Optimizing Breast Pocket Irrigation: The Breast Implant–Associated Anaplastic Large Cell Lymphoma (BIA-ALCL) Era

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Abstract

Background: Specific antimicrobial breast pocket irrigations have been proven over the past 20 years to reduce the incidence of capsular contracture by a factor of 10, and the emergence of breast implant–associated anaplastic large cell lymphoma (BIA-ALCL) and its link to bacteria/technique has created renewed interest in different antimicrobial breast pocket preparation agents. Our previous studies have identified that both Betadine-containing and non-Betadine-containing antimicrobial irrigations provide excellent broad-spectrum bacterial coverage. The current science of BIA-ALCL has implicated the Gram-negative microbiome as a key in pathogenesis.

Objectives: The aim of this study was to revisit the antimicrobial effectiveness of clinically utilized Betadine and non-Betadine solutions, along with other antimicrobial agents that have not yet been tested, against multiple organisms, including additional common Gram-negative bacteria associated with chronic breast implant infections/inflammation.

Methods: Current and new antimicrobial breast irrigations were tested via standard techniques for bactericidal activity against multiple Gram-positive and Gram-negative strains. Test results are detailed and clinical recommendations for current antimicrobial irrigations are provided.

Results: Betadine-containing irrigations were found to be superior according to the testing performed.

Conclusions: There are quite few misconceptions with regard to antimicrobial breast pocket irrigation. These are discussed and final evidence-based recommendations for practice are given.

An association between anaplastic T cell lymphoma and breast implants was first reported 20 years ago. The World Health Organization classified anaplastic large cell lymphoma (ALCL) as a unique form of lymphoma in 2001, and in 2016 recognized breast implant–associated ALCL (BIA-ALCL) as a distinct entity from other forms of ALCL. Although the etiology of this disorder remains under investigation, there is strong evidence correlating chronic breast implant inflammation with increased T cell stimulation from bacterial infection. Establishment of a sustained T cell inflammatory response may contribute to the development of BIA-ALCL. In support of the suggestion that BIA-ALCL development is caused by a bacterially associated T cell response, Hu et al found an increase of Ralstonia spp. in the microbiome of implants associated with the development of BIA-ALCL. Subsequent analysis

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has confirmed a definite shift in the microbiome of BIA-ALCL specimens with Gram-negative bacteria. Textured breast implants harbor significantly more bacteria than smooth implants due to their increased surface area, and are associated with a greater lymphocytic infiltrate. This may explain why all confirmed cases of BIA-ALCL to date have occurred exclusively in patients receiving textured breast implants. There is also substantial evidence to suggest that the presence of bacteria and biofilm on the implant correlates with capsular contracture.

Preventing bacterial contamination on an implant surface, or in the breast pocket, at the time of placement requires a comprehensive aseptic process. An important component of reducing the bacterial load at the time of placement is the use of antibacterial irrigation solutions in the breast implant pocket. We have previously reported that broad-spectrum antimicrobial breast pocket irrigations are effective at reducing bacterially associated adverse outcomes associated with breast implants. The purpose of this study was to revisit the antimicrobial effectiveness of clinically utilized Betadine and non-Betadine solutions, along with other antimicrobial agents that have not yet been tested for this purpose, against multiple organisms, including common Gram-negative bacteria associated with chronic breast implant infections/inflammation.

