Comparison of the Antimicrobial Effect of Chlorhexidine and Saline for Irrigating a Contaminated Open Fracture Model

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OBJECTIVES: The objective of this study is to compare antimicrobial effect of irrigation with chlorhexidine gluconate (CHG) to saline in an animal model.

METHODS: This study used a segmental defect rat femur model contaminated with Staphylococcus aureus and treated 6 hours after injury with debridement and irrigation with 60 mL of fluid delivered at low pressure. In study groups of 10 animals each, 3 concentrations of CHG (0.5%, 0.05%, and 0.005%) were used and a group irrigated with 0.05% CHG and then saline and a control group treated with saline only. After irrigation the wounds were closed, and the rats were recovered. Fourteen days later, bone and implants were harvested for separate microbiological analysis.

RESULTS: There was no statistical difference detected between the subsequent presence or quantity of bacteria after irrigation, with aqueous CHG at a range of concentrations comparing irrigation with saline alone.

CONCLUSIONS: This study does not support the use of CHG as an irrigant. This may be due to the bacterial effect of CHG being offset by the associated host tissue toxicity. Host tissue damage from high irrigation pressures and cytotoxic solutions has been shown to allow bacteria to thrive. We believe this is due to a “rebound” of bacteria growth in a wound bed containing small quantities of necrotic tissue damaged by CHG exposure.

Key Words: open fractures, infection, injury, irrigation, chlorhexidine, antiseptic, lavage, debridement, wounds

ORIGINAL ARTICLE

INTRODUCTION

After Lister1 demonstrated the beneficial effects of irrigating open fracture wounds with an antiseptic solution, surgeons experimented with a variety of fluids up to and during the First World War.2 The colossal number of casualties from this conflict provided Fleming3 with sufficient opportunity to carefully compare the effect of different irrigation solutions. His findings were that the damage to host tissue caused by antiseptics outweighed the benefit of their initial antimicrobial effect. In a lecture to the Royal College of Surgeons of England after the War, he concluded that, “it also makes it necessary in the estimation of the value of an antiseptic to study its effects on the tissues more than its effects on bacteria.”3 Saline remains the accepted norm for irrigating open fracture wounds.4,5 Chlorhexidine gluconate (CHG) is an antiseptic that might potentially combine the mechanical action of an inert fluid in physically removing bacteria with an active chemical antimicrobial effect without damaging host tissue. CHG was synthesized in the 1950s.6 It was quickly recognized as being active against a broad spectrum of microbes including gram-negative and gram-positive bacteria and fungi via its effect on prokaryotic cell membranes7 while exhibiting low toxicity to mammalian tissue.8 For this reason, CHG is used extensively in healthcare as a surgical scrub,9 bladder irrigation fluid,10 and periodontal rinse.11

Interestingly, despite its reputation as an effective antiseptic with low toxicity and its ubiquitous use in most modern hospitals, CHG has not been evaluated as an open fracture irrigation fluid in either an appropriate animal model or a clinical trial. Despite this lack of evidence, many orthopaedic surgeons believe CHG to be a more effective open fracture irrigation solution than saline, and a small number of orthopaedic surgeons continue to irrigate open fracture wounds with CHG solution in preference saline.5 There is no clear guidance in the literature as to which concentration of CHG is most appropriate to use in this way.

The aim of this study was to compare the antimicrobial effectiveness of a range of CHG concentrations and saline as wound irrigation solutions at removing bacteria in a contaminated rat femur model of open fracture. The null hypothesis was that there would be no difference between CHG and saline as wound irrigation solutions in reducing bacteria in a model of contaminated open fracture.

