Efficacy of Common Antiseptic Solutions Against Clinically Relevant Planktonic Microorganisms

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abstract

Prosthetic joint infections (PJIs) are among the most devastating complications after joint replacement. There is limited evidence regarding the efficacy of different antiseptic solutions in reducing planktonic microorganism burden. The purpose of this study was to test the efficacy of different antiseptic solutions against clinically relevant planktonic microorganisms. We designed an experiment examining the efficacy of several antiseptic solutions against clinically relevant planktonic microorganisms in vitro. Regarding planktonic microorganisms, povidone-iodine had 99.9% or greater reduction for all microorganisms tested except for methicillin-resistant *Staphylococcus aureus,* which was reduced by 60.44%. Irrisept (Irrimax Corp) had 99.9% or greater reduction for all microorganisms except *Staphylococcus epidermidis* (98.31%) and *Enterococcus faecalis* (48.61%). Bactisure (Zimmer Surgical Inc) had 99.9% or greater reduction for all microorganisms tested. Various measures exist for PJI prevention, one of which is intraoperative irrigation. We tested irrigants against clinically relevant planktonic microorganisms in vitro and found significant differences in efficacy among them. Further clinical outcome data are necessary to determine whether these solutions can impact PJI in vivo. [*Orthopedics*. 202x;4x(x):xx-xx.]

Prosthetic joint infection (PJI) is one of the most devastating complications of joint replacement surgery. The incidence of PJI ranges from 0.5% to 2.5% after primary total hip arthroplasty (THA) and total knee arthroplasty (TKA), and PJI accounts for up to 25% of the revision surgeries performed.[1-7] Prosthetic joint infection contributes to significant patient morbidity and mortality, and health care expenditures related to PJI are estimated to exceed $1.6 billion annually.[4] The overall survival rate 5 years after PJI was found to be 67.2% for THA and 71.7% for TKA among Medicare patients.[3] Prosthetic joint infection is typically treated surgically with extended antimicrobial therapy; however, even with aggressive treatment, variable success rates are seen. Surgical options most commonly include single- or two-stage exchange arthroplasty, vs debridement with implant retention.[8-10]

As arthroplasty surgery has become more prevalent,[11] the clinical and economic ramifications of PJI have become more important. Therefore, various measures have been attempted for PJI prevention.[12] One common modality used for prophylaxis against surgical site infection, and

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Table 1

Proposed Mechanism of Action of Irrigation Solutions

Irrigation solution	Mechanism of action
Chlorhexidine gluconate	Bactericidal through binding to bacterial cell walls. This causes an alteration in osmotic equilibrium and electrolyte leakage at low concentrations, with cytoplasmic content leakage and cell death at higher concentrations.
Povidone-iodine	Combination of iodine and polyvinylpyrrolidone. Iodine disassociates and penetrates microbial cell membranes to interact with proteins, nucleotides, and fatty acids in the cytoplasm, resulting in rapid cell death.
Benzalkonium chloride	Likely due to dissociation of cellular membrane lipid bilayers, this leads to cellular permeability and leakage of cellular contents.
Sodium hypochlorite	Alters cellular metabolism through oxidation and enzymatic inactivation, and also causes cell wall phospholipid destruction.
Castile soap	Hydrophobic ends bind bacterial cell walls, while a hydrophilic end binds water for removal.
Irrisept	Contains low concentrations of chlorhexidine gluconate 0.05% in sterile water.
Prontosan	Two effective ingredients: polyhexanide functions as a preservative and betaine functions as a surface-active cleanser.
Vashe	Active ingredient is sodium hypochlorite.
Dankin's solution	A dilute sodium hypochlorite and other stabilizing ingredients.
Bactisure	Contains ethanol, acetic acid (pH modifier), sodium acetate (buffer), and benzalkonium chloride (surfactant) in sterile water.

as an adjunct for the treatment of PJI, is intraoperative irrigation.[13-15]

Numerous lavage solutions exist with various geographic- and surgeon-specific practices. Irrigation fluids used in arthroplasty have included normal saline (NS), antibiotics, antiseptics, detergents, and proprietary solutions.[16] **Table 1** lists some of the common solutions and their proposed mechanisms of action.[17-20] When inappropriately mixed, certain combinations of solutions can alter the chemical structure of a substance and form unwanted toxic or irritative byproducts.[21] It has not been established whether a specific agent, or combination of agents, is better suited for preventing PJI.[22] The purpose of this study was to further understand the efficacy of common clinical lavage solutions for disrupting a variety of microorganisms in vitro.

