluble film remains intact under the same conditions. The implication is that similar results may occur in surgery when saline is used.

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Skin antisepsis prior to surgery has been a standard of practice for decades, although clear criteria for the choice of agents and their effects on surgical site infections and wound healing remain unclear.1-6 Iodophors and chlorhexidine gluconate are currently the most commonly used antimicrobial agents in preoperative skin preparation in the United States.7 Water soluble iodophors and chlorhexidine formulations are variably susceptible to inactivation by organic matter (blood and proteins)8-9 and can be removed by saline and/or irrigation fluids10 The use of wound irrigation fluids, most commonly normal saline, has a long history of use to irrigate the wound, keep the exposed tissue moist, and maintain visibility of the operative site11,12 A natural question arises as to whether there is an effect of wound irrigation fluids on the ability of a given antiseptic agent to remain on the skin and to remain active throughout the procedure. Because chlorhexidine has shown water solubility, the possibility exists that products based on chlorhexidine may be lost during surgical irrigation. The specific chlorhexidine product used in the present study was ChloraPrep With Tint skin preparation (MediFlex Inc., Leawood, KS), which contains 2% chlorhexidine gluconate in 70% isopropyl alcohol (IPA). The iodine used in the present study was 3M DuraPrep Surgical Solution (3M, St. Paul, MN). It is an iodine povidone/alkohol solution with 0.7% available iodine in 74% IPA, which forms a water-insoluble film upon drying. Previous work has indicated that the film retains active antimicrobial activity in a model using seeded bacteria.13

This study compares the persistence of antimicrobial activity of 2 commercially available antiseptic agents against transient organisms (modeled using a marker organism applied to the skin) following exposure to saline irrigation or a saline-soaked sponge, measured by a modification of the American Society for Testing and Materials Standard Test Method for Evaluation of Antibacterial Wipes by Scrub Cup Technique.14,15
Table 1. Example sampling schema for assessment of antimicrobial agent performance

<table>
<thead>
<tr>
<th>Sample</th>
<th>Site</th>
<th>Antiseptic agent applied?</th>
<th>Saline applied?</th>
<th>Bacteria applied?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Wrist</td>
<td>No</td>
<td>Rinse</td>
<td>Yes</td>
</tr>
<tr>
<td>2</td>
<td>Forearm</td>
<td>Yes</td>
<td>None</td>
<td>Yes</td>
</tr>
<tr>
<td>3</td>
<td>Forearm</td>
<td>Yes</td>
<td>Rinse</td>
<td>Yes</td>
</tr>
<tr>
<td>4</td>
<td>Forearm</td>
<td>Yes</td>
<td>Gauze</td>
<td>Yes</td>
</tr>
<tr>
<td>5</td>
<td>Above elbow</td>
<td>No</td>
<td>Gauze</td>
<td>No</td>
</tr>
</tbody>
</table>

The primary objective of the study was to evaluate the resistance to wash off of chlorhexidine in a chlorhexidine/alcohol solution compared with iodine in an iodine povidone/iodine solution as measured by antimicrobial activity following contact with saline using a seeded bacteria method. The secondary objective was to determine the removal of chlorhexidine and iodine by the saline-soaked gauze, which was collected after application to the skin at the site of the antiseptic agent. The presence of either antiseptic agent was assessed visually.

METHODS

This was a single-center, blinded, randomized study using a paired comparison design in which each subject received all study materials. Because the investigator could not be blinded to the study materials because of product characteristics, the personnel conducting microbiologic assessments of samples were blinded to the treatment assignments. A local institutional review board reviewed and approved the conduct of this study.

Subject criteria for inclusion/exclusion

The 36 subjects in the study were healthy volunteers of either gender and any race aged 18 to 65 years. Subjects followed study instructions, satisfied inclusion/exclusion criteria, and signed an informed consent statement prior to enrollment. Subjects’ volar forearm requirements were a minimum of 8 inches long; minimal hair; and no evidence of dermatitis, acne, open wounds, or other skin disorders.

