Evaluation of Alternative Uses of Commercial Antimicrobials

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FINAL REPORT

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14. ABSTRACT-
Methods for sanitization of medical tools are sought and efficacy of existing materiel sourced to austere medical facilities should be examined. This study tested the efficacy of commercially available, FDA-approved wound cleansers for alternative use as a potential sanitizer of stainless-steel medical devices found at improvised medical facilities. Escherichia coli and Staphylococcus aureus cultures were inoculated onto sterile stainless-steel carriers. After cleaner treatment, samples were held at different temperatures for different lengths of time. Additional ex vivo challenges were performed using slurry harvested from porcine cecum. Colony forming units and log reduction were calculated. Significance was determined using one-way ANOVA and multiple comparisons between treatment groups was calculated using Tukey's multiple comparison's test. All wound cleansers demonstrated statistically significantly bactericidal activity against lab bacteria and ex vivo cecal slurry. E. coli and S. aureus resulted in approximately a 5-6 log reduction on average. While treatment of ex vivo samples did not result in total kill, significant reductions in bacterial load was observed in all groups. These results indicate that FDA approved wound cleaners show promise as a potential sanitizer in resource limited environments.

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1.0 EXECUTIVE SUMMARY

Service members experience unique circumstances when providing medical care in austere environments. Some challenges include supply shortages and the need to perform surgery in extreme temperatures. As such, methods for sanitization of medical tools are sought and efficacy of existing materiel sourced to austere medical facilities should be examined for this purpose. This study tested the efficacy of commercially available, FDA-approved wound cleansers for alternative use as a potential sanitizer of stainless-steel medical devices found at improvised medical facilities.

Escherichia coli and Staphylococcus aureus cultures were inoculated onto sterile stainless-steel carriers. After cleaner treatment, samples were held for 2-hours, 24-hours, or 7 days to represent different turn-around times between uses at ambient (25℃), cold (-20℃) and hot (50℃) temperatures. Additional ex vivo challenges were performed using slurry harvested from porcine cecum. Colony forming units and log reduction were calculated. Significance was determined using one-way ANOVA and multiple comparisons between treatment groups was calculated using Tukey's multiple comparison's test.

All wound cleansers demonstrated statistically significantly bactericidal activity against lab bacteria and ex vivo cecal slurry. *E. coli* and *S. aureus* resulted in approximately a 5-6 log reduction on average, resulting in no growth after treatment for all cleaners at 2 hours and 24-hours. Similarly, 7-day post exposure results in a 6-log reduction after treatment for all groups at 25℃ and -25℃. While treatment of ex vivo samples did not result in total kill, significant reductions in bacterial load was observed in all groups.

Wound cleansers cleared for use in surgical settings demonstrated antimicrobial effects against bacteria deposited on metal surfaces. These cleansers decreased bacterial viability when challenged against extreme temperatures and few bacteria were harvested from treated surfaces even after 7 days. FDA approved wound cleaners show promise as a potential sanitizer in resource limited environments.

2.0 INTRODUCTION

Due to predicted breaches in communications and projected inability to immediately transport casualties out of theater during near-peer conflict, there is an increased likelihood military care providers will face prolonged care scenarios and severe resource constraints. Battlefield wound infections will increase, and surgical-associated infections will also pose a significant risk to care providers working in an austere environment. Initial data collected from a 5-year prospective observational cohort study of infectious complications associated with traumatic injury sustained during deployment revealed that approximately 25% of patients with combat-related injuries transported to U.S. military treatment facilities had infections[1]; another study recovered and characterized microbial pathogens from severe extremities in ten individuals and found all had bacteria retrieved from their wounds.[2] In mass-casualty situations, it is possible that equipment, tools, devices, and other items might be disinfected and stored for a time before use on another patient without full sterilization processes. In role 3 or higher, steam autoclave is routinely used for this purpose. However, this gold standard of sterilization is impractical closer to the battlefield due to the autoclave size, weight, expense, maintenance, and requiring a constant purified water with steady electrical power. It is imperative that far forward medical personnel have a rapid and effective means of quickly sanitizing soiled instruments, and this solution must also be economical, lightweight, easy to transport, and easy to store. Providing a means of disinfection that meets these criteria is paramount to the success of our troops in a war theater.

Various hard surface disinfectants are currently in use, but no standard operating procedure or method has been developed for use of disinfectants in the field. Previous studies have found that hard surface disinfectants such as Cidex OPA, CaviCide, and Neutral Disinfectant Cleaner have the capacity to reduce bacterial load after a debridement step. [3] Additionally, the same series of hard surface disinfectants were studied and found to reduce bacterial load without harming mechanical function of plastic endotracheal tubes. [4] However, while hard surface disinfectants are effective against biofilms and bacterial spores at high concentrations, they are harmful to human health and the environment.[5,6] Hard surface disinfectants can cause corrosion on metallic surfaces. As such, other methods for sanitization of surfaces and medical tools are sought, and efficacy of existing materials and reagents sourced in austere medical facilities should be examined for this purpose.