**METHODS**

The bacteria tested include *Staphylococcus epidermidis* (ATCC 14990), *Escherichia coli* (ATCC 25922), *Pseudomonas aeruginosa* (ATCC 9027), *Ralstonia pickettii* (ATCC 27511), and *Mycobacterium fortuitum* (ATCC 6841). The antibiotic agents studied were 10% povidone-iodine (“stock” Betadine, Purdue Frederick/Medex, Stamford, CT), Betadine Triple (50 mL Betadine, 1 g cefazolin (Millipore Sigma, Burlington, MA), and 80 mg gentamicin (Millipore Sigma), 500 mL normal saline), Non-Betadine Triple antibiotic (NB-TAB) [50,000 units bacitracin (Pfizer, New York, NY), 1 g cefazolin, and 80 mg gentamicin, 500 mL normal saline], 0.025% hypochlorous acid [HOCl, PhaseOne (Medline, Northfield, IL)], 2% chlorhexidine gluconate in 70% isopropyl alcohol (CHG/IPA, Chloraprep, Becton Dickinson, Franklin Lakes, NJ), 0.05% chlorhexidine [IS, Irrisept (Irrimax, Gainesville, FL)], and a 55 ppm silver ion gel (SG, SilverGel, EltaMD, Dallas, TX). Betadine was tested at 95%, 50%, 25%, 12.5%, and 6.25% concentrations. Betadine Triple, NB-TAB, HOCl, CHG/IPA, and IS were tested at 95% and 50% concentrations. All testing was performed in Mueller-Hinton II (MHII) broth. Due to difficulties with accurately diluting the gel, SG was only tested at 95% strength.

All isolates were stored at −80°C in MHII broth with 25% glycerol. Isolates were grown aerobically on tryptic soy agar with 5% sheep blood and incubated for 18 to 92 hours (depending on organism) at 37°C. Single colonies were grown in MHII broth or Middlebrook OADC Enrichment media (*Mycobacterium* only) for 18 to 48 hours at 37°C. Dilutions were performed with the same growth media to achieve OD600 values that resulted in final bacterial concentrations of approximately 2 × 10⁹ CFU/mL. Further dilutions were performed with the tested antibiotic agents to achieve bacterial concentrations of approximately 10⁸ CFU/mL at targeted antibiotic concentrations in 1.5-mL microcentrifuge tubes. Each sample was cultured aerobically at 37°C for a period of 15 minutes, 2 hours, or 18 hours. At the specified time point each tube was centrifuged at 10,000 × g for 5 minutes and the supernatant aspirated. The bacterial pellet was resuspended with 400 μL Dulbecco’s phosphate-buffered saline (DPBS). Serial dilutions were performed and streaked on tryptic soy agar with 5% sheep blood, and colonies were enumerated.

To examine the bactericidal ability of Betadine and non-Betadine solutions on bacteria attached to silicone, a 3-mm sterilized disc of smooth silicone breast implant obtained by punch biopsy was added to each tube and incubated in the antimicrobial solutions containing the bacteria of interest. At each time point, the silicone discs were removed from each tube and placed onto sheep blood agar plates and washed with 100 μL of DPBS to remove any nonadherent bacteria. Plates were incubated at 37°C for 24 to 96 hours and colonies counted. Plates containing the silicone discs were observed for the presence or absence of growth. The minimum bactericidal concentration was determined for all tested solutions. At least 2 independent assays were conducted in each strain for every time point and antibiotic solution. Each assay contained 1 tube for each condition.