Subjects were excluded from the study if they (1) had a history of skin allergies or known sensitivity to natural rubber latex (surgical gloves), medical tape, alcohol, chlorhexidine, propylene glycol (hand lotions and baby wipes), acrylates (adhesive tapes), paraben preservatives (cosmetics), or iodine-containing products; (2) had damaged or altered skin within the volar forearms (including previous skin cancer, sunburn, tattoos, scars, or other disfiguration); (3) had an existing thyroid condition, were diabetic or immunocompromised, had hepatitis or an organ transplant, or were on steroid therapy; (4) had sensitivities to any antibiotic; (5) were exposed to topical or systemic antimicrobial-containing products such as antibacterial soaps, lotions, dandruff shampoos, creams, ointments, perfumes, colognes or aftershave, hot waxes, depilatories, solvents, acids, bases, or other household chemicals; (6) bathed in chemically treated swimming pools and hot tubs or used tanning beds; or (7) were pregnant, thought they may be pregnant, were nursing, or attempting pregnancy.

Application procedures and site preparation

The volar surfaces of the forearm were randomized to receive either the iodine povidone/iodine solution or the chlorhexidine/alcohol solution, applied according to manufacturer’s directions. To prepare the site, 70% IPA was applied to the forearm and allowed to dry. This step removed normal skin flora that might mask the marker organism. The iodine povidone/iodine solution was applied by painting a uniform coat, starting in the center and working outward, to cover a 2 x 6-inch area (leaving 2 inches unprepped at the wrist). The chlorhexidine/alcohol solution was applied using repeated back and forth strokes for 30 seconds over a contralateral 2 x 6-inch area (leaving 2 inches unprepped at the wrist). Timing of each prep application was recorded. The antiseptic agents were allowed to dry for 10 minutes ± 1 minute before any surgical simulation (ie, saline treatment) occurred.

Each arm had 5 treatment sites: the wrist served as a control for the saline rinse, 3 sites on the volar surface of the forearm were exposed to the antiseptic agent, and an area above the elbow was used as a control for the saline-soaked gauze. The schema is summarized in Table 1.

Surgical simulation (saline treatments), application of marker organism, and bacterial sampling

The saline rinse consisted of 250 mL of sterile saline slowly poured over the 2 x 2-inch randomized site. The site was then blotted dry with sterile gauze. The saline soak consisted of a sterile 2 x 2-inch gauze saturated with sterile saline laid over the site for 5 minutes. After removal of the soaked gauze, the site was blotted dry with a sterile gauze. Sterile winged glass cylinders (“scrub cups”) were attached to each site using medical tape. Within each scrub cup, 50 μL of a marker organism inoculum (tetracycline-resistant Staphylococcus aureus, ATCC 27217) at 10⁶ colony-forming units (CFU)/mL was dispensed in tiny droplets and then spread with a sterile glass rod over the skin.
within the scrub cup. This inoculum was allowed to reside in situ for 30 minutes ± 1 minute. Surviving bacteria were recovered via the cup scrub technique using sampling solution containing sodium thiosulfate, lecithin, and Tween 80 as neutralizers. After all samples were collected, the forearms were decontaminated using 70% IPA and Hibiclens antiseptic skin cleanser (Regent Medical, Norcross, GA). Subjects returned within 4 to 8 days for skin condition observation to ensure no presence of infection.

Chemical testing of saline-saturated gauze

Bleach was added to the skin-contacting surface of the gauze removed from the site on which the chlorhexidine/alcohol solution had been applied. In the same manner, starch was added to the gauze removed from the site on which the iodine povacrylexalcohol solution had been applied. Both samples were tested within 5 minutes of removal from the skin. A test was interpreted as positive for chlorhexidine when application of bleach resulted in a color change to brown. A test was interpreted as positive for iodine if application of starch resulted in a blue-black color change. Visual assessment was used to determine positivity, and digital images for each sample were created immediately after application of the chemical test substance.

Microbial evaluation methods

Quantitative cultures were obtained from sample sites using the cup scrub method of Williamson and Kliger. In addition to taping the scrub cups to the arm, pressure was exerted onto the wings to prevent leakage of the scrub solution. All samples were collected as follows: Scrub 1: 2.5 mL of Sampling Solution (a 0.04% KH₂PO₄, 1.0% Na₂HPO₄ buffer containing 0.1% Triton X-100, 0.3% lecithin, 3% Tween 80, and 0.1% sodium thiosulfate; pH 7.9 ± 0.1) was aseptically pipetted into the scrub cup. The skin was scrubbed in a circular motion with moderate pressure for 1 minute using a sterile rubber policeman. Using a sterile transfer pipette, the scrub solution was transferred to a sterile collection tube. Scrub 2: The 1-minute scrub was repeated with another 2.5 mL of Sampling Solution. The solution from both scrubs was pooled into 1 sample tube for a total sample volume of approximately 5 mL per site. The sample tube was vortexed immediately.

