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Optimal Irrigation and Debridement of Infected Joint Implants:

An In Vitro Methicillin-Resistant *Staphylococcus aureus* Biofilm Model

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Abstract

Acute postoperative and acute, late hematogenous prosthetic joint infections have been treated with 1-stage irrigation and debridement with polyethylene exchange. Success rates, however, are highly variable. Reported studies demonstrate that detergents are effective at decreasing bacterial colony counts on orthopedic implants. Our hypothesis is that the combination of a detergent and an antiseptic would be more effective than using a detergent alone to decrease colony counts from a methicillin-resistant *Staphylococcus aureus* biofilm-coated titanium alloy disk simulating an orthopedic implant. In our study of various agents tested, chlorhexidine gluconate scrub (antiseptic and detergent) was the most effective at decreasing bacterial colony counts both prereincubation and postreincubation of the disks; pulse lavage and scrubbing were not more effective than pulse lavage alone.

Keywords

joint infection; debridement; optimal irrigation; MRSA; biofilm

Infection remains one of the most devastating and challenging complications in total joint arthroplasty. It is estimated that 1% to 2% of total knee and hip replacements become infected [1]. The standard of care for infected total hip or knee replacements is a 2-stage reimplantation protocol, including hardware explantation, synovectomy, irrigation, debridement, placement of an antibiotic-loaded cement spacer, and a course of intravenous antibiotics, followed by reimplantation [2]. Associated morbidity is often substantial; and the complications, including bone loss, soft tissue defects, and fibrosis, are problematic. Long-term joint function is often unpredictable, and the economic burden is high [3]. However, the success rate of this protocol is excellent, with greater than 90% achieving infection eradication [4].

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In an attempt to obviate the need for 2-stage exchange and its associated morbidity, 1-stage irrigation and debridement, synovectomy, and polyethylene exchange has been used for acute postoperative and acute, late hematogenous infections, followed by antibiotic administration. The success rates, however, are widely variable, ranging from 18% to 83% [5], with recent reports at the lower range [6].

Despite the unsatisfactory salvage success rates, single-stage irrigation and debridement with prosthetic retention remains an attractive low-morbidity option in selected cases if success rates were improved to approximate those of a 2-stage reimplantation protocol.

In general, salvage procedures fail because of incomplete infection eradication. Biofilm formation, with adhesion to implant surfaces, is the main cause of this failure because bacteria sequestered in biofilms have poor antibiotic susceptibility. Some bacteria detach from the biofilm become planktonic (“free floating”) and also serve as a nidus of infection. Biofilms can form quickly on implant surfaces and are difficult to eradicate completely [7].

Many different in vitro debridement techniques and irrigation solutions have been used to disrupt biofilms. Anglen et al [8], in studying a *Staphylococcus epidermidis* biofilm on stainless steel screws, found that (1) power irrigation was better than bulb syringe at bacterial removal, (2) antibiotic-impregnated normal saline irrigation was no better than normal saline alone, and (3) castile soap (a detergent) irrigation was more effective than normal saline alone. Moussa et al [9], in a separate study, found that benzalkonium chloride, also a detergent, was effective against *S epidermidis*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* biofilms.

Based on these findings, we hypothesized that combining an antiseptic and a detergent would be more effective in decreasing the bacterial colony count even more than when a detergent was used alone. In addition, we hypothesized that scrubbing the metal surface, in addition to pulse lavage, would decrease the bacterial colony count more than pulse lavage alone.

We chose methicillin-resistant *S aureus* (MRSA) as our experimental bacteria because of its increasing incidence as a cause of total joint infection [2] and its propensity to form biofilm [10]. Our model prosthetic surface was a titanium alloy (Ti-6Al-4V) allergy disk (Stryker, Mahwah, NJ). Chlorhexidine gluconate scrub solution (Cardinal Health, McGaw Park, Ill) and povidone iodine scrub solution (Biomed Systems, Norwalk, Conn) served as experimental scrubbing agents that contain both an antiseptic and a detergent (cocamide diethanolamine and nonoxynol-9, respectively). Castile soap (Triad, Franklin, Wis) served as the experimental detergent scrub.