Surgical wound cleansers are routinely used to irrigate body cavities during surgical procedures. They have also demonstrated antimicrobial activity in vitro at non-toxic concentrations in challenges against skin fibroblasts.[7] Chlorhexidine is commonly used for infection prevention, and a recent meta-analysis and review revealed that it did not prevent surgical site infection, deep wound infection or urinary tract infection but did protect against sepsis, nosocomial infection, superficial surgical site infection and pneumonia in cardiac surgical patients.[8] Knox et al. investigated different strategies to rapidly disinfect surgical instruments that could be applicable in far forward surgical environments by evaluating chlorhexidine scrubbing and ultraviolet C irradiation[9]; the authors demonstrated full decontamination of instruments previously immersed in a prepared bacterial solutions. Wound irrigation is a common practice with numerous products available, although their use is not standardized.[10] Antimicrobial wound irrigates and cleansers are available as liquids and gels, and come in pre-fixed volumes, making it a convenient resource and suitable to test as an alternate means for disinfection of surgical tools. Since they are widely found in operating rooms, their suitability as a multifunctional medical resource and utility as a potential disinfectant was studied.

The purpose of this project was to test three commercially available off-the-shelf wound cleanser liquid and gel products for their practicality and efficacy as disinfectants when required in resource constrained, temperature-extreme environments where steam sterilization is not available to decontaminate surgical instruments.

3.0 METHODS, ASSUMPTIONS AND PROCEDURES

3.1 Experiment 1: Cytokine and Myoglobin Filtration

3.1.1 Hypothesis

The hypothesis tested in Experiment 1 is that the wound cleanser liquid and gel products will be effective in killing the standard clinical isolated bacterium at various temperatures.

3.1.2 Methods

In this study, to determine if routine Food and Drug Administration (FDA) approved wound cleanser irrigates and surgical infection control gels could be used as an alternative surgical tool disinfectant, stainless-steel carriers or stainless steel peni-cylinders were soiled with laboratory bacterial strains and a more real world scenario (GI cecal contents from swine; Figure 1A), sanitized with one of three surgical wound cleansers candidates and subjected to storage times of 2 hours, 24 hours, and 7-days at ambient temperature (25℃) extreme cold (-20℃) and extreme hot (50℃) conditions Figure 1B). After the designated storage time was completed, the stainless-steel materials were then processed, and bacterial burden quantified using traditional colony count methodology. All experiments were performed with five biological samples with three plating replicates.

In vitro studies were performed using commercially available *Escherichia coli* (*E. coli*) and *Staphylococcus aureus* (*S. aureus*) independently to test the efficacy against gram-negative and gram-positive bacteria, respectively. Stainless steel carriers or stainless steel peni-cylinders (Fisher Scientific cat. Number 07-907-5Q) were prepared based on the methods described in US EPA Number MP-05-16, "Standard Operating Procedure for AOAC Use Dilution Method for Testing Disinfectants."[11] In vitro testing was performed using overnight cultures of *E. coli* and *S. aureus* grown in 50 mL sterile Luria broth (LB MP Biomedicals) in a 125 mL Erlenmeyer flask, at 37℃, 180 revolutions per minute overnight. The cultures were then diluted the next morning. *E. coli* was regrown to OD_{600} 0.3 (1.5×10^7 CFU/mL), and *S. aureus* was grown to OD_{600} 0.7 (5.6×10^6 CFU/mL) respectively to ensure logarithmic growth phase. 100 µL of each culture was inoculated onto the polished side of sterile stainless-steel carriers in a thin coating using a pipette and allowed to dry in a biosafety cabinet for 2-hours with air circulating. Negative controls were inoculated with 100 µL of sterile LB.

After the inoculated carriers dried, they underwent a 10-minute disinfection treatment by submersion in specified FDA cleared surgical wound cleanser irrigates (referred to herein as Treatments 1 and 2) commonly available in operating rooms. An FDA cleared wound infection control gel (referred to as Treatment 3) was also tested and applied in a thin coating across

individual carriers. Stainless-steel carriers were then removed from treatment and then stored in individual, dry, sterile conical tubes with loose caps for 2-hours, 24-hours, or 7-days at 25℃, -20℃, or 50℃ to determine if the treatment was efficacious at reducing bacterial burden between patients without being subjected to normal sterility procedures (e.g., autoclave). After storage times were completed, 25 mL of sterile LB broth was added to each tube. The long storage periods (24-hour and 7-days) appears to result in bacteria that is viable but not culturable (VBNC).[12] Initial pilots resulted in no bacterial growth when immediately processed and plated after storage periods longer than 2 hours. Submersion in sterile LB broth at room temperature provides the nutrients and environment that allows the recovery of the bacteria and culturing on classic tryptic soy agar. Pilot studies determined that 2-hour recovery time was adequate to end torpor without increased population, while 4-hour recovery resulted in increased population size. 24-hour and 7-day samples were stored in broth for 2 two additional hours for a bacterial recovery period to assess viability versus dormancy. Samples were then sonicated at 40 kHz (Branson ultrasonic CPXH series bath 3800) for 5 minutes and vortexed (scientific industries vortex-genie 2) for 3-minutes to recover bacteria from carriers. This process is frequently used to recover bacteria from solid surfaces such as stainless-steel carriers to increase detection and consistency without undermining reproducibility.[13]

Recovered bacterial liquid culture samples were serially diluted into aliquots that were plated and incubated for 24 hours at 37℃ before being counted to assess bacterial growth. These tests were repeated at a range of temperatures from extreme cold (-20℃) and extreme hot (50℃). All studies were performed with an n=5 and in triplicate.