A 1.0-mL aliquot of each sample was immediately diluted into sterile tubes containing 9.0 mL phosphate-buffered water (PBW). Serial 10-fold dilutions were performed in PBW. 0.1 mL aliquots of selected dilutions were spread plated in duplicate onto Trypticase Soy Agar containing 4 μg/mL tetracycline. For 10³ samples, 1.0-mL aliquots (0.3, 0.5, and 0.4 mL across 3 plates) were plated in duplicate. Samples were plated within 20 minutes of collection. Spread plates were allowed to dry prior to inversion and incubation. After 48 (±4) hours of aerobic incubation at 35°C ± 2°C, surviving colonies were counted, and viable cells in the original sample were calculated using standard methods.

The adequacy and effectiveness of the neutralizer solution were validated prior to study initiation to demonstrate that the neutralizers effectively inactivated any active antimicrobials and exerted no effect on the growth of the marker microorganism (data not shown).

Evaluation criteria

The primary variable measured was the log reduction of the bacterial challenge applied to the prepped/treated surface of the skin. The log reductions were evaluated after both saline treatment and a bacterial residence time of 30 minutes. Log reduction was calculated as the log counts from the recovery control site minus the log counts from each of the prepped/treated sites. Safety was assessed by recording observed and reported adverse events.

Statistical methods

The test laboratory reported raw data from all treatments as average CFU/mL per test site. Log reductions for each condition studied were calculated by subtracting the recovery log count of the treated sample from that of the appropriate untreated recovery control.

A detailed algorithm was used to convert bacterial counts from CFU/mL to CFU/cm² as follows:

- The CFU/mL data recorded on the case report form (CRF) (eg, 0 or 0.5 CFU/mL) were entered into the database.
- CFU/mL were converted to CFU/cm² using the following formula: (CFU/mL × 5 mL)/5.07 cm² = CFU/cm².
- CFU/cm² values of <1.0 were programatically set to 1.0 CFU/cm² in SAS software (SAS Institute, Cary, NC).
- Log transformations were carried out by applying the log₁₀ of the values in CFU/cm².

The paired difference in log reduction between the test products for control, saline rinse, and saline soak sites was calculated for each subject. Significance of the difference in log reduction between treatments was assessed using a paired t test. Significance was assessed at α = 0.05 (2-sided). In addition, the 95% confidence limits on the paired difference between treatments were calculated. A nonparametric analysis (Wilcoxon signed-rank test) was conducted to verify the results.
Table 2. Posttreatment log recovery

<table>
<thead>
<tr>
<th>Treatment/prep</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine poviarylex/alcohol solution</td>
<td>36</td>
<td>2.17</td>
<td>0.85</td>
<td>2.2</td>
<td>0.7</td>
<td>3.8</td>
</tr>
<tr>
<td>Saline soak</td>
<td>35</td>
<td>1.74</td>
<td>0.80</td>
<td>1.7</td>
<td>0.2</td>
<td>3.3</td>
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<tr>
<td>Prepped control</td>
<td>36</td>
<td>1.95</td>
<td>0.92</td>
<td>1.9</td>
<td>0.3</td>
<td>4.8</td>
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<tr>
<td>Saline rinse</td>
<td>36</td>
<td>2.58</td>
<td>0.62</td>
<td>2.6</td>
<td>0.9</td>
<td>4.1</td>
</tr>
<tr>
<td>Chlorhexidine/alcohol solution</td>
<td>36</td>
<td>1.67</td>
<td>1.36</td>
<td>1.6</td>
<td>0.0</td>
<td>4.6</td>
</tr>
<tr>
<td>Saline soak</td>
<td>36</td>
<td>2.10</td>
<td>0.79</td>
<td>2.0</td>
<td>0.6</td>
<td>4.3</td>
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Table 3. Posttreatment log reduction