Materials and Methods

Bacterial Strain

A clinically isolated MRSA strain (USA300 subtype) was chosen because of its known propensity to form biofilm. The MRSA was suspended in a liquid culture of trypticase soy broth (TSB) and incubated at 37°C overnight.

Biofilm Production and Verification

Biofilms were grown by inoculating 1:40 dilution of overnight MRSA culture (~1×10^8 cells) in 3-mL TSB onto each of 6 titanium alloy disks in polystyrene 6-well plates. The plates were incubated at 37°C overnight with rotation at 50 rpm. Each biofilm adherent disk was then removed and washed 3 times with 2-mL phosphate-buffered saline (PBS) solution to remove planktonic bacteria, after which each disk was placed in a new 6-well plate. Biofilm on the disks was verified by both observation of a filmy glycocalyx matrix as well as by XTT reduction assay with spectrophotometric measurement of optical density changes [11]. This process was done in triplicate, and the disks were then divided into 7 groups as follows:

Group A: Positive control (PBS wash)

Group B: Normal saline irrigation

Group C: Normal saline plus bacitracin (NS + B) irrigation

Group D: NS + B irrigation then povidone iodine scrub

Group E: NS + B irrigation then chlorhexidine gluconate scrub

Group F: NS + B irrigation then castile soap scrub

Group G: NS + B irrigation then normal saline scrub

Irrigation and Debridement of Biofilm Disks

Each disk (groups C–G) was uniformly irrigated, by the same technician, with 300 mL of normal saline containing bacitracin at a concentration of 100 000 U bacitracin per 3-L bag of normal saline. Group B was irrigated with normal saline alone. A Pulsavac Plus (Zimmer, Warsaw, Ind) irrigation system was used on “high” setting at a distance of 6 in from each disk. After irrigation, 4 groups were selected for scrubbing (groups D–G). In these groups, immediately after irrigation, each disk was scrubbed for 1 minute, front and back, by the same technician alternating between sponge and bristle sides, with standard, commercially available surgical scrub brushes. Each brush contained 20 mL of 1 of 4 different solutions as described above.

Assay for Efficacy of Debridement Protocols

To assay for the differential efficacy of irrigation and debridement protocols, the titanium disks were placed into 3-mL PBS and scraped thoroughly with plate scrapers. This was done immediately after irrigation and debridement of the biofilm disks. The resulting suspension of any residual biofilm bacteria in PBS was then homogenized, serially diluted, and plated onto trypticase soy agar plates. The plates were grown at 37°C overnight, and colony-forming unit (CFU) values were then derived from colony counts (group 1, without reincubation). This was repeated for groups B to G. In addition, a second group (group 2, with reincubation) was designed to assess for regrowth of bacteria after irrigation and debridement. This second group was composed of disks that were incubated in 3-mL TSB at 37°C and 50 rpm overnight after irrigation and debridement of the disks. Then, the assay to obtain CFUs proceeded as previously described.

Results

Group 1: Without Reincubation

The residual mean CFU values with SDs are shown in Table 1. All groups were compared by analysis of variance (ANOVA) ($F < 0.0001$) and Tukey pairwise comparison test. All differences in the mean were significant ($P < .05$) except when comparing groups C and G.

Group A had the most CFUs, whereas group E had the least. Groups that were irrigated (groups B and C) or irrigated and scrubbed (groups D, E, F, and G) each had fewer CFUs than the positive control (Fig. 1). Groups that were irrigated and scrubbed (groups D, E, and F), excluding G, which did not reach significance, had fewer CFUs than the groups that were irrigated alone (groups B and C). Normal saline plus bacitracin irrigation (group C) had fewer CFUs than normal saline irrigation (group B). Normal saline plus bacitracin with normal saline scrub had fewer CFUs than normal saline irrigation. Finally, NS + B with chlorhexidine gluconate scrub (group E) had the fewest CFUs, followed by povidone iodine, castile soap, and normal saline scrubs.