**RESULTS**

The study was performed from December 2017 to May 2018. The results from all tested Betadine- and non-Betadine-containing solutions are summarized in Figure 1. Bactericidal activity was defined in standard fashion as a greater than 99.9% (>3-log) reduction in the organism at a specific time point and concentration. The color coding demonstrated bactericidal vs nonbactericidal activity—basically “blue is bad,” and especially if it remains blue over the different contact times, the dark maroon is bactericidal to the limit of detection (6 logs) and the beige is bactericidal at 4- or 5-log reductions. Betadine alone and Betadine Triple (Betadine, cefazolin, and gentamicin) solutions were bactericidal at all tested time points and concentrations for each bacterium. These Betadine-containing solutions reduced the bacteria down to the limit of detection for all organisms.
NB-TAB at 95% showed bactericidal activity for all bacteria except *Mycobacterium fortuitum*, although *R. picketti* required 18 hours of exposure to reach the bactericidal breakpoint. HOCl, at 95%, was effective at eradicating bacteria at all time points; however, when HOCl was diluted at the first level the bactericidal effect was significantly reduced for 4 out of our 5 organisms. Additionally, although 50% HOCl caused a CFU reduction at early time points, bacterial regrowth was observed by 18 hours, which was confirmed by additional experiments. *M. fortuitum* was effectively eradicated at all time points by HOCl. CHG/IPA eliminated all bacterial growth within 15 minutes of exposure for both the 95% and 50% concentrations. Full-strength IS also exhibited bactericidal activity at each time point for all bacterial strains, with the exception of *M. fortuitum*, which required more than 15 minutes of solution exposure time, but also exhibited some issue with reduced killing at the first dilution as well as some evidence of bacterial regrowth with Gram-negative organisms similar to HOCl. Bactericidal activity of SG was observed after 2 hours of exposure against *P. aeruginosa*, *E. coli*, and *S. epidermidis*, and by 18 hours for *M. fortuitum*. However, SG was notably ineffective at killing *R. picketti*, as regrowth was observed by 18 hours. SG was particularly difficult to work with in our experimental setting due to its viscosity, and therefore only 1 concentration was examined and *R. picketti* data exhibiting regrowth after 18 hours of SG exposure were also obtained in quadruplicate. Regarding the presence of smooth silicone there was no change in the results; in all cases where no bacterial growth was detected on the agar plates or the result demonstrated bacterial growth,
the same was observed on the plates containing the cor-responding silicone discs.

**DISCUSSION**

We have actively researched and educated on the use of proper breast pocket irrigations and techniques for 20 years, and it is ironic that it has taken the BIA-ALCL crisis for surgeons and patients to revisit these important concepts. An important factor in evaluating the use of solutions for breast implant pocket irrigation is any damage that may occur to the implant surface. In 2000, the Food and Drug Administration (FDA) stated that contact of an implant with Betadine is contraindicated, and this statement has remained in effect for 17 years. The rationale for this was based on reports of delamination when Betadine was used for intraluminal filled saline implants, as well as weakening of the silicone filling tube with sustained exposure to Betadine; however, the silicone implant shell is manufactured through a different process than the tubing, and numerous studies have failed to show alterations to the mechanical integrity of the implant shell from extraluminal exposure to Betadine even when bathed in Betadine for 4 weeks. Additionally, we are not aware of any studies (basic science or clinical) describing the effects of HOCl, chlorhexidine, or silver compounds on breast implant surfaces. In August 2017, the FDA removed the Betadine restriction label for Allergan Corporation’s implants based on submitted data. Betadine implant pocket irrigation also has a long track record of reducing capsular contracture. Many surgeons, including ourselves, continue to use Betadine-containing solutions for implant pocket irrigation, and we specifically included off-label use in each patient’s informed consent.

In the current study, we evaluated the irrigation solution exposure time vs 5 different bacterial species found in breast implant infections, including the more recently emerging *M. fortuitum* and *R. picketti*. Exposure times of 15 minutes, 2 hours, and 18 hours were chosen due to their relevance in the surgical setting, commensurate with the length of time that the antibacterial solutions would be expected to retain efficacy on the implant surface and breast pocket. In further support of this investigation, we have observed remnants of Betadine-containing breast pocket irrigation in a patient re-explored at 18 hours (unpublished observations).

Contamination from skin bacteria is generally felt to be the most likely source of *S. epidermidis* during the implant placement process, and we chose a strain likely to represent nonmicrobial-resistant skin flora. Nasal swabs are commonly used to assess skin flora bacteria such as in determining carriers of methicillin-resistant *Staphylococcus aureus*. We did not test bacteria with known antimicrobial resistance as that was outside the scope of this study. Although strain-specific virulence factors certainly may affect the likelihood of developing an implant-related infection, we did not specifically test the ability of various antibiotic agents to prevent implant infection, but rather the ability of these agents to inhibit bacterial growth of strains anticipated to commonly occur in the patient-implant environment.