3.2 Experiment 2: Ex vivo Challenge Methods

3.2.1 Hypothesis

The hypothesis tested in Experiment 2 is that the wound cleanser liquid and gel products will be effective in killing the microorganisms in ex vivo cecum samples.

3.2.2 Methods

The most common anaerobic infections in surgical patients are: Bacteroides fragilis, Prevotella spp, Porphyromonas spp, Fusobacterium spp, peptostreptococcus spp, clostridium spp, and actinomyces spp.[14] Bacterioides fragilis displays minimal aerobic tolerance, and is rendered inviable after 4 hours or less of oxygen exposure.[15] Prevotella spp displays unique adaptive aerotolerance.[16] For a more true-to-life experiment, an ex vivo portion was also performed where the antimicrobial wound infection control products were challenged to enteric contents collected directly from porcine cecum. The cecal contents were collected from euthanized swine utilized from other experiments, as a means to reduce unnecessary utilization of animals. The experiments reported herein for cecal content collection were conducted according to the principles set forth in the National Institute of Health Publication No. 80-23, Guide for the Care and Use of Laboratory Animals and the Animal Welfare Act of 1966, as amended.

The anaerobic methodology was developed primarily to examine the efficacy of disinfectants against aerotolerant anaerobes. Swine cecal contents were applied to peni-cylinders and allowed

to dry for one hour inside of a biosafety cabinet with the door open and air circulating. Penicylinders were then removed from treatment and then stored in individual, dry, sterile conical tubes with loose caps for 2-hours, 24-hours, or 7-days to represent different turn-around times. During storage, challenged samples were kept in 15 mL conical tubes without caps inside the anaerobic chambers to maintain sample isolation, but also allow oxygen removal. No rinse or mechanical wash step was utilized during any portion of the experiment to provide the most stringent efficacy test possible. It is well documented that washing procedures are vital for complete disinfection of soiled equipment and results in drastic reduction of bacterial colonies.[17]

After storage times were completed, 25 mL of sterile brain heart infusion broth was added to each tube. As with the in vitro procedure above, 24-hour and 7-day samples were stored in broth for 2 two additional hours for a bacterial recovery period to assess viability vice dormancy. Recovered bacterial liquid culture samples were then sonicated as described above and serially diluted into aliquots that were plated and incubated for 24 hours at 37℃ before being counted to assess bacterial growth. These tests were repeated at a range of temperatures from extreme cold (-20℃) and extreme hot (50℃). All studies were performed with an n=5 and in triplicate.

3.3 Data Analysis

After counting, colony-forming units (CFU's) were calculated for each plate: plate count x (1/amount plated (mL)) x (1/sample volume (mL) x dilution. The average CFU of all 3 replicates for each treatment group were then calculated (CFUavg). The survival fraction, percent killed, and log reduction were calculated using standard equations using the average CFU for all 3 replicates for each treatment group.[18] The survival fraction (SF) was then calculated for each treatment by dividing the CFUavg of the treated carriers (T) by the untreated (UT):

$$SF = TCFUavg/UTCFUavg$$

Percent killed was calculated: $PK = (1-SF) \times 100\%$

Log Reduction was calculated: $LR = \log10(1/SF)$[19]

Significance was determined using one-way Analysis of Variance and multiple comparisons between treatment groups was calculated using Tukey's multiple comparison's test.

4.0 MAJOR EVENTS/MILESTONES/SUCCESS

- IACUC approval (animal exempt) – Jan 2020
- Project laboratory technologist hired – Nov 2019
- Project Scientist hired – Nov 2019
- CRADA with Next Science - Nov 2019
- CRADA with Parasol Medical – June 2020
- In vitro evaluation completion – June 2021
- Ex vivo evaluation completion – Dec 2021
- Publication in Military Medicine – Nov 2023

5.0 RISK ASSESSMENT

5.1 Risk Analysis

Delays during the project:

- FY20Q1 - Risk in not having the project funds loaded into the DMLSS account to allow for purchasing of supplies/equipment. Risk mitigation plan is to supplement some of the project funds with CAMD ODC funds for supply purchasing while awaiting the money to load in DMLSS account. Risk in not having the CRADA completed with Parasol Medical LLC company to evaluate anti-microbial coating product. Risk mitigation will be to re-arrange the order of product evaluations while the agreement is pending.

- FY20Q2 - Work stoppage due to COVID19 has impacted the availability of animals from CIRS, as well as the performance other forms of non-essential lab work.

- FY20Q3 - Risk in not having the CRADA completed with Parasol Medical LLC company to evaluate anti-microbial coating product. The Parasol product was replaced by another product (Biakos from Rochal) in the evaluation queue.

- FY20Q4 - Work stoppage due to delay in receiving funds.

- FY21Q1 - New funds received, working to get the funds on contract.

- FY21Q2 - Funds for supplies in house, staffed new technician, working on making up on experimental schedule due to staffing shortfall. Anticipate being on track by May 21.

- FY21Q3 - Still progressing to make up for staffing delays; anticipate on time experimental completion by end of FY21.

- FY21Q4 - Delays in consumable procurement caused timeline shift.

- FY22Q2- Despite residuals not loading funds to FY22 contract, project data collection completed.

5.2 Technical Challenges

None.