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MATERIALS AND METHODS

Procedure
A previously described, contaminated open fracture model was used to evaluate the effects of irrigation with CHG. This study was conducted under a protocol in compliance with the Animal Welfare Act and the implementing Animal Welfare Regulations and in accordance with the principles of the Guide for the Care of Use of Laboratory Animals. Briefly, a contaminated open femoral defect was created by using the following technique: Adult male Sprague Dawley rats (Harlan Laboratories, Indianapolis, IN) were anesthetized with isoflurane and prepared for surgery; their right femoral shafts were exposed and stabilized internally with a custom-made polyoxymethylene plate secured with 6 threaded K-wires. A 6-mm defect was then created in the mid-shaft with a reticulating saw, cooled with saline (Fig. 1). The defect was contaminated with 30 mg of sterile bovine collagen soaked with $1 \times 10^2$ colony forming units of \textit{Staphylococcus aureus} in 0.5 mL of saline. The Xenogen 36 strain of \textit{S. aureus} used derived from American Type Culture Collection (ATCC) 49525 originally from a septic human patient (Caliper Life Sciences, CA).

The wounds were closed in layers and the animals recovered. The animals were reanesthetized 6 hours after the initial “injury,” and their wounds opened, debrided with careful removal of all contamination, and irrigated with a total of 60 mL of fluid delivered at low pressure from a hand-held syringe approximately 10 cm from the surgical field. Their wounds were again closed in layers. The animals were recovered and allowed full mobility, food, and water.

The animals were euthanized 14 days after simulated injury. The femur and implants were stripped of soft tissue and separated. The bone tissue was snap-frozen in liquid nitrogen and crushed. Bone and implant samples were then sent separately for standard quantitative microbiological analysis. Crushed bone samples were homogenized with 10 mL of saline in an agitator, and implant specimens were similarly rinsed with 10 mL of saline in an agitator; then aliquots from individual specimens were sequentially diluted and spread onto tryptic soy agar plates. After overnight incubation at 37°C, bacterial colonies were counted and recorded; the threshold of detectability was 30 colony forming units per gram.

Study Groups
There were 10 animals in each group. The treatments in each study group are detailed in Table 1.

Outcome Measures
The outcome measures were the presence and quantity of bacteria in the femur or attached to the implants (polyoxymethylene plate and K-wires).

Statistics
Statistical analysis was performed using SAS software (SAS Institute, Inc., Cary, NC). For comparing rates of bacterial presence in each group, Fisher exact test was used. For detection of variance between bacterial quantities across all groups, a Kruskal–Wallis test analysis of the log mean of the sum of quantity of the bacteria on the bone and implant was used. The threshold for significance was set at 0.05; if this threshold was met, paired comparisons of test groups to the control group would be individually performed with a Mann–Whitney analysis of the log mean of the sum of quantity of the bacteria on the bone and implant.

RESULTS
There was no statistical difference detected between irrigation with aqueous CHG at a range of concentrations on the proportion of sample with detectible bacteria (Fig. 2) or the quantity of bacteria in the wound (Fig. 3) at 14 days compared with irrigation with saline alone. $P$ values for the proportion of samples with detectable bacteria in each test group compared with the control group are shown in Table 2. The results of the Kruskal–Wallis test of the log sums of the bacteria recovered from the wounds of the animal across all groups were not significant ($P = 0.21$), and therefore pairwise comparison between individual test groups and the control was not performed.

DISCUSSION
In this study, a commonly used antiseptic, CHG, was evaluated as an irrigation fluid for reducing infection in open fractures. The study group with the lowest infection rate or

TABLE 1. Study Groups Detailing Irrigation Fluids Used in Each, After Surgical Debridement, 6 Hours After Initial Injury and Contamination With $1 \times 10^2$ Colony Forming Units of Bacteria