Materials and Methods

Microorganisms included in this study were methicillin-resistant *Staphylococcus aureus* ATCC 33592 (MRSA), *Staphylococcus epidermidis* ATCC 12228, *Pseudomonas aeruginosa* ATCC 9027, vancomycin-resistant *Enterococcus faecalis* ATCC 51299 (VRE), *Enterobacter cloacae* ATCC 13047, *Candida albicans* ATCC 10231, and *Candida tropicalis* ATCC 750. **Table 2** lists growth agar, neutralization broth, and incubation requirements for all microorganisms tested. Surgical lavage solutions tested included the following: (1) Bactisure (lot Q1895322; Zimmer Surgical Inc), a solution containing sodium citrate, citric acid, benzalkonium chloride, and ethanol; (2) Irrisept (lot 18DA0181; Irrimax Corp), a solution containing low-concentration chlorhexidine gluconate 0.05%; and (3) povidone-iodine at 0.35% (lot 0354225; Vi-Jon Inc),

Table 2

Microorganism Preparation

	Mean±SD			
Microorganism	**Incubation temperature**	**Incubation time, h**	**Growth supporting agar**	**Neutralization broth**
Staphylococcus aureus	36°±1° C	24±4	TSA	2×Dey/Engley
Staphylococcus epidermidis	36°±1° C	24±4	TSA	2×Dey/Engley
Pseudomonas aeruginosa	36°±1° C	24±4	TSA	2×Dey/Engley
Enterococcus faecalis	36°±1° C	24±4	TSA	2×Dey/Engley
Enterobacter cloacae	30°±2° C	24±4	TSA	2×Dey/Engley
Candida albicans	30°±2° C	48±4	PDA	2×Dey/Engley
Candida tropicalis	30°±2° C	48±4	PDA	2×Dey/Engley

Abbreviations: PDA, potato dextrose agar; TSA, sterile trypticase soy agar.

prepared by mixing 0.35 mL into 100 mL of sterile water.

Planktonic Microorganism Timed-Kill Analysis

Planktonic microorganism timed-kill analysis was performed using a timed suspension, in vitro test method in accordance with the Clinical and Laboratory Standards Institute guidelines. The different antiseptic solutions used were exposed to the microorganisms for 120 seconds and then the colony forming units (CFU)/milliliter (mL) were determined and compared with a control solution. First, a loop of target microorganism was transferred to a tube containing 10 mL of sterile trypticase soy agar (TSA) or potato dextrose agar (PDA), and these were incubated at a temperature appropriate for the microorganism (**Table 2**). Fungal and bacterial inocula were harvested and prepared with sterile phosphate-buffered saline (PBS) to approximately 1×10^8 CFU/mL. For each microorganism tested, control substances were prepared as well. For the various antiseptic solutions, 9.90 mL was aseptically aliquoted into a 50-mL centrifuge tube, in triplicate, along with 9.90 mL of control PBS. Control substances were used to determine the starting concentration of microorganism prior to contact with the test substance and were termed time-zero.

Inoculation of the test substance was performed at 23°±2° C. For each test substance, a 0.100-mL volume of each microorganism inocula was added to the separate test vessels, immediately after which a timer was started for contact time. The test vessel was vortex mixed. After 120 seconds, a 1.0-mL aliquot of the inoculated test substance was harvested and neutralized in 9.0 mL of the appropriate neutralizer broth. Additionally, 1:10 dilutions were performed in the broth and plated in duplicate to the appropriate growth supporting agar. Time-zero control was created in a similar fashion using 9.90 mL of sterile PBS inoculated with 0.100 mL of the test microorganism. The CFU/mL values were calculated for tests and controls after 120 seconds of exposure to lavage solution and were compared.