6.0 TRANSITION PLAN

6.1 Military Relevance

Battlefield wound infections and surgical-associated infections are expected to increase and pose a significant risk to care providers with the rise in austere environment missions. A recent study

showed 25% of patients with combat-related injuries transported to military treatment facilities had infections.[1] It is critical to provide extensive antimicrobial policies and procedures to limit morbidity and mortality of servicemembers. This study supported the following requirements: AFMS ICL #20; Prevent wound infection, sterilizer limitations. Expeditionary Medicine Research Development Document priority #2, prevent wound infection; #20 decontamination methods for far forward medical teams.

6.2 Transition Strategy

Currently, evaluations of disinfectant products performed by laboratories follow the Association of Official Analytical Chemists (AOAC) Use-Dilution Method.[11] However, the results from these test standards do not necessarily translate into real world application. Literature shows that the AOAC method was not efficiently reproduced across multiple laboratories. Variability in these results discredits the choice to implement disinfectant use strictly on the basis of this standard testing method. Our effort proposes to resolve this gap by testing the efficacy of high-level disinfectants in a manner that differs from the established AOAC method. Namely, by using a testing design that simulates the real-world scenarios in which far forward military surgical teams operate.

Capability Description: Evaluate efficacy of commercially available EPA-registered, FDA-approved, and over the counter novel antimicrobials (Biakos, Mediclean, and the Next Science product TorrentX as well as Next Science's hard surface disinfectant) for disinfecting surgical instruments for forward deployed surgical teams. These antimicrobials are less toxic/reactive than existing antimicrobials because the reagent penetrates and dissolves the biofilm and lyses the bacteria.

The impact of this work: 1) addresses deficiency for disinfection in austere environments, 2) inclusion of the appropriate & cost-effective products in allowance standards of operational medical kits and processes; 3) reduced risk of infections, associated costs, and lost duty time. This study moved the knowledge readiness for the tested materials from a KRL3 at the beginning of the study to end at KRL5.

7.0 RESULTS

7.1 Experiment 1: In Vitro Experimental Approach

Stainless steel surfaces inoculated with *E. coli* were found to be sensitive to all of the wound cleansers tested (Figure 2A). When stored at 25℃, 6-log reductions were found for Treatment 1, 2, and 3 after 2-hour storage, >4-log reduction was calculated after a 24- hour storage, and a 6-log reduction was noted for all treatments stored for 7 days (Figure 2 left). For all treated surfaces and samples stored at -20℃ for 2 hours, a 6-log reduction was found; for 24- hour storage, 4- to 5-log reductions were recorded, and for 7-day storage, >5.5-log reductions were observed (Figure 2, right).

Stainless steel surfaces inoculated with *S. aureus* (Figure 2B) had variable responses to treatments. When stored at 25℃ for 2 hours, a 6-log reduction was observed for surfaces regardless of treatment (Figure 2B, left). For 24-hour storage times at 25℃, >5-log reductions were observed for all treatment surfaces stored at both 24 hour for 7-day time points (Figure 2B, left). When treated surfaces were stored at -20℃, Treatments 1 and 2 displayed significantly different log reductions compared with Treatment 3 after 2-hour storage ($p<0.0001$, Figure 2B middle). The same effect was seen after 24-hour storage, where Treatment 1

log reduction was significantly different from Treatment 3 ($p<0.05$), and Treatment 2 log reduction was also found to be significantly different from Treatment 3 ($p<0.01$, Figure 2B, middle). After 7-day storage times, all treatments resulted in a log reduction of >5, and there were no significant differences in Treatment treatment performance (Figure 2B, middle). Treated surfaces stored at 50°C were found to have a profound effect against *S. aureus* viability, with all treatments resulting in >5.5-log reductions at all storage times (Figure 2B, right). Overall kill percentages are shown in Table 1.

7.2 Experiment 2: Ex Vivo Experimental Approach

To assess the real-world utility of these wound cleansers/irrigates, cecal contents from swine were uniformly applied using a dip-coat method to stainless-steel surface and dried. Contaminated surfaces were then exposed to the three candidate treatments and stored in the same conditions as the lab strain studies. Overall, longer storage times at 25°C were found to increase log reductions, independent of treatment type (Figure 3, left). Significant differences were found between Treatment 1 and Treatment 2 after 2-hour storage ($p<0.05$), as well as between Treatment 1 and 2, and Treatment 2 and 3 ($p<0.0001$ and $p<0.001$, respectively) after a 7-day storage period (Figure 3, left). When treated surfaces were stored at -20°C for 2 hours, Treatment 1 had significant differences in log reductions between Treatment 1 and 2, and Treatment 1 and 3 at 2 hours ($p<0.05$ and $p<0.01$, respectively) and Treatment 1 and 3 (Figure 3, middle). Treatment 1 reached 4-log reductions in bacterial viability after 24 hour and 7-day storages for Treatment 1, and comparisons between Treatment 1 and Treatment 2 as well as Treatment 1 and Treatment 3 were found to have significant differences ($p<0.0001$) at those same times at -20°C (Figure 3, middle). The impact of heat on log reductions was also noted, where Treatment 1 was found to have >3-log reduction in bacterial viability after 2 hours at 50°C, and this reduction was significantly different from Treatments 2 and 3 ($p<0.0001$, Figure 3, right). Log reductions increased to above 3 log for Treatments 1 and 3 after 24-hour storage at 50°C, and for Treatment 3 after 7 days of storage (Figure 3, right). Overall kill percentages are shown in Table 1.

