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Determining the efficacy of sanitizers against virulent nosocomial infections

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Abstract

The goal of this study was to determine the effectiveness of 0.13% benzalkonium chloride (BAC) (Steirolotion™), 100% ethanol, and 70% ethyl alcohol gel (Purell™) hand sanitizer in inhibiting the growth of nosocomial bacteria - methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant Enterococcus (VRE), and *Pseudomonas aeruginosa* (PA) - when plated on optimal culture media over an extended period of time. In addition, our objective was to quantify the length of time these hand sanitizer agents remained effective. 50 microliters of either BAC, 100% ethanol, or 70% ethyl alcohol hand gel sanitizer were pipetted onto Trypticase soy agar with 5% sheep blood plates that were cultured with either MRSA, VRE, or Pseudomonas. The plates were then incubated at 37.0°C and the zone of inhibition (ZOI) was measured daily for 5 days, noting whether or not regrowth occurred in areas where growth had initially been inhibited. BAC was found to be superior to both 100% ethanol and 70% ethyl alcohol in the inhibition of MRSA over all time points (p<0.05). BAC was found to be superior to 70% ethyl alcohol in the inhibition of VRE over all time points (p<0.05) and BAC was found to be superior to 70% ethyl alcohol and 100% ethanol in the inhibition of pseudomonas over multiple time points (p<0.05). The results of this study demonstrate superior *in vitro* efficacy of BAC of preventing growth of common nosocomial bacteria over a 5-day time period compared to ethyl alcohol. Further study is warranted to determine *in vivo* effectiveness of this formulation of BAC.

Introduction

Hospital-acquired or healthcare-associated infections (HAIs) are a common complication in hospitalized patients [1]. They occur with an estimated incidence of 4.5 HAIs per 100 hospital admissions, and amount to an additional burden of $35 to $45 billion dollars on the healthcare system [2]. They are responsible for significant hardship accounting for more than 90,000 deaths each year, putting HAIs among the top 5 leading causes of death in the United States of America (USA) [3-5]. Transmission of pathogens from healthcare surfaces and staff are an important source of HAIs. Personal and environmental hygiene is a crucial aspect of reducing transmission, and hand washing, cleaning surfaces and/or sanitizing is required with patient and environmental contact [6].

Both alcohol-based and alcohol-free sanitizers are available options when washing is not available or efficient. Alcohol-based sanitizers containing 60-95% alcohol is most often used in hospitals. Benzalkonium chloride (BAC) is the active ingredient contained in most alcohol-free hand sanitizer products available today. It has been theorized that BAC possesses an extended killing time of bacteria when the solution has dried compared to alcohol-based agents [7-10].

The goal of this study was to determine the duration of efficacy of 0.13% BAC, 70% ethyl alcohol, and 100% ethanol in decreasing methicillin-resistant Staphylococcus aureus (MRSA), vancomycin-resistant Enterococcus (VRE), and Pseudomonas aeruginosa (PA) colonization and regrowth over an extended period of time when plated on optimal growth culture media.

Materials and methods

BD BBL™ Trypticase™ soy agar slants prepared media of nosocomial bacteria MRSA, VRE, and PA were grown on agar plates for 24 hours at 37°C and used to establish a reservoir.

A 0.5 McFarland standard solution was created for the MRSA, VRE, and PA bacteria strains, by using a calibrated inoculating loop to transfer bacteria from the incubated blood agar plates to a vial of saline with 0% absorbance until the absorbance of the vial solution was between 0.08 and 0.1%.

50 microliters of 0.13% benzalkonium chloride (BAC) (Steirolotion™, Germcure, Houma Louisiana), 100% ethanol (Sigma-Aldrich Inc., St. Louis, Missouri) and ethyl alcohol 70% (Purell™, Gojo, Akron, Ohio) solution were pipetted onto each of the eight 5% sheep blood agar plates. Reverse pipetting was used to ensure accurate amounts of viscous solution were pipetted onto the plates. Plates were left for one hour to dry.