Our results demonstrate the bactericidal effects of multiple antibacterial solutions on the tested organisms; however, the effectiveness of these preparations was variable, and depended on the bacterial strain, solution exposure time, and dilution. Dilutional testing of antibiotics and antiseptics are a basic and critical technique for assessing standardized activity. Betadine, Betadine Triple, and CHG/IPA were particularly effective across all pathogens studied and showed stability of bactericidal activity even at dilution. IS was bactericidal at 95% and 50% concentrations but was less effective at 50% against *M. fortuitum*, requiring extended exposure time, and was not able to fully eliminate the *P. aeruginosa* or *R. picketti*, resulting in some regrowth. NB-TAB was effective, and consistent with our prior data, but required a longer contact time to have bactericidal effects compared to Betadine- or chlorhexidine-containing solutions; however, our new data indicate that NB-TAB is ineffective against *M. fortuitum*. Conversely, HOCl became largely ineffective against *P. aeruginosa*, *E. coli*, and *S. epidermidis* when diluted. The results in our study support a recent trial by Becker et al where they found excellent antibacterial activity with NB-TAB, chlorhexidine (0.5%), Betadine, and Betadine Triple against *Staphylococcus* spp. and *P. aeruginosa*.

Betadine has rapid broad-spectrum activity against bacteria, mycobacteria, fungi, viruses, and protozoa. In addition to the previous concerns discussed above, fibroblast toxicity has been demonstrated in animal and human tissue culture. Nevertheless, Betadine has been found to be effective against bacteria, without impairing human foreskin fibroblasts at 10% dilution; however, clinical findings have been inconsistent. Our current study suggests that Betadine is bactericidal even at 6.25% concentrations with 15 minutes of exposure time. Our previous results found that least a 50% Betadine solution was necessary for *E. coli* elimination, and straight Betadine was necessary for *P. aeruginosa* elimination, although the methodology, bacterial strain, and contact time (2 minutes) differed in our previous study. It is important note that in the wound-healing literature and disciplines povidone-iodine is still contraindicated secondary to negative wound-healing effects—hence our development of Betadine Triple, a broad-spectrum breast pocket irrigant with a lower Betadine concentration. Also relevant to this discussion is the preference for a combination irrigation, such as Betadine Triple: gentamicin has demonstrated beneficial anti-infective and local angiogenesis effects, thus
providing another reason to use Betadine Triple over 50% Betadine. 32-34

HOCl is reportedly effective against a wide range of pathogens. 35 Our data demonstrate HOCl to be sensitive to the effects of dilution. This is predictable as HOCL is a highly oxidative agent and a mechanical wound irrigant not an antiseptic. Similar to other mechanical wound irrigants with HOCl preparations, very dilute HOCl is added to prevent bacterial growth in the bottle. This distinction is important as these preparations are not FDA approved as anti-infectives, antiseptics, internal use, and are off-label for breast pocket irrigation. Furthermore, Hu and Vickery 36 recently compared Betadine to HOCl where a TGA/FDA standard protein soil testing was performed that completely inactivated HOCl bactericidal activity. Although we did not add a protein soil to our testing in this study, our results are consistent with HOCl being a less effective option due to inactivation by multiple clinical factors that are typically in play. With regard to both HOCl and IS, we would also caution against claims of clinical effectiveness that are not backed by long-term clinical studies around breast implants, especially because the proven alternatives with better performance and long-term breast pocket irrigation data make off-label use of one of these alternative products problematic.