<table>
<thead>
<tr>
<th>Study Group</th>
<th>Irrigation Fluid</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>Irrigation with 60 mL 0.9% saline</td>
</tr>
<tr>
<td>0.5% CHG</td>
<td>Irrigation with 60 mL 0.5% CHG aqueous solution</td>
</tr>
<tr>
<td>0.05% CHG</td>
<td>Irrigation with 60 mL 0.05% CHG aqueous solution</td>
</tr>
<tr>
<td>0.005% CHG</td>
<td>Irrigation with 60 mL 0.005% CHG aqueous solution</td>
</tr>
<tr>
<td>0.05% CHG and saline rinse</td>
<td>Irrigation with 50 mL 0.05% CHG aqueous solution followed by rinsing with 10 mL 0.9% saline</td>
</tr>
</tbody>
</table>
quantity of bacteria in the wound was irrigated with 0.05% CHG followed by removal of antiseptic residue by rinsing with saline. This difference did not reach significance. A post hoc power analysis indicated that to have an 80% chance of detecting a statistical difference between the log-transformed sums of the amount of bacteria recovered from this group and the control group, groups of at least 36 animals each would be required.

This rat femur model can obviously only mimic an open fracture and does not encompass all the features of the clinical situation, including soft tissue damage, surgical defect rather than fracture, single surgical treatment, and immediate primary closure. It is reasonable to speculate that the effects observed might be peculiar to the type of bacteria. Unpublished work on this model has used sonification techniques to determine whether a bacterial biofilm might be present in instances where conventional microbiology fails to detect bacteria. However, biofilms were only detected in samples where bacteria in planktonic form were also detectable; therefore, a false-negative result due to bacteria persisting in biofilm form only is unlikely. This finding is different from clinical studies that used sonification techniques to detect biofilm bacteria on recovered arthroplasty prosthesis that conventional microbiological techniques had found to be sterile. This apparent discrepancy may be due to the use of antibiotics in the clinical setting that eliminates bacteria not in a biofilm; animals in this study were not exposed to antibiotics, therefore conventionally detectible planktonic bacteria persist. The concentration of 0.05% CHG was selected for the “irrigate and rinse” group because this is the concentration that has been most thoroughly studied in the literature.

Lister’s practice of irrigating open fracture wounds with carbolic acid resulted in an unprecedented reduction in infections compared with the dire rates typical of the 1860s. In hindsight, much of his improved results might be attributed to the application of carbolic acid by the surgeon and his instruments and the development of antiseptic practice rather than the application of antiseptic directly into the wound. “Listerism,” as it became known, was the clinical standard until Fleming’s first great contribution to the management of open fractures: The recognition that the use of antiseptics in open fracture wounds actually increased bacteria loads. He ascribed this counterintuitive observation to the toxicity of chemical antiseptics to the host immune system, which he thought was the most important factor in wound infection.

The reason that many seemingly innocuous antiseptics are toxic in traumatic wounds can be explained by using the model proposed by Jackson in 1953. This model divides the wounds into the inner zone of coagulation (necrotic tissue) and the peripheral zone of hyperemia (inflamed tissue) divided by the zone of stasis, which is potentially viable but vulnerable to secondary insult. The tissue in this zone of stasis is believed to be very sensitive to damage by antiseptics. This model also explains the rebound phenomenon of bacteria load in a wound after irrigation with irrigants other than saline previously observed in our laboratory. Although the antiseptics and soap solutions removed more bacteria from the

![FIGURE 2](image1.png)

**FIGURE 2.** Number of bone and hardware samples with detectible bacteria 14 days after inoculation with $1 \times 10^2$ colony forming units of *S. aureus* and irrigation with various fluids or combinations. No significant differences between groups.

![FIGURE 3](image2.png)

**FIGURE 3.** Mean bacterial quantification of bone and hardware samples 14 days after inoculation with $1 \times 10^2$ CFUs of *S. aureus* and irrigation with various fluids or combinations. Error bars show the SEMs. No significant difference between groups. CFU, colony forming units; SEM, standard error of the mean.

<table>
<thead>
<tr>
<th>Test Group</th>
<th>$P$ of Comparison With Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.5% CHG</td>
<td>0.74</td>
</tr>
<tr>
<td>0.05% CHG and saline rinse</td>
<td>0.20</td>
</tr>
<tr>
<td>0.005% CHG</td>
<td>0.74</td>
</tr>
</tbody>
</table>

$P$ generated by Fisher exact test.

