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A Time-Kill Kinetic Study of Four Antiseptic Active Ingredients Versus Strains of Twenty-six Species of Medically Relevant Bacteria and Yeast

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Abstract

In recent years, the Food and Drug Administration (FDA) has redefined the requirements for demonstrating the effectiveness and safety of topical antiseptic ingredients. To provide *in vitro* efficacy data fulfilling the FDA requirements, a large-scale Time-Kill study was undertaken using ASTM E2783-16, *Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure*. Three active ingredients used in antiseptic hand washes (Benzalkonium Chloride, Benzethonium Chloride, and Chloroxylenol) were evaluated against 269-272 strains of Gram-negative and Gram-positive bacterial species and 2 yeast species after a 30-second exposure. Ethyl alcohol, which is only used in antiseptic hand rubs (hand sanitizers), was evaluated after a 15-second exposure time against 43 strains of bacterial and 2 yeast species. The four antiseptic active ingredients each demonstrated rapid microbicidal efficacy against this broad range of microorganisms. With minor exception, the test materials produced reductions of at least 3 Log_{10} and, most often, significantly greater reductions (to the limit of detection) in the challenge populations within 15 seconds (ethyl alcohol) or 30 seconds of exposure. These data provide evidence that the four antiseptic active ingredients are effective against a broad range of potentially infectious bacteria that may be encountered in healthcare, institutional, food-handler, and consumer settings. Further studies are underway to corroborate these data using *in vivo* clinical simulation methods.

Introduction

- Gram-positive and Gram-negative bacteria, as well as yeasts can cause disease in healthcare, food-handler and consumer settings (e.g., prisons, daycares, assisted living facilities)
- Effective antiseptics must treat diverse microorganisms with short contact times (seconds)
  - The FDA stated in the Consumer Tentative Final Monograph (TFM) that a consumer antiseptic drug product would be considered bactericidal at the concentration and contact time that produces a reduction of ≥ 3 log_{10} (99.9 %) in viability
- Over-the-counter topical antiseptics are available for use by healthcare personnel, food handlers and consumers and are inclusive of hand washes and hand rubs
  - Hand washes are rinsed from the hands after use and hand rubs are left on the skin
  - Active ingredients that represent major products on the market today are Benzalkonium Chloride for hand washes and hand rubs, Benzethonium Chloride and Chloroxylenol for hand washes and Ethanol for hand rubs

Overall Objective: To assess the efficacy of the antiseptic ingredients, Benzalkonium Chloride, Benzethonium Chloride, and Chloroxylenol used in hand washes and the antiseptic ingredient, Ethanol used in hand rubs for decreasing the viability of laboratory cultured microorganisms that are known to cause disease in healthcare, food-handler and consumer settings

Methods

- Preliminary neutralization study per ASTM Standard Method E1054[1]
- American Society for Testing and Materials Method E2783-16: Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure[2]

Organism Preparation
Initial titers of 7 to 10 log_{10} CFU/mL

↓

Exposure
3 replicates
Duration of 30 sec for BZK, BZT and PCMX and 15 sec for Ethanol

↓

Active Ingredient Neutralization
Neutralizing solution - 0.5% Tween 80 and 0.07% lecithin

↓

Viable Organism Enumeration

Test Materials

Number	Active Ingredient	Percent Active Ingredient	Inactive Ingredients	Form
1	Benzalkonium Chloride (BZK)	0.12%	1% propylene glycol (v/v)	Aqueous solution
2	Benzethonium Chloride (BZT)	0.2%	1% propylene glycol (v/v)	Aqueous solution
3	Chloroxylenol (PCMX)	0.485%	1.5% Castor Oil Soap (w/w) 0.8% α-terpineol (w/w) 0.95% isopropanol	Aqueous solution
4	Ethanol	60%	--	Aqueous solution

Microorganisms Tested	ATCC Numbers of Species Tested	Setting for Disease Causing Organism: Healthcare[1]	Food-Handler[2]	Consumer[3]
Acinetobacter baumannii	19606	X		
Bacteroides fragilis	25285	X		X
Burkholderia cepacia†	25416 / 25608			X
Campylobacter jejuni*	33291 / 49943		X	X
Candida albicans	10231	X		
Candida tropicalis	750	X		
Enterobacter aerogenes	13048	X		X
Enterococcus faecalis*	19433		X	X
Enterococcus faecalis	29212	X	X	X
***Enterococcus faecalis* VRE, MDR**	51575	X		
***Enterococcus faecium* VRE**	700221			
Escherichia coli*	11229 / 25922 / 31705	X	X	X
Escherichia coli	11229 / 31705	X		
***Escherichia coli* O157:H7**	35150		X	
Haemophilus influenza	19418	X		X
Klebsiella pneumoniae	13883 / 27736	X		X
Listeria monocytogenes*	7644 / 19115		X	X
***Micrococcus yunnanensis* (formerly *M. luteus*)**	7468	X		
Proteus mirabilis	7002	X		
Pseudomonas aeruginosa*	15442 / 27853	X	X	X
Salmonella enterica*	13076 / 14028		X	X
Serratia marcescens	14756	X		X
Serratia marcescens†	8100			X
Shigella sonnei*	9290 / 25931		X	X
Staphylococcus aureus*	6538 / 29213	X	X	X
***Staphylococcus aureus*, Community-Acq. MRSA**	BAA-1683			
***Staphylococcus aureus*, MRSA**	33591 / 33592		X	X
Staphylococcus epidermidis	12228	X		
***Staphylococcus epidermidis*, MRSE**	51625			
Staphylococcus haemolyticus	29970	X		
Staphylococcus hominis	27845	X		
Staphylococcus saprophyticus	15305	X		
Streptococcus pneumoniae	6303 / 49619	X		X
Streptococcus pyogenes*	14289 / 19615	X	X	X