8.0 CONCLUSION/DISCUSSION

The main initiative of this study is to determine if FDA approved wound cleansers/irrigates could be used to disinfect surfaces commonly found in surgical settings. This report is the first to our knowledge that tested ability of wound cleansers and irrigates and wound infection control gels for their utility to disinfect stainless-steel surfaces; most other studies investigated use of hard surface disinfectants.[3,4] An interesting technical phenomenon was uncovered during the study where bacteria seemed to require a resuscitation phase after the treatment hold times. This 2-hour resuscitation step was imperative to conduct because without it, it cannot be discerned if the treatments merely had a bacteriostatic effect, where residual cells became dormant during the hold periods or if the treatments were fully bactericidal, and the treatments were able to render the cells completely nonviable. *E. coli*, a Gram-negative bacterium, was chosen as a challenge organism since it is normal flora of the gastrointestinal tract, however, some strains are pathogenic and can cause disease in individuals and as well as contribute to nosocomial infections. Abdominal injuries disrupt an otherwise sterile cavity and can result in septicemia particularly in complex polytraumatic injuries.[18] In the current study, *E. coli* was highly sensitive to all the treatments irrespective of hold times. There were no recoverable bacteria even after a 7-day hold time post treatment. Notably, the *E. coli* strain chosen for these experiments was found to be heat sensitive, and therefore a challenge at a 50°C hold temperature could not be conducted since control bacterium were not able to survive at 50°C, even for only 2 hours. While not all *E. coli* demonstrate heat sensitivity, it is important to report that

increased temperature holds may contribute to reduced microbial bioburden. Samples that were treated with wound cleansers and held at ambient and -20℃ did not harbor viable *E. coli* at any hold time tested compared to control surfaces that went through the same procedure; this is an important finding as storage in cold is known to have a preservative effect and can institute a state of dormancy within the bacterium. It is posited that the treatment induced a cellular response that utilized valuable energy and therefore made the bacterium more susceptible to cold exposure. In addition, the ambient temperature held treated surfaces also did not result in any bacterial preservation. *E. coli* and *S. aureus* have different cellular structures including lipid bilayer components; therefore, antimicrobial molecules may enter or damage cells in a manner that is not recoverable, hence resulting in a completely non-viable state after cleanser treatment at all hold times tested. This is promising data for the applicability of off the shelf surgical wound cleansers and irrigates that are commonly available in a surgical setting. One feature that should be recognized is that the liquid wound cleansers/irrigates (Treatments 1 and 2) were not viscous. An FDA cleared antimicrobial wound gel (Treatment 3) was also tested and found to reduce bacterial bioburden completely, with no surviving cells. However, it was difficult to maintain uniform application since the gel was viscous and required a lather to be applied to the surface.

In the challenges with *S. aureus*, all treatments were effective at significantly reducing bacterial viability, though only stainless-steel surfaces exposed to Treatment 2 reached 100% antibacterial efficacy against *S. aureus* for all hold times (Table 1). Greater than 99.90% reduction was found for conditions that *S. aureus* was challenged with (Table 1). Interestingly, *S. aureus* is heat resistant, so holds at 50℃ were conducted in an effort to simulate a desert environment where surgical tools may not be stored in environmentally controlled locations. Likewise, as stated above, extreme cold (<-20℃) may act in a preservative manner and create a sort of dormancy where bacteria might be sustained, which again underscored the importance of the resuscitation step employed in these studies. The results from the present study suggest that with the physical differences between Gram-positive and Gram-negative bacteria, selected for this study may impact their responsiveness to antimicrobial treatments; alternatively, the overall cell responses displayed by *S. aureus* could be as simple as ability to mount a more protective stress response when challenged with antimicrobial treatments and temperature fluctuations outside if its normal growth conditions. The overall goal of this study was to examine the efficiency of antimicrobial cleanser/irrigates and wound gels as a tool for disinfection in resource limited environments. It is noted that much more extensive studies into the mechanism of the antimicrobial effects of these products is needed, but beyond the scope of this work.

Finally, *ex vivo* studies with cecal contents were conducted to better ascertain the performance of the off the shelf antimicrobial wound cleaners/irrigates in a more realistic setting which contained a myriad of gastrointestinal flora that would contaminant surgical surfaces. Here, cecal contents were isolated from swine via tissue sharing protocols. The cecal contents were made into a slurry and applied to the stainless-steel surfaces. The samples were processed identical to the lab strain bacterial challenges and held for the same amount of time and at the same temperatures. While the responses were not as uniform as the pure lab bacterial culture challenges, the antimicrobial efficacy was found to be ≥ 94.3% after 2-hour hold times, >84% for samples held for 24 hours and >83% for samples held for 7 days (Table 1). Interestingly, the samples held at -20℃ were found to have the most bacteria recovered, lending some credibility that bacterial survival may be enabled by entering a dormant state when exposed to colder temperatures. All of the products had some level of antibacterial activity, although Treatment 1, a liquid wound cleanser/irrigant did seem to have the most consistent antimicrobial efficacy compared to the other wound cleanser (Treatment 2) and the wound gel (Treatment 3). Several attempts to recover and culture anaerobic bacteria were made, however, growth was not uniform despite holding the treated surfaces under anaerobic

conditions. Future studies could investigate the recovery and identification of anaerobic bacteria and their susceptibility to these and other surgical wound cleaners and irrigates.

