Persistence of Activity of Hand Sanitizers against Antibiotic Resistant Microorganisms

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1.0 OBJECTIVE

The overall purpose of this study is to investigate the ability of a hand sanitizer (my-shield Hand Sanitizer with Aloe Vera) to prevent attachment and growth of antibiotic resistant microbial strains (Methicillin-resistant *Staphylococcus aureus*, Vancomycin-resistant *Enterococcus*, and Carbapenem-resistant *Enterobacteriaceae*) up to 4 hours after treatment.

2.0 PROTOCOL OVERVIEW

Samples of porcine skin (used as a human skin analog) were prepared and sanitized with 70% alcohol. A test group was additionally treated with a hand sanitizer (my-shield Hand Sanitizer with Aloe Vera) and left at room temperature for up to 4 hours. At predetermined time intervals, one of three inocula was applied to the samples. After inoculation, samples were enumerated. Counts for the samples not treated with sanitizer were compared to the treated samples to determine the effect of each sanitizer at preventing attachment of the organism. Effectiveness over time from initial treatment was also determined.

3.0 MATERIALS AND METHODS

3.1 Challenge organisms and stock solution preparation

The following challenge organisms were prepared for this study:

Methicillin-resistant *Staphylococcus aureus* (MRSA; ATCC #33592)
Vancomycin-resistant *Enterococcus faecalis* (VRE; ATCC #51575)
Carbapenem-resistant *Enterobacteriaceae* (CRE; *Klebsiella pneumonia* ATCC #BAA-1705)

Each culture was prepared from a lyophilized preparation (KWIK-STIK™, Microbiologics, St. Cloud, MN) according to manufacturer's instructions. The cultures were transferred into Tryptic Soy Broth (TSB, Neogen, Lansing, MI) and incubated at 35 ± 2°C for 24 ± 2 hours. These preparations were plated onto Standard Methods Agar (SMA, Neogen) at appropriate dilutions to determine the actual concentration of each inocula.

3.2 Preparation and treatment of samples

Porcine skin samples were obtained and used as human skin analogs. The skin samples were aseptically cut into pads measuring approximately 3" x 6" inches. Each pad had two holes cut in it to allow for aseptic handling as discussed below. All pads were sanitized using 2.0 mL of a 70% alcohol solution. A total

of 38 pads were prepared (1 treated pad, in triplicate, for each of 4 time points, and for each of 3 inocula, plus 2 additional untreated pads left as controls).

Samples in the treatment group were treated with my-shield Hand Sanitizer with Aloe Vera in a manner designed to simulate use on human skin. The skin pad was held using sterile latex gloves, held through the precut holes with the non-dominant hand. Using the dominant hand, 1.0 mL of sanitizer was applied to the interior portion of the pad (an area of approximately 1.5" x 4"), and rubbed onto the surface for 30 to 45 seconds. After treatment, samples were left at ambient temperature. Samples were held for the following time periods before inoculation: 2 minutes, 1 hour, 2 hours, and 4 hours.

3.3 Sample inoculation

After each holding time had elapsed, 3 of the treated samples were surface inoculated with one of the inoculation organisms. A 1.0 mL volume of overnight TSB culture (containing approximately 1 x 10^8 cfu) was applied and spread onto the interior portion of the sample, in a manner similar to the sanitizer application. Samples were allowed to air dry for 5 minutes before enumeration of the sample. After inoculation of one culture was completed, an additional set was inoculated with one of the remaining cultures until all three organisms had been applied to the samples. After the 4 hour samples were inoculated and enumerated, the 2 remaining untreated, uninoculated controls were also enumerated as below.

3.4 Sample plating and enumeration

Samples were rinsed with 100 mL of Butterfield's Phosphate Buffer (BPB) and vigorously hand massaged to remove any remaining viable culture. The rinsate was collected and spread plated at appropriate dilutions onto Baird-Parker Agar (BP, Neogen) for the MRSA samples or Violet Red Bile Agar (VRB, Neogen) for the VRE or CRE samples. All plates were incubated at 35 ± 2°C for 24 ± 2 hours.

After incubation, plates were enumerated using a Quebec colony counter. The number of observed typical colonies was multiplied by the dilution factor to determine the total count. Representative isolates were confirmed to ensure the recovered counts represent the inoculated culture. Counts for all samples were recorded.

3.5 Data analysis

The raw count observed for each treated sample was compared to the untreated control. The percent reduction between the treated and untreated samples was defined as the efficacy of each treatment at each time point.

4.0 RESULTS

4.1 Challenge organism concentrations

Results of the enumeration of the challenge organisms are shown in Table 1, below.

Table 1. Challenge organism enumeration

Challenge organism	Sample 1	Sample 2	Sample 3	Average	Log_{10}
MRSA (cfu/mL)	218,000,000	153,000,000	147,000,000	172,666,667	8.24
VRE (cfu/mL)	144,000,000	175,000,000	81,000,000	133,333,333	8.12
CRE (cfu/mL)	195,000,000	77,000,000	58,000,000	110,000,000	8.04

The overnight culture suspensions were at a concentration of 1.72 x 10^8 cfu/mL for MRSA, 1.33 x 10^8 cfu/mL for VRE, and 1.10 x 10^8 cfu/mL for CRE. All cultures were used undiluted as inocula in the study.

4.2 Porcine skin sample results

Results of the challenge samples are shown in Tables 2 through 4, below, including holding time, type of sample, sample replicate, amount of inoculum recovered (in cfu/mL of rinsate), and the percent reduction seen between the treated and untreated samples at each time point.

