Introduction

Warrior medics experience unique circumstances when performing surgery in austere environments which sometimes require immediate re-use capability, efficacy in extreme temperatures, and rapid, long-lasting disinfection of surgical tools. Surgical-associated infections pose a significant risk to war-fighters in an austere theater. Mass casualty situations require re-use of surgical instruments, necessitating swift and effective disinfection methods.

Hypothesis: This study tests the efficacy of commercially-available, FDA approved wound disinfectants for alternative use of sterilizing surgical instruments, medical devices/items, and surfaces found within improvised medical facilities for far-forward deployed surgical teams.

Methods

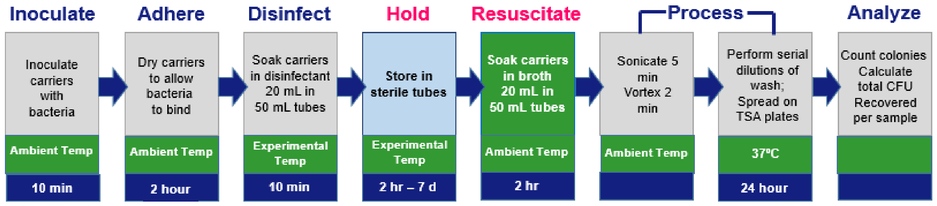
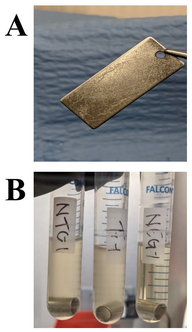


Figure 1: Experimental Schematic: Boxes represent procedure steps including steps, temperature, and time allocation per step



- Samples were inoculated onto stainless steel carriers and submerged into 20 mL of wound disinfectants for 10-minutes each
- Post treatment, the carriers were stored in sterile conical tubes for 2-Hours, 24-Hours, and 7-days to test different turn around times
- Conical tubes were refilled with 20 mL sterile media. 24-hour and 7-day treatments were kept at room temperature in media for 2 hours for a resuscitation period.
- Samples were sonicated at 40 kHz for 5-minutes and vortexed for 2 minutes
- Samples were serially diluted and plated onto sterile TSA plates. Colonies were counted after 24-hours and CFU calculated

Figure 2: Stainless steel carriers. A. Tag covered in treatment chemical 3; B. Peni-cylinders in conical tubes and broth for processing steps

In Vitro: Commercially available *Escherichia coli* and *Staphylococcus aureus* were grown in an overnight culture of Luria Broth, then diluted and grown back up into logarithmic phase. *E. coli* was inoculated at 1.5 x 10^7 CFU/mL and *S. aureus* was inoculated at 5.6 x 10^6 and inoculated directly onto a room temperature sterile stainless-steel tag.

Ex Vivo: Intestinal contents were harvested from the cecum of expired swine and immediately inoculated onto sterile stainless steel peni-cylinders. Bacterial recovery was performed using sterile Luria Broth. Samples were inoculated onto sterile blood agar plates

Following the 2-hour adherence step, carriers were treated; placed into 20 mL of treatment chemical. Treatment 3 is a gel and was covered in a thin coating evenly spread. All carriers were stored in 50 mL conical tubes during the 10-minute treatment duration. Ambient temperature samples were kept in a biosafety cabinet, -20º C were kept in freezer, and 50º C in an incubator set to respective temperatures. Following treatment, carriers were removed from chemicals, tapped to dry, and placed in sterile 50 mL conical tubes. Treated carriers were stored for the designated time-frame (2-hours, 24-hours, or 7-days at experimental temperature.

After holding period, 20 mL Luria broth was added to each tube. 2-hour samples were processed immediately. 24-hour and 7-day samples were held at room temperature for 2-hours to resuscitate viable but non-culturable bacterium (VBNC). Samples were then sonicated at 40 khZ for 5 minutes then vortexed for 3-minutes to recover bacteria from the surface. All samples were serially diluted and plated on Tryptic Soy Agar (TSA) plates.

Statistical Analysis : All tests were run in triplicate with an n=5. Following overnight incubation at 37º C, plates were counted individually and CFU, percent kill, survival fraction, and log reduction were calculated. Raw data were compared using ANOVA and Tukey's Multiple Comparisons.