Application and ease of use are important factors to consider when assessing antimicrobial cleaners/irrigates outside of their intended use. We believe that consistent gel surface coverage and coating thickness would be difficult to recreate during standard operating procedure drafting and therefore, the preferred media for decontamination of surgical stainless-steel surfaces in resource constrained environments were the liquid wound cleansers/irrigates as they came in prefixed volumes, were easy to open and apply to vesicles containing soiled surfaces. In line with results and scoring criteria outlined in Hune, where a decontamination process resulting in over 90% kill was considered highly effective and over 70% kill was considered effective, all treatments and conditions could be rated as highly effective, and only two conditions (Treatment 2 and 3, held at -20℃ for 2 hours) were given an effective rating. [3]

In Conclusion, this is the first study investigating the utility of FDA-cleared antimicrobial wound cleanser/irrigates and wound gels as a potential strategy to disinfect surgical surfaces, which extends to stainless-steel surgical tools. The antimicrobial wound cleansers/irrigates and the gel tested in these studies appear to be effective surface disinfectants against pure and mixed cultures of bacterium. While the diverse colonies in cecal contents were not totally killed, a significant reduction in their bioburden was observed. All treatments tested were effective at ambient and representative extreme temperatures faced by military medical care providers in austere settings; treatments appeared most effective in extreme heat. The experimental strategy presented here served as a stringent test to the wound cleansers since there was no pre-wash step in any of the experiments, which is known to significantly reduce bacterial load on its own. The results from this study suggest that while wound cleansers found in surgical environments are in no way equivalent to gold standard sterilization practices for surgical tools, surfaces and instrumentation, their use can provide some protection against cross contamination when presented with resource limitations and large patient volumes that are likely to occur in a mass casualty situation.

9.0 DELIVERABLES

9.1 Publications

- Baker K, Sandoval M, Ervin MD, Rall J, Nagy A. Effectiveness of FDA-cleared Wound Cleansers for Disinfection of Surgical Surfaces in Resource Limited Environments. Mil Med. 2023 Nov 8;188(Suppl 6):545-552.

9.2 Presentations

- Poster Presentation at the 2022 San Antonio Military Health and Universities Research Forum. (SURF), San Antonio, TX, Jun 2022. Title: Effectiveness of FDA-Cleared Wound Cleansers to Sanitize Surgical Tools and Surfaces in Austere Environments
- Poster presentation at the 2022 Military Health System Research Symposium (MHSRS), Kissimmee, FL, Sept 2022. Title: Effectiveness of FDA-Cleared Wound Cleansers to Sanitize Surgical Tools and Surfaces in Austere Environments

10.0 COST

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12.0 TABLES AND FIGURES

12.1 Tables

12.2 Figures

A.

B.

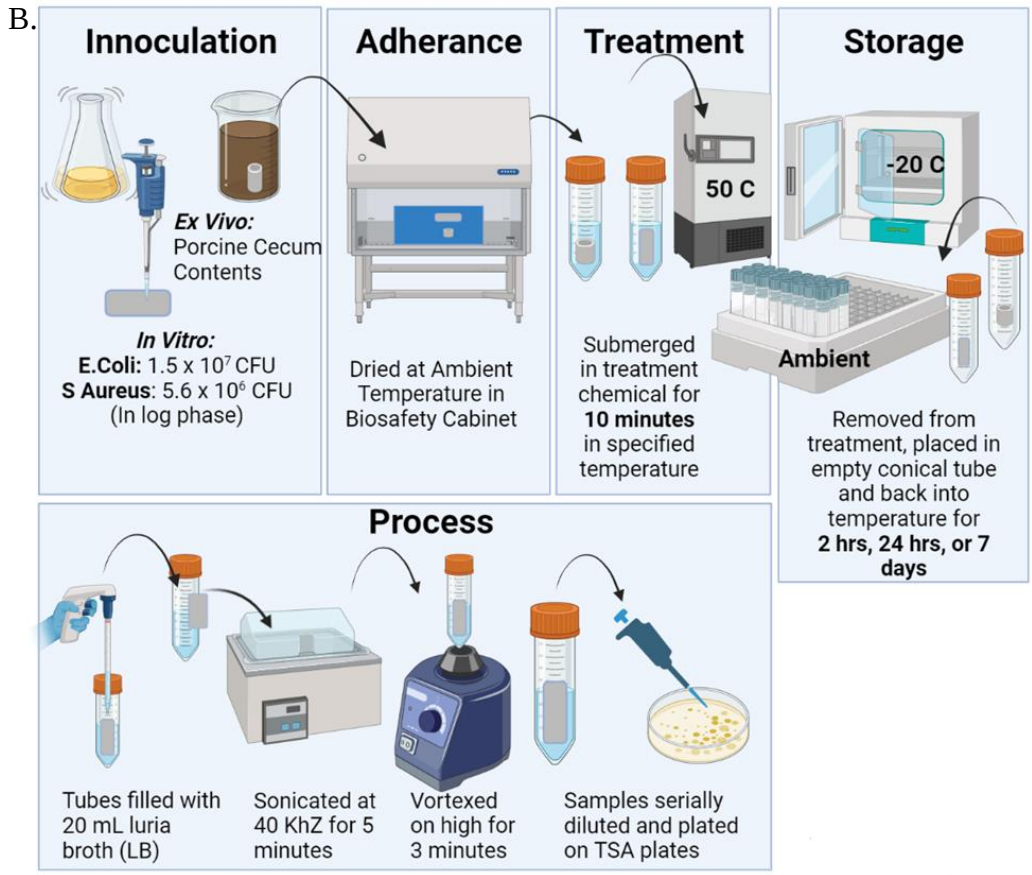

Figure 1. A. Stainless steel test coupons (left) and stainless steel peni-cylinders submerged in bacteria containing broths (right) used in the experiment. B. Schematic of experimental design created using BioRender.com.