Table 2. Porcine skin sample enumeration (MRSA)

Holding Time (Sample Type)	Sample 1	Sample 2	Sample 3	Average	Log_{10}	% Reduction
2 minutes (Untreated)	104,000,000	45,000,000	22,500,000	57,166,667	7.76	
2 minutes (Treated)	52,000	65,000	56,000	57,667	4.76	99.9%
1 hour (Untreated)	14,400,000	3,600,000	6,400,000	8,133,333	6.91	
1 hour (Treated)	46,000	79,000	61,000	62,000	4.79	99.2%
2 hours (Untreated)	19,800,000	21,900,000	12,200,000	17,966,667	7.25	
2 hours (Treated)	600,000	610,000	208,000	472,667	5.67	97.4%
4 hours (Untreated)	17,600,000	14,700,000	6,300,000	12,866,667	7.11	
4 hours (Treated)	850,000	360,000	650,000	620,000	5.79	95.2%
24 hours (Uninoculated)	<1	<1	<1	<1	n/a	n/a

Table 3. Porcine skin sample enumeration (VRE)

Holding Time (Sample Type)	Sample 1	Sample 2	Sample 3	Average	Log_{10}	% Reduction
2 minutes (Untreated)	10,600,000	63,000,000	71,000,000	48,200,000	7.68	
2 minutes (Treated)	90,000	19,800	16,200	42,000	4.62	99.9%
1 hour (Untreated)	10,300,000	20,500,000	11,600,000	14,133,333	7.15	
1 hour (Treated)	157,000	28,000	66,000	83,667	4.92	99.4%
2 hours (Untreated)	18,500,000	6,300,000	6,500,000	10,433,333	7.02	
2 hours (Treated)	162,000	107,000	330,000	199,667	5.30	98.1%
4 hours (Untreated)	11,300,000	3,400,000	21,200,000	11,966,667	7.08	
4 hours (Treated)	1,690,000	970,000	620,000	1,093,333	6.04	90.9%
24 hours (Uninoculated)	<1	<1	<1	<1	n/a	n/a

Table 4. Porcine skin sample enumeration (CRE)

Holding Time (Sample Type)	Sample 1	Sample 2	Sample 3	Average	Log_{10}	% Reduction
2 minutes (Untreated)	15,500,000	65,000,000	18,100,000	32,866,667	7.52	
2 minutes (Treated)	126,000	122,000	145,000	131,000	5.12	99.6%
1 hour (Untreated)	19,100,000	6,400,000	2,800,000	9,433,333	6.97	
1 hour (Treated)	169,000	149,000	177,000	165,000	5.22	98.3%
2 hours (Untreated)	7,400,000	17,300,000	7,400,000	10,700,000	7.03	
2 hours (Treated)	1,110,000	970,000	176,000	752,000	5.88	93.0%
4 hours (Untreated)	20,900,000	8,300,000	16,100,000	15,100,000	7.18	
4 hours (Treated)	2,120,000	2,160,000	1,190,000	1,823,333	6.26	87.9%
24 hours (Uninoculated)	<1	<1	<1	<1	n/a	n/a

Efficacy of the treatment dropped from a maximum of 99.9 – 99.6% after 2 minutes of storage to a minimum of 95.2 – 87.9% after 4 hours of the study. The treatment was most effective against MRSA, with efficacy ranges from 99.9% to 95.2%, while the treatment was least effective against CRE, with efficacy ranges from 99.6% to 87.9% over the course of the study. Representative isolates were confirmed as their respective inocula. No background organisms were detected in the uninoculated samples over the course of the study.

5.0 CONCLUSIONS

The data in this study indicates that the hand sanitizer (my-shield Hand Sanitizer with Aloe Vera) was able to prevent the attachment of the test organisms (Methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC #33592, Vancomycin-resistant *Enterococcus faecalis* (VRE) ATCC #51575, and Carbapenem-resistant *Enterobacteriaceae* (CRE); *Klebsiella pneumonia* ATCC #BAA-1705) for short time periods, especially against MRSA and VRE. The calculated percent efficacy of the treatment peaked at 99.9% (i.e. 3 logs of

reduction) for MRSA and VRE at 2 minutes after application. For these organisms, percent efficacy remained above 99.0% (2 logs of reduction) for up to an hour after application, and remained above 90.0% (1 log of reduction) for the full course of the study (4 hours after application). Efficacy was less for the CRE inoculated samples, with an initial reduction at 2 minutes after application of 99.6% (over 2 logs of reduction), and above 90.0% (1 log of reduction) for up to 2 hours after application. For the CRE inoculated samples, the treatment was not able to provide additional effective protection after 4 hours of ambient storage.

6.0 REFERENCES

ASTM E2897-12 Standard Guide for Evaluation of the Effectiveness of Hand Hygiene Topical Antimicrobial Products Using *ex-vivo* Porcine Skin

ASTM WK36911 New Guide for Measuring the Inactivation of Persistent Activity of Topical Antimicrobial Products Using *ex-vivo* Porcine Skin

National Advisory Committee on Microbiological Criteria for Foods. 2009. Parameters for Determining Inoculated Pack/Challenge Study Protocols.

7.0 FINAL REPORT APPROVAL

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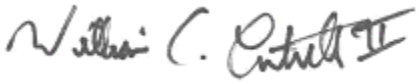

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