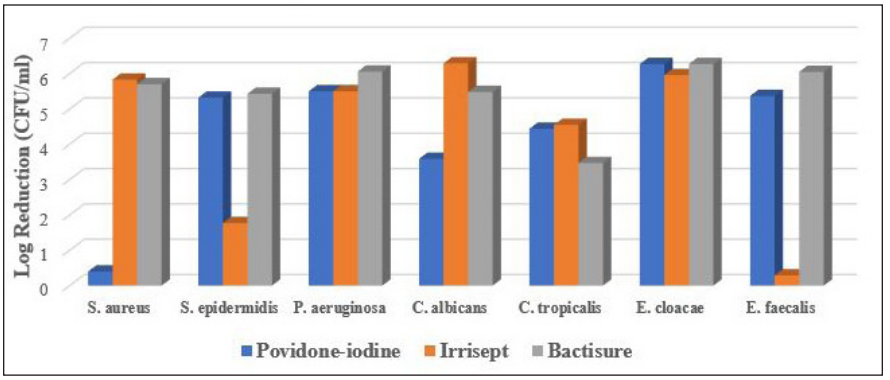

Figure 1: Lavage treatment of planktonic bacteria. Abbreviation: CFU, colony forming units.

A neutralization control was performed for each test microorganism per test substance wherein 1.0 mL of the test substance was added to 9.0 mL of the appropriate broth to represent the "neutralization test" tube. A 1.0-mL volume of sterile PBS was added to a separate neutralization tube to represent the "neutralization control" tube. The test samples were inoculated with a 0.1-mL aliquot of test microorganism suspension representing 10 to 1000 CFU with 9.9 mL of the test substance, vortex mixed, and allowed to sit for the allotted time (120±5 seconds). Neutralization was validated if the test counts were 70% or greater when compared with controls. A control of 0.1-mL aliquots of neutralization broth and PBS plated on TSA and PDA was incubated at 36°±1° C and 30°±2° C to verify media sterility. A full cycle of the test microorganism was plated to their respective growth media, incubated at the appropriate temperature to determine purity for each microorganism, and served as the enumeration media growth control. Growth and purity control testing for each test microorganism showed positive microorganism growth and pure cultures for all samples. Study media including PBS, Dey/Engley neutralization broth, TSA, and PDA controls showed no growth of microorganisms, confirming their sterility.

Results

Planktonic Microorganism Timed-Kill Analysis

Figure 1 and **Table 3** present the average planktonic microorganism suspension reduction after 120-second exposure to the test solutions. These results demonstrate how well the different antiseptic irrigants were able to reduce microorganism burden in their planktonic form. For povidone-iodine, there was 99.9% or greater reduction in burden for all microorganisms tested except for MRSA, which was reduced by only 60.44%. Irrisept had 99.9% or greater reduction in microorganism burden in all specimens tested except *Staphylococcus epidermidis* (98.31%), and *Enterococcus faecalis* (48.61%). Bactisure had 99.9% or greater reduction in microorganism burden for all specimens tested.

Discussion

Current management of PJI is multifaceted and typically involves surgical intervention with antibiotics. Intraoperative irrigation is used to prophylax against surgical site infection and as an adjunct for the treatment of PJI.[13-15] It has not been established whether a specific agent, or combination of agents, is better suited for preventing surgical site infection or PJI.[22] Specific information regarding the type of, amount of, and duration of exposure to antiseptic solution is lacking and variable among surgeons and in the literature. The purpose of this study was to increase

Table 3

Planktonic Microorganism Kill Results After Lavage Exposure

Test microorganism	Time-zero mean, CFU/mL	Post-treatment mean, CFU/mL	Reduction compared with time-zero	Mean log reduction
Povidone-iodine				
Staphylococcus aureus (MRSA)	3.35E+06	1.33E+06	60.44%	0.40
Staphylococcus epidermidis	1.37E+07	<6.50E+01	>99.99952%	>5.32
Pseudomonas aeruginosa	1.57E+06	<5.00E+00	>99.99968%	>5.50
Candida albicans	9.78E+06	2.54E+03	99.974%	3.59
Candida tropicalis	2.43E+06	8.83E+01	99.996%	4.44
Enterobacter cloacae	9.25E+06	<5.00E+00	>99.99995%	>6.27
Enterococcus faecalis	3.12E+06	<1.33E+01	>99.99957%	>5.37
Irrisept				
Staphylococcus aureus (MRSA)	3.35E+06	<5.00E+00	>99.9998%	>5.83
Staphylococcus epidermidis	1.37E+07	2.31E+05	98.31%	1.77
Pseudomonas aeruginosa	1.57E+06	<5.00E+00	>99.99968%	>5.50
Candida albicans	9.78E+06	<5.00E+00	>99.99995%	>6.29
Candida tropicalis	2.43E+06	6.83E+01	>99.997%	>4.55
Enterobacter cloacae	9.25E+06	<1.00E+01	>99.99989%	>5.96
Enterococcus faecalis	3.12E+06	1.60E+06	48.61%	0.29
Bactisure				
Staphylococcus aureus (MRSA)	2.48E+06	<5.00E+00	>99.9998%	>5.70
Staphylococcus epidermidis	1.36E+06	<5.00E+00	>99.9996%	>5.43
Pseudomonas aeruginosa	5.68E+06	<5.00E+00	>99.99991%	>6.06
Candida albicans	3.53E+06	1.17E+01	>99.9997%	5.48
Candida tropicalis	3.55E+06	1.20E+03	99.97%	3.47
Enterobacter cloacae	9.27E+06	<5.00E+00	>99.99995%	>6.27
Enterococcus faecalis	5.58E+06	<5.00E+00	>99.99991%	>6.05