<table>
<thead>
<tr>
<th>Prep/treatment</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Median</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodine poviarylex/alcohol solution</td>
<td>36</td>
<td>3.67</td>
<td>0.86</td>
<td>3.7</td>
<td>1.9</td>
<td>5.1</td>
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<tr>
<td>Saline soak</td>
<td>35</td>
<td>4.13</td>
<td>0.76</td>
<td>4.2</td>
<td>2.8</td>
<td>5.6</td>
</tr>
<tr>
<td>Prepped control</td>
<td>36</td>
<td>3.89</td>
<td>0.91</td>
<td>4.0</td>
<td>1.1</td>
<td>5.9</td>
</tr>
<tr>
<td>Saline rinse</td>
<td>35</td>
<td>3.20</td>
<td>0.65</td>
<td>3.2</td>
<td>1.6</td>
<td>4.8</td>
</tr>
<tr>
<td>Chlorhexidine/alcohol solution</td>
<td>35</td>
<td>4.13</td>
<td>1.31</td>
<td>4.2</td>
<td>1.4</td>
<td>6.1</td>
</tr>
<tr>
<td>Saline rinse</td>
<td>35</td>
<td>3.67</td>
<td>0.86</td>
<td>3.7</td>
<td>1.6</td>
<td>5.3</td>
</tr>
</tbody>
</table>

The number of gauze samples that were positive for color change was analyzed using the Fisher exact test. Significance was assessed at $\alpha = 0.05$ (2-sided).

RESULTS

Thirty-six subjects were enrolled in the study. Among these subjects, 44.4% (16/36) were females, 55.6% (20/36) were male. The distribution of race was 94.4% (34/36) white, 2.8% (1/36) African American, and 2.8% (1/36) Asian. The mean (standard deviation) for the numerical demographic variables were: age, 36.6 (13.12) years; height, 5.72 (0.38) feet; and weight, 183.7 (55.23) lb. No adverse events occurred in this study.

Only randomized subjects who had at least 1 pair of efficacy measurements from the iodine poviarylex/alcohol solution and the chlorhexidine/alcohol solution were considered evaluable. Two subjects (No. 25 and No. 29) had missing efficacy data. Because the design of the study was paired, if the data from a treatment pair were not available, the data from a single treatment were not included in the analysis. For this reason, 34 subjects were evaluated for efficacy for log reduction for prepped control, and 35 subjects were evaluated for efficacy for log reduction of saline soak and saline rinse.

The log counts recovered from the control site were compared. The counts were statistically equivalent between treatment groups ($P = .222$). The posttreatment log counts recovered at each treatment site are summarized in Table 2.

The log-count reduction data are summarized for each prep and treatment in Table 3. These data are also shown graphically in Fig 1. The log reductions were compared between the antiseptic agents for each of the 3 treatment types (control, saline soak, and saline rinse). The iodine poviarylex/alcohol solution had a log reduction that was statistically higher than the chlorhexidine/alcohol solution for the saline soak condition ($P = .006$ for the paired t test and $P = .003$ for the Wilcoxon signed-rank test). The log reductions were not statistically different between the treatments for the other 2 conditions ($P$ value $= .756$ using a paired t test for the prepped control condition, $P$ value $= .275$ using a paired t test for the saline rinse condition). The paired differences in log reduction along with the 95% confidence limits are given in Table 4.
Fig 2. Iodine povacrylex/alcohol solution gauze after starch test for presence of iodine.

Fig 3. CHG/alcohol solution gauze after bleach test for presence of chlorhexidine.

The chemical testing indicated that the iodine in the iodine povacrylex film was not removed by saline-soaked gauze (Fig 2). None of the gauze samples from 36 iodine povacrylex/alcohol solution-treated sites showed visible color, whereas 35 of 36 gauze samples from the chlorhexidine/alcohol solution-treated sites exhibited a visible brown color, indicating presence of chlorhexidine gluconate (Fig 3). One gauze sample from a chlorhexidine/alcohol solution-treated site was inadvertently discarded before chemical testing could
be conducted. The $P$ value using the Fisher exact test is $<.0001$.