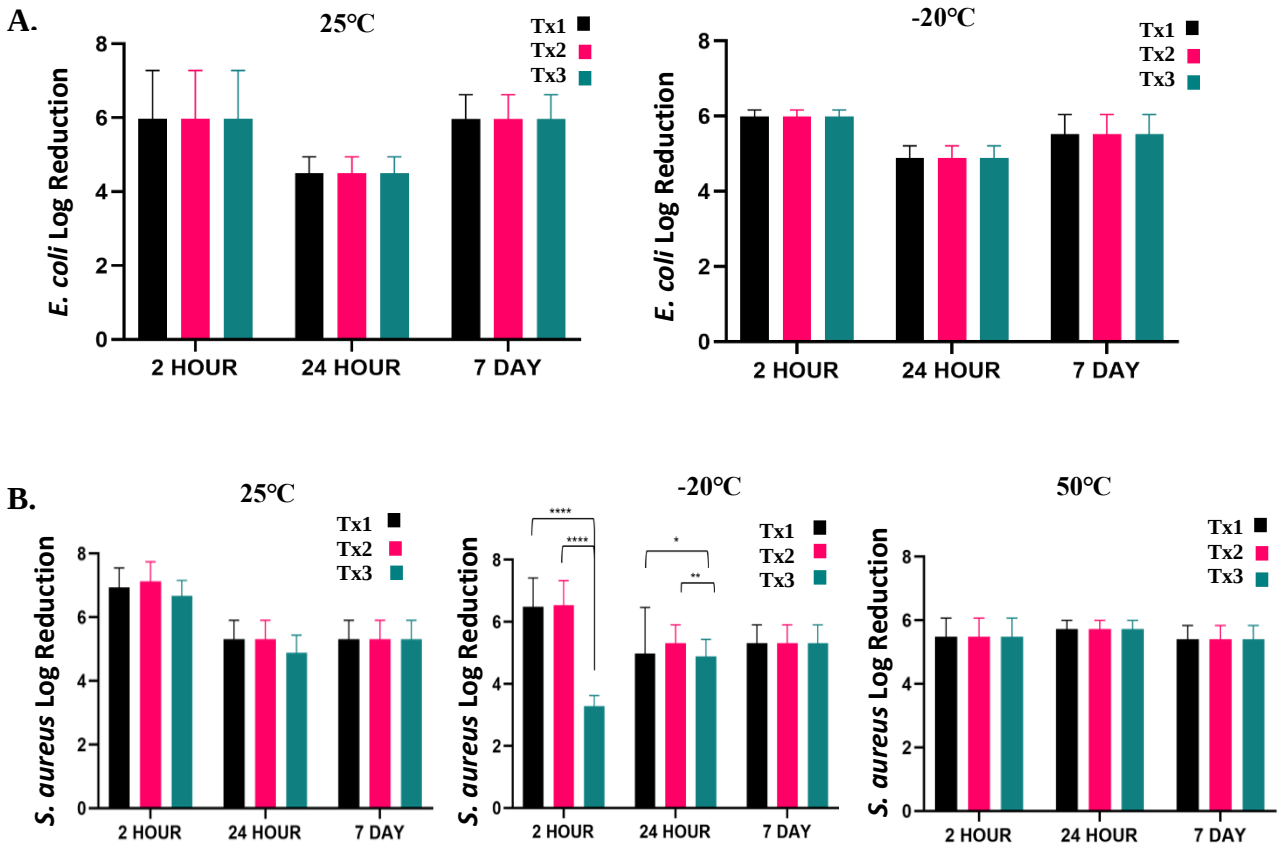

Figure 2. A. Log reduction of *E. coli* after a 10-minute treatment with liquid wound cleaner candidate 1 (Tx1; black bars), liquid wound cleanser candidate 2 (Tx2; pink bars), or wound cleanser gel (Tx3; teal bars) followed by 2-hour, 24-hour, or 7-day hold conditions at 25°C (top left panel) and -20°C (top right panel). **B.** Log reduction of *S. aureus* after 10-minute treatment with liquid wound cleaner candidate 1 (Tx1; black bars), liquid wound cleanser candidate 2 (Tx2; pink bars), or wound cleanser gel (Tx3; teal bars) followed by 2-hour, 24-hour, or 7-day hold conditions at 25°C (bottom left panel), -20°C (bottom middle panel), and 50°C (bottom right panel). Log reduction was calculated following CFU quantification from TSA no treatment control plate growth. Data is expressed in n=5 in triplicate with standard deviations. Asterisks denote significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

