In vitro Antimicrobial Activity Assessment of Zymox® Topical Cream Against Methicillin-Resistant *Staphylococcus aureus* (MRSA)

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ABSTRACT

The goal of this study was to determine the antibacterial effectiveness of Zymox® Topical Cream against Methicillin-Resistant Staphylococcus aureus (MRSA). The product was tested at 100% concentration using a log reduction method. Twenty mL of Zymox® Topical Cream (Product) and 20 mL of Phosphate buffer 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. 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 Cream, elicited a 100% reduction at 5 minutes. This represents a >3.0 log reductions at 5 minutes. The results of the study demonstrate Zymox® Topical Cream has antibacterial activity against MRSA at 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 buffer 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, was added to 20 mL of Zymox® Topical Cream (Product) and then to 20 mL of PBS (Control) in separate sterile centrifuge tubes. Each 20 mL of Zymox® Topical Cream (Product) and PBS (Control) 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 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 were calculated at each interval. These numbers are expressed in terms of scientific notation with the log reduction calculation being used to express the change (reduction or increase) of the microorganism population relative to a starting inoculum.

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 achieves more than 3 log reductions at the 5 minute time interval for MRSA. The results indicate that the Zymox® Topical Cream has antibacterial activity against Methicillin-Resistant Staphylococcus aureus (MRSA) at 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 Cream - Hydrocortisone Free		

Table 2. *Results*

Exposure Time	Concentration of Organism (CFU/mL)		Percent Reduction		Log Reduction		Dilution countable
	Control	Product	Control	Product	Control	Product	Product
Initial	2.0E+05	2.0E+05	N/A	N/A	N/A	N/A	N/A
30 sec	2.0E+05	7.7E+04	0.0%	61.5%	0.0	0.4	1:1000
1 min	1.7E+05	3.2E+04	15.0%	84.0%	0.1	0.8	1:1000
5 min	1.6E+05	<10	20.0%	100.0%	0.1	4.3	1:10

[1] *Disinfection, Sterilization, and Preservation. Fourth Edition. Seymour S. Block, pg. 1035*