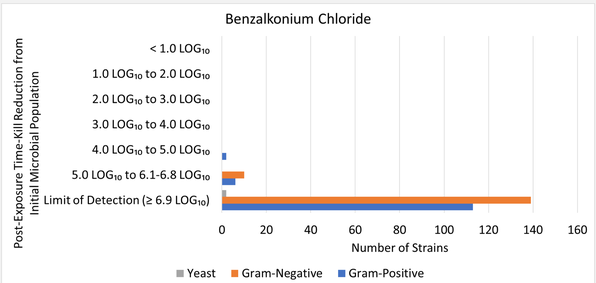
*25 clinically-isolated strains of these species were tested against Test Materials 1, 2, and 3; for *S. aureus*, both MSSA and MRSA were included. †Species tested against Test Materials 1 and 4.

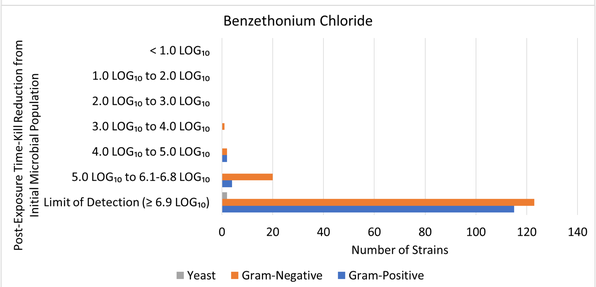
[1] Microorganisms included in FDA's Healthcare Antiseptic Proposed Rule 17 June 1994[3] and 01 May 2015[4]

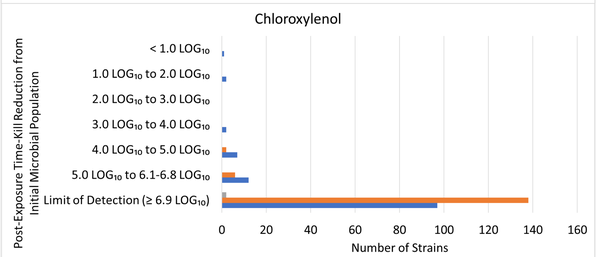
[2] Microorganisms identified via personal communication with industry experts

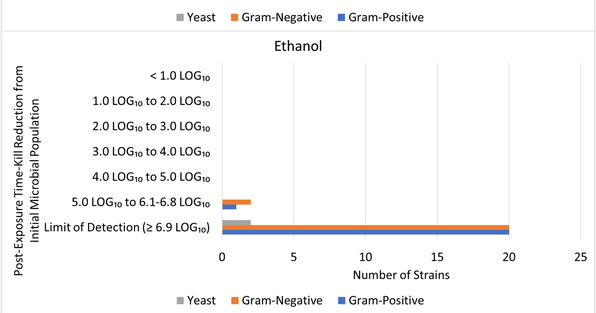
[3] Microorganisms included in FDA's Consumer Antiseptic Hand Wash Proposed Rule 17 December 2013[5] and 30 June 2016[6]

Results









Conclusions

- Results demonstrate broad-spectrum, and rapid microbicidal efficacy of 4 active ingredients versus a large variety of Gram-negative and Gram-positive bacteria and two yeast species, including many clinical and/or multi-drug resistant strains
- Significant decreases in microbial viability were achieved in 15 and 30 seconds
- The test materials produced at least 3-Log_{10} or greater reductions for 100% of the microorganisms exposed to Benzalkonium Chloride, Benzethonium Chloride and Ethanol and 99% of the microorganisms exposed to Chloroxylenol
- The data resulting from this study fulfill one of FDA's new requirements for demonstration of *in vitro* efficacy of antiseptic ingredients for healthcare and consumer settings

The results from this study demonstrate that these active ingredients provide rapid and broad-spectrum microbicidal efficacy. Their use in antiseptic hand products may play a key role in the reduction of bacteria and yeast on hands and provide less opportunity for the spread of infectious microorganisms in healthcare, food-handler and consumer settings.

References

[1] American Society for Testing and Materials International. 2018. *Annual Book of ASTM Standards*: Section 11, Volume 11.08. E1054-13: Standard Test Methods for Evaluation of Inactivators of Antimicrobial Agents. pp. 61-67.

[2] American Society for Testing and Materials International. 2018. *Annual Book of ASTM Standards*: Section 11, Volume 11.08. E2783-16: Standard Test Method for Assessment of Antimicrobial Activity for Water Miscible Compounds Using a Time-Kill Procedure. pp. 370-375.

[3] Federal Register 59(116), 17 June 94: *Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Tentative Final Monograph for Health-Care Antiseptic Drug Products*. pp. 31444-31452.

[4] Federal Register 80(84), 01 May 15: *Safety and Effectiveness of Health Care Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record*; Proposed Rule. pp. 25165-25205.

[5] Federal Register 78(242), 17 Dec 13: *Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record*; Proposed Rule. pp. 76443-76478.

[6] Federal Register 81(126), 30 Jun 16: *Safety and Effectiveness of Consumer Antiseptics; Topical Antimicrobial Drug Products for Over-the-Counter Human Use; Proposed Amendment of the Tentative Final Monograph; Reopening of Administrative Record*; Proposed Rule. pp. 42912-42937.

Acknowledgement

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