**DISCUSSION**

The testing performed to address persistence of antiseptic agents is based on national guidelines, and these guidelines do not mandate the use of rinse fluids. The current study was designed to examine one aspect of persistence measured by susceptibility to saline rinse. We have demonstrated that saline removes chlorhexidine from the skin following its application, and this removal is reflected in a decreased effectiveness of the chlorhexidine to kill seeded bacteria. The reduction in effectiveness is greater with saline-soaked gauze than with a saline rinse. By comparison, the iodine in the iodine povidocynex film demonstrated a statistically significant difference in the ability to maintain bactericidal activity, and no removal of iodine could be demonstrated.

Chlorhexidine is known as a persistent antiseptic agent, and it has been widely accepted for surgical hand antisepsis and as a skin antiseptic agent for intravenous catheter insertion and care and blood culture collection. Chlorhexidine is recommended by the Centers for Disease Control and Prevention to prevent catheter-related bloodstream infections.\(^{16}\) By extension, the use of chlorhexidine as a preoperative antiseptic agent also has gained momentum. However, there are no scientific data that support the use of one antimicrobial over another\(^ {1,17}\) in the preoperative setting.\(^ {21}\) Likewise, it is often stated that iodophors have little or no persistent activity. These data are based on surgical hand scrub studies in which the iodophor is washed away.\(^ {21}\) In patient antiseptic preparation studies in which the iodophor remains on the skin, iodophors have been shown to have comparable persistent activity to chlorhexidine.\(^ {22,23}\) This may be why the CDC Guideline for the Prevention of Surgical Site Infection does not recommend one antimicrobial agent over another for preoperative patient prepping.

The literature on chlorhexidine inactivation by "organic matter" is mixed, and the variability in the literature reflects, in part, the inability to describe a fixed composition of "organic matter." Although there is evidence in the literature\(^ {10}\) that chlorhexidine is inactivated by saline, our study was not designed to investigate chlorhexidine inactivation. However, we were able to demonstrate visually that chlorhexidine is removed by saline-saturated gauze and by inference suggest that chlorhexidine could also be removed by a saline rinse, but we are unable to address whether this is accompanied by inactivation of the remaining antiseptic.

Because there is no standardized method for surgical rinse, nor any standard surgical rinse fluid, there are no objective guidelines against which to compare the present study. However, empirically, saline is commonly used as either a rinse or a major component of a rinse fluid, so the use of saline carries a sense of realism, albeit operationally. The practice of surgical rinsing is believed to reduce contamination by bacteria, by other microorganisms, and by malignant cells that might have been removed from a primary lesion during excision or biopsy. That the present study incorporated a saline challenge implies that it resembled more closely the surgical environment than in other studies in which no fluid challenge was provided. Patient exposure to fluids in the form of irrigation or saline-saturated sponges differs considerably from those situations encountered in intravenous line placement and in blood collection. The reality of removal of water-soluble skin preps by irrigation fluids, wet gauze, or dry gauze sponges during surgery has the potential to impact negatively the antimicrobial persistence of the skin prep as demonstrated by the data from this study. Although the differences were statistically significant, their absolute values were small. Therefore, differences at this level may only achieve clinical significance when a small inoculum is capable of causing significant morbidity, eg, in the case of implanted devices.\(^ {18-20}\) Until definitive prospective studies clearly define a superior antiseptic agent and antiseptic process, the choice of agent will require consideration not only of the infection risk resulting from patient factors and virulence components but also whether removal of these agents as a result of standard therapy is an acceptable risk.

**CONCLUSION**

The study was conducted to determine the effects of saline as a rinse or soak on the persistent activity of skin antiseptic agents using a seeded bacteria method. The iodine povidocyrex/alcohol solution had significantly higher log reductions of seeded organisms compared with the chlorhexidine/alcohol solution for the saline-soak condition ($P = .006$). The chemical testing results indicated that the iodine in the iodine povidocyrex water-insoluble film was more resistant to removal by saline-soaked gauze than the chlorhexidine/alcohol solution ($P < .0001$). Chlorhexidine from the chlorhexidine/alcohol solution was removed from the application site in 35 of 36 subjects, whereas the iodine in the iodine povidocyrex film was removed in 0 of 36 subjects.

**References**

17. Parks PJ. Patient preoperative skin preparations to reduce surgical site infections. Touchbriefings 2006;March:84-7.