Chlorhexidine solution is primarily used for skin cleansing at concentrations of 0.5% to 2%, typically in an alcohol base, and is not recommended for internal wound irrigation. An advantage of chlorhexidine for skin cleansing is its prolonged residual activity through binding to skin and mucous membranes. 37 For preoperative skin cleansing, chlorhexidine appears to be more effective than Betadine as a skin preparation for preventing infections at the surgical site. 38-40 Our data indicate that chlorhexidine is effective against all 5 bacteria species we examined, and may continue to be effective against all but M. fortuitum. Dilute chlorhexidine (IS) is also approved as a mechanical wound irrigant; it performs better in dilutional testing than HOCl, although not as well as Betadine-containing irrigations. For this reason, we would not recommend chlorhexidine or any other irrigation that is touted as being effective/superior until they have accrued comparable 20+-year data around breast implants. Furthermore, given the FDA warning bulletin in February 2017 regarding allergic reactions to chlorhexidine, the future of this product may be in serious doubt. 41

CONCLUSIONS

Our current clinical recommendation for breast pocket irrigation is to use Betadine-containing irrigations. Our preference over the past 20 years can be summarized as follows:

1. We use Betadine Triple with the goal of minimizing the negative wound healing effects of povidone-iodine. This should be prepared at the time of the surgery by an educated operating room nurse. For some implants this is an off-label practice and should simply be disclosed in the patient consent. Patients in our practice are universally accepting over the past 20 years of Betadine with informed consent as to the benefits of pocket irrigation in reducing potential complications and/or reoperation.

2. Surgeons unfamiliar with the science and without proper knowledge have claimed that the use of anti-biotic irrigations for breast pocket irrigation promotes the development of resistance – this is untrue as the mechanism for resistance for a local wound application is very different, and unlikely compared to systemic administration. In fact, local antimicrobial wound therapies have been proven to have better antimicrobial effects/kill rates, enhanced wound angiogenesis, and less resistance development. 32-34

3. For some patients with cephalosporin or aminoglycoside allergy we will use 50% Betadine (10% povidone-iodine mixed with an equal volume of saline). In rare cases, for patients with a true iodine or Betadine allergy, we utilize NB-TAB.

4. Misconceptions about povidone-iodine (PVPI or Betadine) abound – the following statements are accurate:
   a. Betadine does not need to dry to work.
   b. There is no known resistance to Betadine.
   c. Extraluminal Betadine does not harm the silicone elastomer shell of implants.
   d. Betadine does not harbor bacteria in solution. The origin of this legend stems from repeated use of the same stock bottle, and a manufacturing contaminant that was identified over 25 years ago and promptly corrected. 42-44 Sterile single-use bottles are available and should be used per case.

We would like to stress that clinical practice of breast pocket irrigation should utilize a standardized or systematic approach. In our surgical procedures, we perform a series of 2 irrigations with 75 mL normal saline, each of which are completely evacuated. This is followed by irrigation with 75 mL antimicrobial solution, which is completely evacuated, and a second 75-mL irrigation, of which only the excess that drains from the incision is removed. This ensures the maximal concentration of breast pocket solution remains in the pocket prior to implant placement. We also clean the peri-incision skin with a small chlorhexidine prep stick that as shown above kills all surface bacteria almost immediately.

We did not test the ability of these solutions to treat established biofilm, as that will form a second part of this
study. It should be noted that activity of planktonic bacteria is more important with antimicrobial breast pocket irrigations because biofilms in most cases will not have time to establish in under 2 hours; however, there is clear evidence that S. aureus and Enterococcus faecalis, for example, can form a biofilm on an abiotic urinary catheter in < 6 hours and S. epidermidis can form biofilms on breast implant surfaces within 18 to 20 hours. All of our recommended final antimicrobial breast pocket irrigations achieve their full effect in under 2 hours and are therefore effective against planktonic bacteria; however, surgeons should remember that in secondary cases there may be established biofilms. Our future studies will more thoroughly investigate the use of these antimicrobial solutions in treatment of biofilm associated with multiple pathogens.

Finally, we recommend adhering to a strict 14-point protocol to minimize the chance of device associated infection during surgery. This practice has been proven to minimize capsular contracture rates, and recent evidence to suggest that it minimizes the risk of BIA-ALCL presumably by reducing the bacterial load and formation of bacterial biofilm on the implant surface.

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