TABLE 2. The Similarity of the Effect of Various Concentrations of CHG in on the Rate of Detectable Bacteria Compared With Saline

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wound than saline initially, the wounds irrigated with solutions other than saline had higher bacteria levels 2 days after debridement and irrigation. It is believed that the bacteria that remained in the wound were able to thrive because of tissue damage caused by the irrigants.

Because of the continuing challenge of infection in open fractures, investigators have continued to evaluate potential irrigation solutions that combine the mechanical action of an inert fluid in physically removing bacteria and contamination with an active antimicrobial effect while not damaging host tissue. CHG has been considered by a small number of studies because it is believed to offer this combination of bactericidal effect with low cytotoxicity. In a study that evaluated the use of CHG in a soft tissue wound, Platt and Bucknall found that irrigation with a 0.05% CHG solution was superior to 1% povidone iodine, 0.1% benzalkonium, and 0.9% saline at removing bacteria in a guinea pig contaminated dorsal wound model.

A concern about using CHG in wounds is the potential negative effect on healing. Brennan et al found that there was no difference in rat healing or collagen production in a rat wound model exposed to saline or 0.05% CHG, whereas an adverse effect was associated with exposure to hypochlorite antiseptic. This group also found that 0.05% CHG and saline exerted a similarly negligible effect on microvascular action of an inert fluid during arthroscopy at both high and low concentrations. A 2007 in vitro study using human cartilage suggests that there is no significant effect of a 1-minute exposure to 0.05% CHG in nonarthritic cartilage.

Of additional concern to orthopaedic surgeons is the chondrolytic effect of CHG that has been reported after the accidental use of CHG as an irrigation fluid during arthroscopy at both high and low concentrations. A 2007 in vitro study using human cartilage suggests that there is no significant effect of a 1-minute exposure to 0.05% CHG in nonarthritic cartilage.

Possibly because of these concerns about the effect on cartilage and wound healing, only one very limited clinical trial of CHG irrigation in orthopaedic trauma has been performed. This trial compared irrigation with 0.05% CHG in closed hip fractures with no irrigation and used bacterial quantification of intraoperative wound swabs as the outcome measure. This very limited study demonstrated a small reduction in recovered bacteria, but statistical analysis was not provided. Small numbers of surgeons do use CHG to irrigate open fractures in their current clinical practice.

In the only clinical randomized trial of different irrigation solutions in open fractures, Anglen compared castile soap solution with bacitracin solution for the irrigation of lower-extremity open fractures in 400 patients. He found an insignificantly lower infection rate in the castile soap group and a significantly higher rate of wound breakdown in the bacitracin group. This study did not include irrigation with saline as a control.

Most surgeons currently irrigate open fractures with low pressure saline, the current phase of the Fluid Lavage of Open Wounds study project is a multicenter randomized trial comparing irrigation with saline to castile soap solution at high and low pressures. This appropriately powered study should finally answer some of the questions regarding the best pressure and whether castile soap or saline is the best irrigant.

This study indicates that CHG at concentrations of 0.05% is not more effective than saline as an irrigation solution for reducing bacteria in open fractures. Because there is evidence that CHG can delay or reduce healing and there have not been any evidence that CHG reduces infection rates, saline remains the best choice of irrigants. However, in cases where surgeons are using CHG to irrigate a wound, this study suggests that a concentration of 0.05% and a final saline rinse to remove any residue before closure/dressing is the best option. The authors would urge particular caution when surgeons are contemplating using CHG in a wound with large amounts of tissue of borderline viability because this type of wound might be particularly vulnerable to the toxic effects of CHG. These results are consistent with the concept suggested by Fleming of a balance between antibacterial effect and tissue damage determining the effectiveness of antiseptics in this setting.

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REFERENCES

1. Lister J. On a new method of treating compound fracture, abscess, and so forth; with observations on the conditions of suppuration. Lancet. 1867; 89:326, 357, 387, 507.