Group 2: With Reincubation

The mean CFU values with SDs are shown in Table 2. All groups were compared via an ANOVA ($F < 0.0001$) and Tukey pairwise comparison test. All differences in the mean were significant ($P < .05$) except when comparing groups C and D, A and B, A and G, and B and G.

The only groups that had fewer CFUs than the positive control were the NS + B plus chlorhexidine gluconate scrub and NS + B plus castile soap scrub groups (Fig. 2). Groups that were irrigated alone (groups B and C) had no significant difference in CFUs than the positive control. The normal saline irrigation group had no significant difference in CFUs compared with the NS + B irrigation group. Finally, NS + B with chlorhexidine gluconate scrub had the fewest amount of CFUs, followed by castile soap scrub, normal saline scrub, and povidone iodine scrub.

The differences within an experimental group for both groups 1 and 2 (without and with reincubation) were statistically significant (Student *t* test, $P < .05$) in all cases. In all cases, except the positive control, there were more CFUs in group 2 when compared with group 1.

Discussion

The results of group 1, without reincubation, demonstrate that any experimental condition, including scrubbing and/or irrigating, was better than the control at decreasing the number of CFUs of MRSA remaining on the titanium alloy allergy disks. In addition, scrubbing and irrigating were better than irrigating alone. When irrigating alone, however, antibiotic-impregnated irrigation (group C) was better than normal saline alone (group B). When scrubbing, however, the chlorhexidine gluconate scrub was significantly better than all other groups (log 0). This confirms our hypothesis, in group 1, that scrubbing a MRSA biofilm-coated titanium alloy disk with an antiseptic (chlorhexidine gluconate) and a detergent

(cocamide DEA) had less bacterial CFUs than scrubbing with a detergent alone (castile soap).

As there was no significant difference between the NS + B irrigation group and the NS + B plus normal saline scrub group, we cannot validate our second hypothesis for group 1 that scrubbing after pulse lavage irrigation had fewer CFU growth than pulse lavage irrigation alone. However, each of the 3 other scrubbing groups (groups D, E, and F) demonstrated an overall trend toward fewer CFUs.

All of the disks that underwent reincubation (group 2), excluding the positive control, experienced a rebound in growth as compared with their counterpart in group 1. Most important, only NS + B irrigation plus either chlorhexidine gluconate scrub or castile soap scrub had fewer CFUs than the positive control. However, the NS + B with chlorhexidine gluconate group (detergent and antiseptic) had significantly fewer CFUs than the castile soap group (detergent alone), thereby confirming our first hypothesis.

Interestingly, in group 2 (with reincubation), irrigating alone (groups B and C) made no difference as compared with the positive control (group A). In addition, scrubbing and irrigating (group G) were not better than irrigating alone (group C). Our second hypothesis is, therefore, rejected for group 2.

Although the results are interesting and subjective, they cannot be extrapolated to an in vivo model. Nevertheless, we feel that a reincubation environment may, in part, mimic what transpires after a single-stage irrigation and debridement. After the surgical wound is closed, the periprosthetic milieu is a rich bacterial culture medium, which may serve as a reincubation environment. Bacterial regrowth may ensue as paralleled in our in vitro study by the large increase in CFUs in all experimental groups after reincubation. In the in vivo environment, antibiotics and normal immune mechanisms would be available, which could be more effective in the presence of a decreased bacterial load. We plan further study of the reincubation group.

Although we validated our first hypothesis—in each case, an antiseptic plus detergent was better than a detergent alone at reducing the number of CFUs—we, in fact, used 2 different antiseptics—chlorhexidine gluconate and povidone iodine. In group 1, both antiseptic/detergent scrubs were better than the castile soap scrub. However, in group 2, only the chlorhexidine gluconate scrub was better; povidone iodine was not statistically different than the NS + B group alone.