The MRSA, VRE, and *P. aeruginosa* inoculated plates were incubated at 37°C overnight. They were all grown in aerobic conditions. The plates

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Table 1. Growth of Pseudomonas *vs*. Ethyl alcohol, Ethanol, and BAC with associated *p*-values. Values denote the zone of inhibition measured in centimeters^2

	Ethyl alcohol (cm^2)	Ethanol (cm^2)	BAC (cm^2)	Ethyl alcohol *vs*. Ethanol	Ethanol *vs*. BAC	Ethyl alcohol *vs*. BAC
				p-value	*p*-value	*p*-value
Initial Zone	5.921	7.078	3.647	0.0388	0.00630	0.0452
Day 1	0.401	2.72	1.971	$2.74e^{-5}$	0.00330	0.000200
Day 2	0	0.694	0.820	0.00720	0.0609	$7.39e^{-7}$
Day 3	0	0.302	0.405	0.0572	0.0572	0.00130
Day 4	0	0.177	0.153	0.0929	0.7186	0.0557
Day 5	0	0.143	0.162	0.0846	1	0.0846

Table 2. Growth of VRE *vs*. Ethyl alcohol, Ethanol, and BAC with associated *p*-values. Values denote the zone of inhibition measured in centimeters^2

	Ethyl alcohol (cm^2)	Ethanol (cm^2)	BAC (cm^2)	Ethyl alcohol *vs.* Ethanol	Ethanol *vs.* BAC	Ethyl alcohol *vs.* BAC
				p-value	*p*-value	*p*-value
Initial Zone	6.161	9.846	5.359	$7.50e^{-6}$	1	$7.50e^{-6}$
Day 1	0	4.894	5.494	$8.25e^{-12}$	0.197	$6.43e^{-10}$
Day 2	0	4.666	5.255	$1.91e^{-12}$	0.165	$4.28e^{-10}$
Day 3	0	4.582	4.961	$3.39e^{-11}$	0.362	$2.68e^{-10}$
Day 4	0	4.645	4.894	$4.13e^{-11}$	0.585	$2.08e^{-9}$
Day 5	0	4.623	4.952	$9.89e^{-10}$	0.486	$4.38e^{-10}$

Table 3. Growth of MRSA *vs.* Ethyl alcohol, Ethanol, and BAC with associated *p*-values. Values denote the zone of inhibition measured in centimeters^2

	Ethyl alcohol (cm^2)	Ethanol (cm^2)	BAC (cm^2)	Ethyl alcohol *vs.* Ethanol	Ethanol *vs.* BAC	Ethyl alcohol *vs.* BAC
				p-value	*p*-value	*p*-value
Initial Zone	1.785	4.035	5.396	$9.56e^{-8}$	$2.41e^{-5}$	$6.49e^{-11}$
Day 1	0	3.477	5.338	$1.0426e^{-11}$	$3.33e^{-8}$	$6.38e^{-17}$
Day 2	0	3.341	5.289	$5.184e^{-12}$	$4.61e^{-8}$	$6.65e^{-18}$
Day 3	0	3.272	5.293	$2.950e^{-12}$	$1.70e^{-8}$	$7.71e^{-18}$
Day 4	0	3.27	5.169	$7.286e^{-12}$	$4.73e^{-8}$	$2.01e^{-18}$
Day 5	0	3.151	5.09	$3.015e^{-11}$	$6.69e^{-8}$	$1.71e^{-18}$

were removed from the incubator every 24 hours for a growth period of 120 hours to take photographs and quantitative measurements of the zone of inhibition (ZOI) of each antiseptic. Measurements were performed for a total of 120 hours for the MRSA, VRE, and PA plates.

ImageJ, an internet software program, was used to perform digital measurements of the ZOI utilizing the pixels of the digital photographs taken and the known standard diameters of the agar plates to quantitatively measure the ZOI. Four researchers made the digital measurements independently to increase validity of the measurements.

Statistical analysis was performed by measuring the difference in area of inhibition between ethyl alcohol, ethanol, and benzalkonium chloride for Pseudomonas, VRE, and MRSA each. *P*-values were obtained using *t*-tests comparing each solution independently.

Results

Results can be seen in Tables 1-3. Ethyl alcohol and Ethanol showed significant regrowth of bacteria within 24 hours against Pseudomonas, VRE, and MRSA. This regrowth of bacteria continued the full 5 days. BAC showed regrowth of only Pseudomonas after the initial 24-hour period had passed. For BAC, no regrowth was noted throughout the 120 hours in MRSA or VRE after the initial ZOI had been established.

The ratio of percent loss of initial zone vs each subsequent zone revealed that BAC was most effective at preventing regrowth of all three bacteria. Ethyl alcohol did not inhibit growth of MRSA and VRE throughout the study period. All three sanitizing agents had significant regrowth of PA with BAC showing the slowest regrowth (Table 1). For VRE by day 5, BAC had 7.6% regrowth of the initial zone vs Ethanol's 53.0% and ethyl alcohol's 100% regrowth. The day 5 regrowth of MRSA *vs.* BAC was shown to be only 5.7% while it was 21.9% and 100% for ethanol and ethyl alcohol, respectively. Overall, BAC did not exhibit any significant regrowth within the initial zone of inhibition vs MRSA and VRE ($p>0.05$).