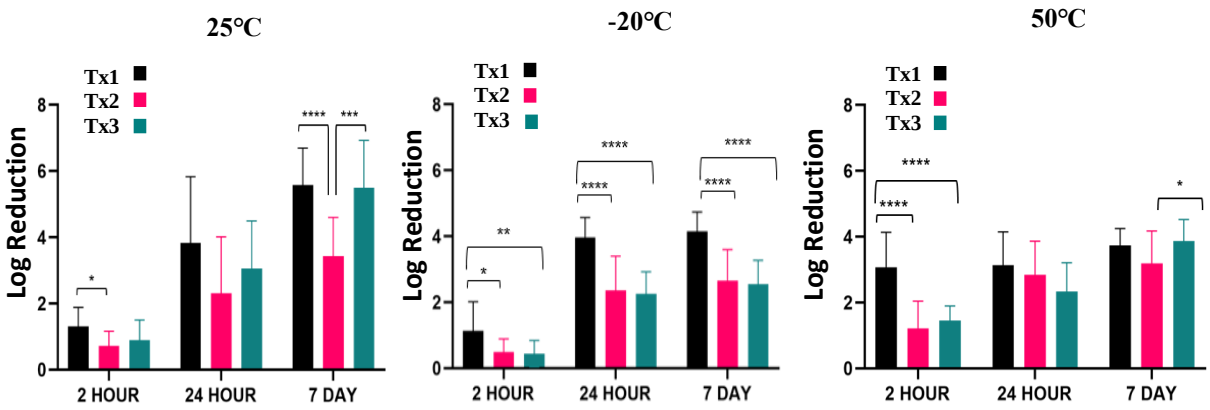

Figure 3. Log reduction of bacteria results from stainless steel samples contaminated with swine cecum contents then treated for 10 minutes with liquid wound cleaner candidate 1 (Tx1; black bars), liquid wound cleanser candidate 2 (Tx2; pink bars), or wound cleanser gel (Tx3; teal bars)for 10 minutes and held for 2-hours, 24-hours, or 7-days at 25°C (bottom left panel), -20°C (bottom middle panel), and 50°C (bottom right panel). Log reduction was calculated following CFU quantification from TSA no treatment control plate growth. Data is expressed in n=5 in triplicate with standard deviations. Asterisks denote significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$).

Table 1. Percent Kill

CONTAMINENT	HOLD TEMPERATURE	HOLD TIME	TREATMENT 1	TREATMENT 2	TREATMENT 3
E. coli	**25°C**	2	100 (± 0)	100 (± 0)	100 (± 0)
		24	100 (± 0)	100 (± 0)	100 (± 0)
		7	100 (± 0)	100 (± 0)	100 (± 0)
	-20°C	2	100 (± 0)	100 (± 0)	100 (± 0)
		24	100 (± 0)	100 (± 0)	100 (± 0)
		7	100 (± 0)	100 (± 0)	100 (± 0)
S. aureus	**25°C**	2	99.99 (± 0.01)	100 (± 0.01)	99.99 (± 0.01)
		24	100 (± 0)	100 (± 0)	100 (± 0)
		7	99.99 (± 0)	100 (± 0)	100 (± 0)
	-20°C	2	99.99 (± 0.01)	99.99 (± 0.01)	99.92 (± 0.05)
		24	99.93 (± 0.01)	99.97 (± 0.2)	99.88 (± 0.7)
		7	100 (± 0)	100 (± 0)	100 (± 0)
	50°C	2	99.99 (± 0)	99.99 (± 0)	99.99 (± 0)
		24	99.99 (± 0)	99.99 (± 0)	99.99 (± 0)
		7	99.99 (± 0)	99.99 (± 0)	99.99 (± 0)
Cecal Contents	**25°C**	2	97.85 (± 12.28)	91.22 (± 22.72)	95.52 (± 20.12)
		24	99.96 (± 0.25)	94.65 (± 30.78)	99.92 (± 1.79)
		7	99.99 (± 0.01)	99.87 (± 0.06)	99.99 (± 0.01)
	-20°C	2	94.39 (± 12.78)	84.87 (± 14.79)	83.09 (± 17.36)
		24	99.98 (± 0.51)	99.19 (± 6.86)	99.75 (± 01.06)
		7	99.99 (± 0.51)	99.61 (± 6.92)	99.88 (± 1.09)
	50°C	2	99.39 (± 0.43)	91.86 (± 3.33)	96.45 (± 2.56)
		24	99.71 (± 1.79)	99.14 (± 3.79)	99.43 (± 2.47)
		7	99.97 (± 0.03)	98.87 (± 0.50)	99.97 (± 0.01)

Percent kill of bacterial inoculated onto stainless steel surfaces after a 10-minute disinfectant treatment period and 2-hour, 24-hour, or 7-day hold period at either 25℃, -20℃, or 50℃.

13.0 LIST OF SYMBOLS, ABBREVIATIONS AND ACRONYMS

AOAC	Association of Official Analytical Chemists
ANOVA	Analysis of Variance
CFU	Colony Forming Unit
CFUavg	Average Colony Forming Unit of three replicates
E. coli	*Escherichia coli*
FDA	Food and Drug Administration
GI	Gastrointestinal
LB	Luria Broth
OD_{600}	Optical Density at wavelength of 600 nm
S. aureus	*Staphylococcus aureus*
SF	Survival Fraction
TSA	Tryptic Soy Agar
Tx	Treatment
VBNC	Viable But Not Culturable