Results

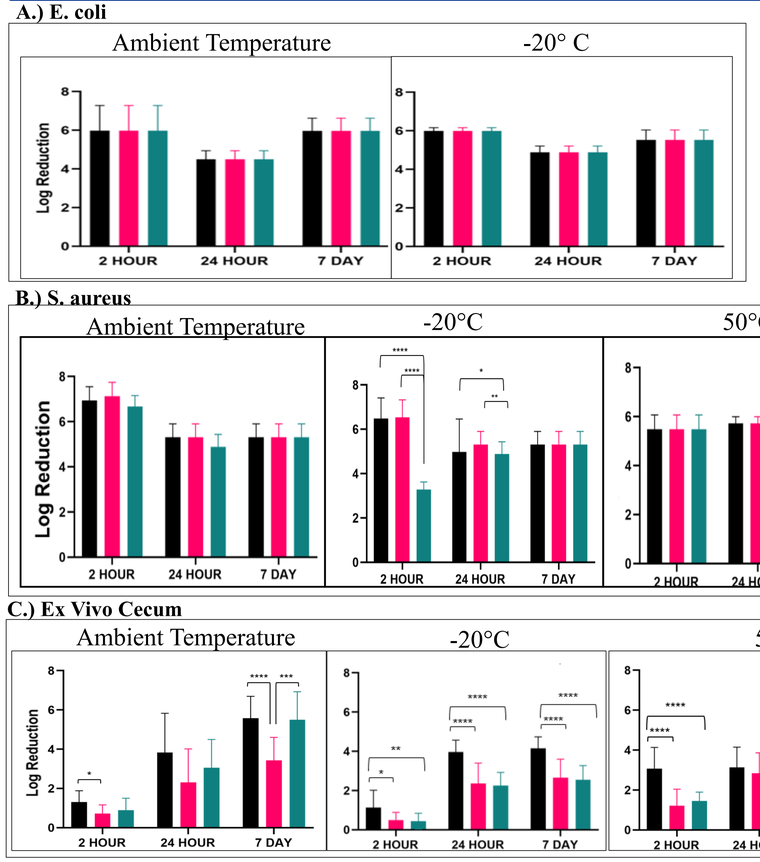


Figure 3: Log reduction of e. coli, s. aureus, and ex vivo cecum samples at 2-hour, 24-hour, and 7-days in ambient, -20, and 50 C. Data is expressed in n=5 in triplicate with standard deviations. Asterisks denote significant differences (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$). Log reduction was calculated following CFU quantification from TSA plate growth. **A**. Treatment of E. coli on stainless steel tags. The ambient temperature treatments at 2-hour and 7-day resulted in a 6-log reduction, and 24-hour resulted in 4.5-log reduction. At -20° C, 2-hour resulted in a 6-log reduction, 24-hour and 7-day, a 5-log reduction. The error bars are the result of differing NTC bacterial density. No significant difference was observed between treatments since they were all complete kill. The experiment was not performed for 50° C because NTC E. coli did not survive at this temperature. **B**. Treatment of S. aureus on stainless steel carriers. Every treatment resulted in significant reduction compared to NTC. At ambient temperature, 2-hour samples resulted in more than a 6-log reduction, and 24-hours and 7-days resulted in about a 5-log reduction. No significant differences were observed between treatments. In -20 C, Treatments 1 and 2 resulted in a 6-log reduction, while treatment 3 resulted in only a 3-log reduction. Both treatment 1 and 2 were significantly different from treatment 3. For 24-hours and 7-days, treatments resulted in around a 5-log reduction. There was large variability in treatment 1 with respect to recovered CFU at 24 hours and there was a significant difference between treatments 1 and 2 and treatment 3. At 7-days, all treatments resulted in about a 5-log reduction, with no significant differences between treatments. **C.** Treatment of ex vivo cecum samples. All treatments resulted in significant reduction versus NTC samples. The bacterial recovery was much more variable, leading to larger standard deviations. In ambient temperature at 2-hours, all treatments results in about a 1-log reduction. Only treatments 1 and 2 were significantly different. At 24-hours, treatments 1 and 3 resulted in about a 3-log reduction, verses the 2-log reduction of treatment 2. Treatments 1 and 3 resulted in a 5-log reduction in the 7-day group, while treatment 2 only showed a 3-log reduction. The treatments 1 and 3 showed significant difference and more effective reduction that treatment 2. At -20 C, All treatments showed minimal log reduction, only about 1-log. There was significant difference between treatment 3 and the other treatments in this group.

