In Vitro Antimicrobial Activity Assessment of Zymox® Topical Spray Against Methicillin-Resistant Staphylococcus aureus (MRSA)

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KEY WORDS: Zymox® Topical Spray, antimicrobial activity, bacteria, microorganism, log reduction

ABSTRACT

The goal of this study was to determine the effectiveness of Zymox® Topical Spray against Methicillin-Resistant Staphylococcus aureus (MRSA). The product was tested at 100% concentration using a log reduction method. 20 mL of Zymox® Topical Spray (The Product) and 20 mL of Phosphate buffered solution (Control) were added into separate centrifuge tubes and inoculated with the bacterial strain. At time intervals of 30 seconds, 1 minute, and 5 minutes the Product and Control were placed into a dilution of neutralizing broth to stop the activity. One milliliter from each of the dilutions was plated and incubated at 32.5 ± 2.5°C to determine the number of microorganisms remaining at each time point. The test material, Zymox® Topical Spray, elicited a >99.99% reduction at all the time intervals. This represents a >4.0 log reductions at all three testing times. This study demonstrates Zymox® Topical Spray has antimicrobial activity against MRSA at 30 seconds, 1 minute and 5 minutes.

MATERIALS AND METHODS

The materials and reagents used in the study are shown in Table 1.

Log reduction is used to determine the effectiveness of a product at reducing a specific microorganism population. The bacterial strain was obtained from American Type Culture Collection (ATCC) and cultured according to the manufacturer's specification. The organism was prepared by inoculating the surface of tryptic soy agar slants. The microorganism was then incubated at 32.5 ± 2.5°C for 24 hours. Following the incubation period, the slants were washed with sterile Phosphate buffered saline (PBS) to harvest the microorganisms. The microbial suspension was adjusted to approximately 107 colony forming units (CFU) per mL and labeled as the stock suspension. The microorganism, MRSA, 20 mL of Zymox® Topical Spray, and 20 mL of PBS were added into separate sterile centrifuge tubes. Each 20 mL of Zymox® Topical Spray and PBS was inoculated with 0.2 mL of the 107 CFU/mL suspension. The inoculum resulted in approximately 105 CFU/mL into the product and PBS control. At the time intervals of 30 seconds, 1 minute, and 5 minutes, 1.0 mL from the inoculated test product was

Table 2. *Results Percentage Reduction*

Exposure Time CRL153407-1	Initial Bacterial population (CFU/mL)		# of Surviving organisms		% Reduction		Dilution countable
	Control	Product	Control	Product	Control	Product	Product
Initial	1.20E+07	1.20E+07	N/A	N/A	N/A	N/A	N/A
30 sec	1.20E+07	1.20E+07	1.20E+07	<10	0.0	>99.99	1:10, 1:100, 1:1000
1 min	1.20E+07	1.20E+07	1.20E+07	<10	0.0	>99.99	1:10, 1:100, 1:1000
5 min	1.20E+07	1.20E+07	1.20E+07	<10	0.0	>99.99	1:10, 1:100, 1:1000

taken and placed into 9.0 mL of neutralizing broth (1:10 dilution). Additional 1:10 serial dilutions were prepared using neutralizing broth to achieve 1:100 and 1:1000 dilutions. One milliliter from each dilution was plated in duplicate. Melted tryptic soy agar with polysorbate 80 and lecithin was added as the growth medium. The plates were incubated at 32.5 ± 2.5°C minimum 48 hours. The same procedure was repeated for the control. After the incubation period, all plates were counted to determine the number of microorganisms remaining at each time point.

The concentration of the microorganism for the control and product are calculated for each interval.

The Log10 reduction is calculated as follows:

Log10 (initial count) - Log10 (x time interval) = Log10 reduction

RESULTS AND DISCUSSION

Minimum bactericidal concentration is defined as 3 log reductions from the initial inoculum[1]. The product elicited a >99.99% reduction at all the time intervals. This represents a >4.0 log reduction at all three testing times.

The results indicate that the Zymox® Topical Spray have antibacterial activity against Methicillin-Resistant Staphylococcus aureus (MRSA) at 30 seconds, 1 minute and 5 minutes.

Table 1. *Materials and Reagents Used in the Study*

Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 33592
Phosphate Buffer Solution
Dey/Engley Neutralizing Broth
Tryptic Soy Agar with 0.07% Lecithin and 0.5% Polysorbate 80
Zymox® Topical Spray - Hydrocortisone Free