Despite the clear efficacy of chlorhexidine gluconate in reducing the number of MRSA CFUs in this in vitro study, much caution should be exercised in extrapolating these results for clinical use. Increasing evidence strongly supports chlorhexidine's preferential use as a skin preparation agent in reducing surgical site infection [12–14]. However, its potential adverse local soft tissue and systemic effects from intra-articular use remain largely uninvestigated and unresolved. Only case reports in which chondrolysis occurred with chlorhexidine use as a lavage during knee arthroscopy exist [15]. Despite its good safety profile and wide range of clinical application, further investigation is warranted to determine

chlorhexidine's systemic and local soft tissue effects before instituting its intra-articular use in clinical practice, especially considering its known toxicity elsewhere [16,17].

This study is limited by its nonblinded design. Furthermore, it used a single-organism/single-strain experimental model that may not be applicable to other types of bacteria.

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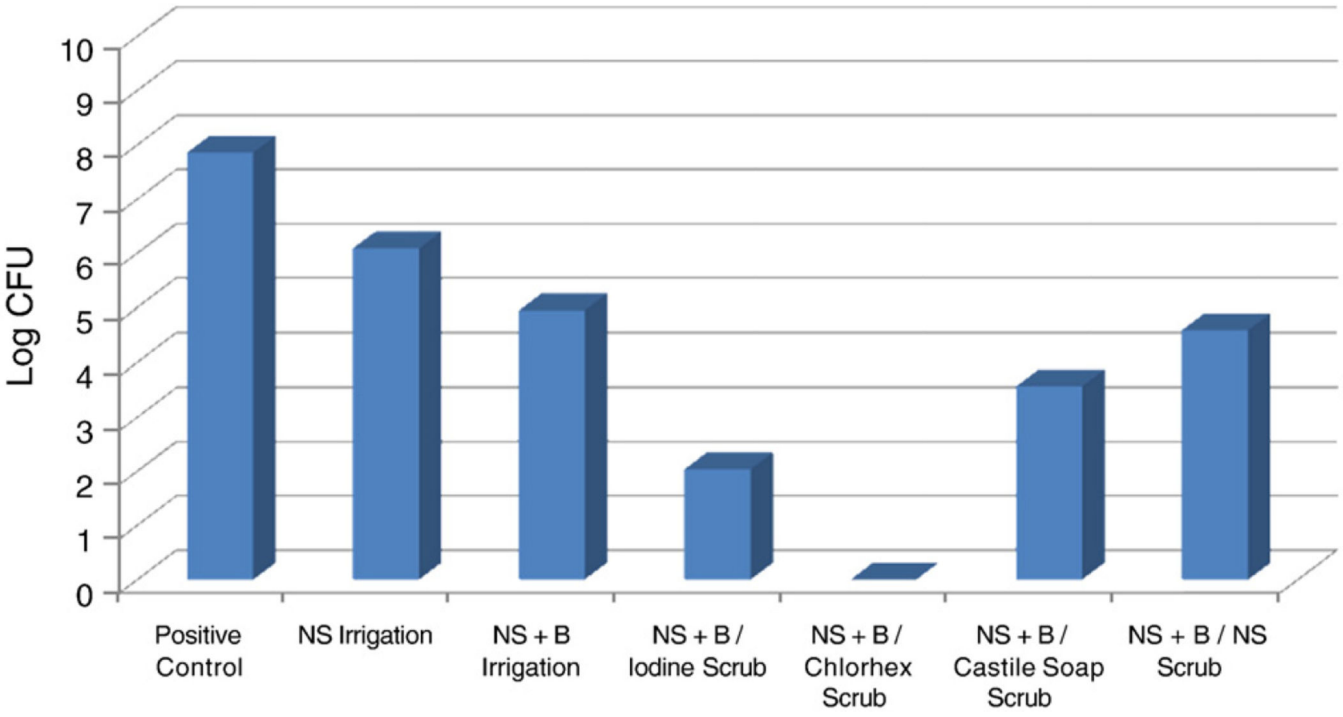

Fig. 1.
Group 1—without reincubation.