Average Recovered Colony Forming Units

		TIME POINT	NTC	Treatment 1	Treatment 2	Treatment 3
E. COLI	AMBIENT	2	1.48 X 10^7	0	0	0
		24	5.41 X 10^5	0	0	0
		7	5.15 X 10^6	0	0	0
	-20° C	2	1.06 X 10^6	0	0	0
		24	9.29 X 10^4	0	0	0
		7	5.79 X 10^5	0	0	0
STAPH	AMBIENT	2	6.27 X 10^7	6.00 X 10^1	6.67 X 10^1	5.33 X 10^1
		24	9.02 X 10^7	0	0	0
		7	4.95 X 10^5	0	0	0
	-20° C	2	7.93 X 10^6	1.07X[2]	4.00 X 10^1	6.26 X 10^3
		24	2.45 X 10^6	1.74 X 10^3	7.93 X 10^2	2.85 X 10^3
		7	2.88 X 10^6	0	0	0
	50° C	2	6.35 X 10^5	0	0	0
		24	6.17 X 10^5	0	0	0
		7	3.45 X 10^5	0	0	0
EX VIVO	AMBIENT	2	5.27 X 10^5	1.14 X 10^4	4.63 X 10^4	2.36 X 10^4
		24	4.53 X 10^6	1.99 X 10^3	2.43 X 10^5	3.84 X 10^3
		7	2.65 X 10^6	7.33 X 10^1	3.41 X 10^3	1.13 X 10^2
	-20° C	2	4.34 X 10^5	2.43 X 10^4	6.57 X 10^4	7.34 X 10^4
		24	4.11 X 10^5	7.33X 10^1	3.33 X 10^3	1.01 X 10^3
		7	8.45 X 10^5	7.33 X 10^1	3.33 X 10^3	1.01 X 10^3
	50° C	2	8.47 X 10^5	5.20 X 10^3	6.87 X 10^4	3.05 X 10^4
		24	5.29 X 10^5	1.53 X 10^3	4.53 X 10^3	3.00 X 10^3
		7	2.59 X 10^5	6.67 X 10^1	2.93 X 10^3	6.67 X 10^1

Percent Kill

		TIME POINT	Treatment 1	Treatment 2	Treatment 3
E. COLI	AMBIENT	2	100	100	100
		24	100	100	100
		7	100	100	100
	-20° C	2	100	100	100
		24	100	100	100
		7	100	100	100
STAPH	AMBIENT	2	9.99	100	99.99
		24	100	100	100
		7	99.99	100	100
	-20° C	2	99.99	99.99	99.92
		24	99.93	99.97	99.88
		7	99.99	99.99	100
	50° C	2	99.99	99.99	99.99
		24	99.99	99.99	99.99
		7	99.99	99.99	99.99
EX VIVO	AMBIENT	2	97.85	91.22	95.52
		24	99.96	94.65	99.92
		7	99.99	99.87	99.99
	-20° C	2	94.39	84.87	83.09
		24	99.98	99.19	99.75
		7	99.99	99.61	99.88
	50° C	2	99.39	91.86	96.45
		24	99.71	99.14	99.43
		7	99.97	98.87	99.97

- Antimicrobial disinfectants appear
  temperatures tested, although no sig
  between treatments at any temperat
- All treatments cause significant red
  bacterial colonies at all temperature
- Significant differences were observ
  aureus at -20 C.

Tukey's Multiple Comparison

		TIME POINT
E. COLI	AMBIENT	2
		24
		7
	-20° C	2
		24
		7
STAPH	AMBIENT	2
		24
		7
	-20° C	2
		24
		7
	50° C	2
		24
		7
EX VIVO	AMBIENT	2
		24
		7
	-20° C	2
		24
		7
	50° C	2
		24
		7

Discu

- Antimicrobial wound disinfectants
  disinfectants against a plethora of b
- While the diverse colonies in cecal
  significantly reduced by treatments
- All disinfectants were effective at a
  effective in extreme heat.
- The methodology presented a strin
  there was no pre-wash step in any
  significantly reduce bacterial load

Conclusions and

- All disinfectants appear effective ag
  samples at all temperatures.
- Overall, Treatment 1 appeared most
  challenges.
- Log reduction appeared more substa
  suggesting that perhaps the disinfec
  bacteria to survive stressful conditio

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The views expressed here are those of the a
policy of the Department of the Defense or
herein were conducted according to the prin
Health Publication No. 80-23, Guide for the
the Animal Welfare Act of 1966, as amende

Warrior Medics – Mission Ready – Patient Focused