Abbreviations: CFU, colony forming units; MRSA, methicillin-resistant Staphylococcus aureus.

understanding of the efficacy of different antiseptic solutions commonly used in arthroplasty for disrupting planktonic microorganisms in vitro to hopefully guide further in vivo investigations.

Clinically, a reduction in planktonic bacteria offers the potential to reduce infection rates by eliminating microorganisms at the time of surgery or during treatment of acute infections where biofilm has not had an opportunity to form. This in vitro study compared the ability of several antiseptic solutions to eliminate planktonic, active forms of various microorganisms. We found that treatment with Bactisure obtained 100,000-fold or greater reduction for all microorganisms tested except *Candida tropicalis,* which had a 99.97% reduction compared with control samples. For povidone-iodine, there was a 1000-fold or greater reduction in burden for all microorganisms tested except MRSA, which was reduced by only 60.44% after a 120-second exposure time. Irrisept had a 10,000-fold or greater reduction in microorganism burden in all specimens tested except *Staphylococcus epidermidis* (98.31%) and *Enterococcus faecalis* (48.61%).

The clinical significance of this information is unclear. A greater reduction in bacterial load may not necessarily correlate with a lower rate of surgical site infection or PJI in vivo. Despite this, one can justify choosing a therapeutic agent that eliminates planktonic microorganisms more effectively in order to lessen the infectious burden overall. Irrigation is only one aspect in the multifaceted treatment of and prophylaxis against infection, and the clinical relevance of these data is not yet known. Successful treatment of PJI is predicated on thorough removal of necrotic tissue and infected hardware, which can serve as a nidus for biofilm formation. More in vivo studies are needed

to directly compare various irrigation solutions. These clinical studies must be performed in a randomized prospective fashion; however, the data presented here are in line with previous studies showing that certain antiseptic agents can reduce microorganism burden in vitro with differences among them.[23-34]

The limitations of this study are well recognized. First, we used a single-species in vitro model, which has inherent limitations for clinical application. Next, we used only one concentration of povidone-iodine and chlorhexidine (Irrisept). It is possible that higher concentrations would have had a more pronounced effect on our in vitro model. However, the concentrations studied are commonly used clinically, and it is possible that higher concentrations could result in significant local tissue toxicity. Typically, irrigation solutions are applied with a certain amount of pressure to mechanically disrupt bacteria, and this is part of the therapeutic effect. However, in this study, the effect of pressure was not tested. Although it is not clear from this study why certain antiseptics have different efficacy against different bacteria, this could be due to differences in the mechanisms of antiseptics. Povidone-iodine has been shown to have greatest efficacy when it is allowed to dry, but our experiment did not permit this. However, in clinical scenarios, it is unlikely that povidone-iodine would be given time to dry within the wound. Further clinical outcome data are necessary to better determine whether different intraoperative antiseptic solutions can impact surgical site infection or PJI incidence and treatment outcome.

Conclusion

Various measures exist for prevention of surgical site infection and PJI, with one being intraoperative irrigation. It has not been established whether a specific agent, or combination of agents, is optimal. We used an in vitro model to assess how efficacious different antiseptic solutions were for eradicating planktonic microorganisms for common pathogens. For planktonic microorganisms, Bactisure demonstrated greater than 99.90% reduction in all those tested. Povidone-iodine reduced MRSA by only 60.44% and Irrisept reduced *Enterococcus faecalis* by only 48.61% after a 2-minute exposure. Further clinical outcome data are necessary to better determine whether different intraoperative antiseptic solutions can impact the incidence and successful treatment of infection.

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