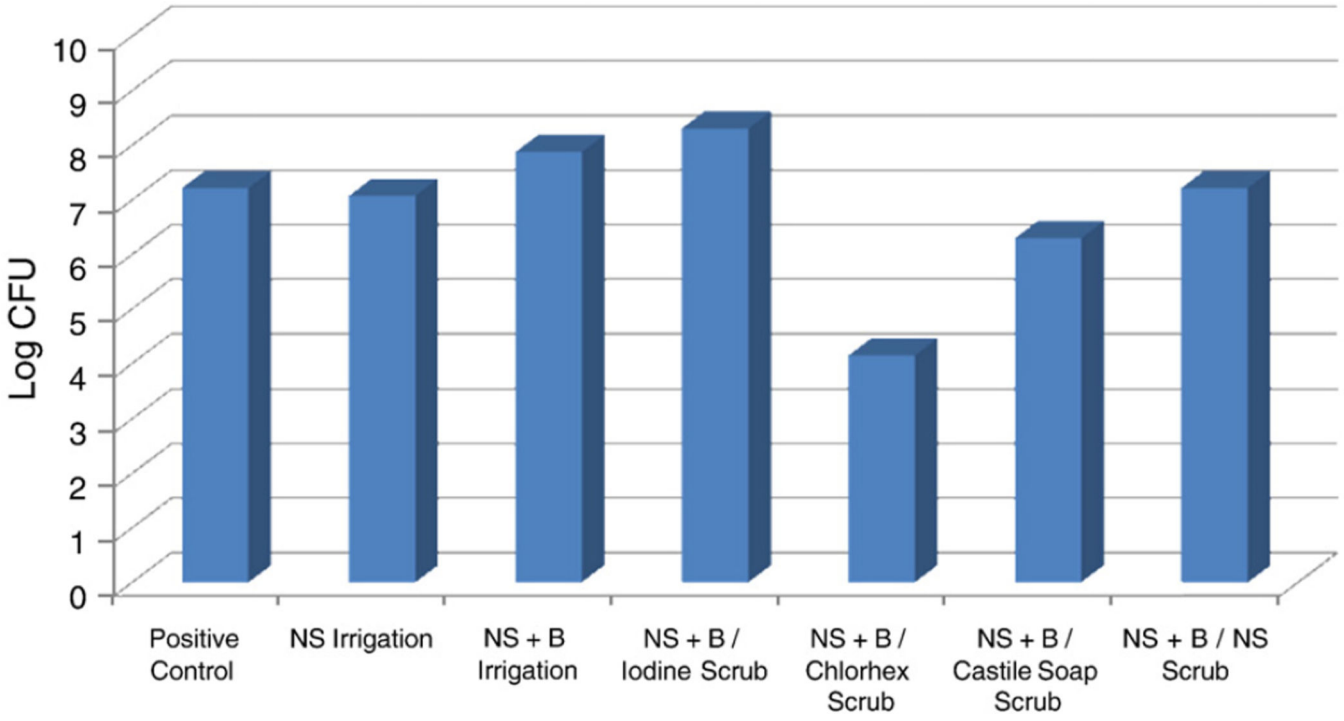

Fig. 2.
Group 2—with reincubation.

Table 1

Log CFUs Per Experimental Group—Without Reincubation Group (Group 1)

Experimental Group	Log CFU Mean
Group A	7.86 ± 0.01
Group B	6.09 ± 0.19
Group C	4.95 ± 0.05
Group D	2.04 ± 0.51
Group E	0 ± 0
Group F	3.57 ± 0.02
Group G	4.59 ± 0.08

Comparisons between groups using ANOVA/Tukey pairwise comparison test all reached significance ($P < .05$), except group C compared with G.

Table 2

Log CFUs Per Experimental Group—With Reincubation Group (Group 2)

Experimental Group	Log CFU Mean
Group A	7.22 ± 0.04
Group B	7.08 ± 0.14
Group C	7.88 ± 0.07
Group D	8.31 ± 0.11
Group E	4.15 ± 0.46
Group F	6.30 ± 0.03
Group G	7.22 ± 0.08

Comparisons between groups using ANOVA/Tukey pairwise comparison test all reached significance ($P < .05$), except group C compared with D, group A compared with B, group A compared with G, and group B compared with G.