Discussion

Causative agents of HAIs and the routes by which they spread have been well documented for over a century. Despite knowledge of the factors that influence the risks of HAIs and means to prevent or control them [11], patients in the healthcare setting continue to acquire HAIs [8]. These infections result in increased morbidity and mortality for the patient, and ultimately higher health care costs for both the patient and the health care system [2,12]. As one example, it's estimated that preventing a single postoperative surgical site infection could potentially save the healthcare system upwards of $60,000 [9].

This study demonstrates the anti-microbial activity of alcohol-based sanitizing agents (70% ethyl alcohol and 100% ethanol) and alcohol-free agents (BAC) against common virulent antibiotic-resistant micro-organisms MRSA, VRE, and *P. aeruginosa in vitro* over a period of 120 hours. The 0.13% BAC formulation used here exhibited superior effectiveness and duration when compared to 70% ethyl alcohol and 100% ethanol against all three micro-organisms used in this study. Specifically, against MRSA and VRE, BAC created clear cut zones of inhibition (ZOI) with minimal bacterial regrowth over a period of 120 hours. Against Pseudomonas, BAC created a clear cut ZOI until 24 hours before allowing some bacterial growth to o

ccur after the 24-hour mark. In contrast 70% ethyl alcohol failed to prevent bacterial regrowth for any micro-organism. Even at the 24-

hour mark no clear cut ZOI could be appreciated on any plate. Likewise, 100% ethanol also failed to prevent bacterial regrowth for any of the micro-organisms used. BAC not only proved efficacious in preventing the regrowth of bacteria over an extended period of time, but against MRSA and VRE also demonstrated an ability to maintain the integrity of the ZOI over the course of 120 hours. Against *P. aeruginosa*, the ZOI created by BAC was noted to shrink in size over the duration of the experiment. These in vitro results suggest that BAC may have longer efficacy than 70% ethyl alcohol as a sanitizing agent that could provide an extended action of anti-microbial protection and potentially reduce the number of HAIs spread from patient to patient by healthcare workers and environmental surfaces.

This study has several important limitations. these agents were tested against in vitro microbial cultures of MRSA, VRE, and Pseudomonas with optimal growth media. It remains to be seen whether similar efficacy will be shown against these pathogens when used *in vivo*. Second, although the BAC formulation used in this study demonstrated a bactericidal effect the results may be variable depending on the particular strain of bacteria isolated, as modes of resistance may vary greatly between strains. Thirdly, only one strain of the bacteria was used, and it is possible that different resistant strains may have differing results.

Benzalkonium chloride is commonly used in the healthcare setting as a bactericidal agent in surface disinfectants, nasal sprays, and eye drops with minimal skin irritation. The mechanism of action of BAC is related to its ability to penetrate the bacterial cell wall that leads to damage and loss of cell membrane integrity. This leads to leakage of molecular components, and eventual cell wall lysis [10]. Alcohol's antimicrobial effect stems from its ability to denature proteins. Concentrations between 60-95% are most effective with higher concentrations losing potency because of the necessity to have water with the alcohol to be most effective [10]. Alcohol is effective at killing microbes but has no lasting effect. BAC is non-volatile and therefore is able to remain on the skin while it dries [13]. This explains the length of duration of BACs anti-microbial effectiveness observed in our experiment. To our knowledge, the long-term efficacy of BAC against MRSA, VRE, and *P. aeruginosa* in comparison to alcohol-based hand sanitizing agents had not been previously investigated. As such, this is the first study to demonstrate a superior ability of BAC compared to alcohol-based hand sanitizers in preventing *in vitro* MRSA, VRE, and Pseudomonas regrowth following application. Further work is necessary to determine whether BAC exhibits similar effectiveness *in vivo*.

Conclusion

In conclusion, BAC was shown to be superior to both 100% ethanol and 70% ethyl alcohol gel sanitizer in subduing the growth of MRSA, VRE and pseudomonas over 5 days. These results suggest a potential for using BAC as a possible sanitizer in the healthcare setting with its long duration of its effect, potentially helping prevent the spread of common nosocomial infections. Further study is warranted to determine *in vivo* effectiveness of this formulation of BAC as well as the appropriate time frame of application for effectiveness against HAIs.

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Conflicts of interest

The following author of this manuscript has the following competing interests: Paul Watson MD owns stock in Steiros LLC and Bioblockade LLC. All other authors declare they have no competing interests.

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