

In Vitro Antimicrobial Activity Assessment of Zymox[®] Otic Solution Against Methicillin-Resistant Staphylococcus Aureus (MRSA)

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ABSTRACT

The goal of this study was to determine the effectiveness of Zymox[®] Otic Solution against Methicillin-Resistant Staphylococcus aureus (MRSA). The product was tested at 100% concentration using a log reduction method. 20 mL of Zymox[®] Otic Solution (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 $32.5 \pm 2.5^\circ\text{C}$ to determine the number of microorganisms remaining at each time point. The test material, Zymox[®] Otic solution, elicited a >99.99% reduction at all the time intervals. This represents a >4.0 log reductions at all three testing times. The results of study demonstrate Zymox[®] Otic Solution has antibacterial 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 $32.5 \pm 2.5^\circ\text{C}$ 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, was added to 20 mL of Zymox[®] Otic Solution and then to 20 mL of PBS in separate sterile centrifuge tubes. Each 20 mL of Zymox[®] Otic Solution

Table 1. Materials and Reagents Used in the Study

Methicillin-Resistant Staphylococcus aureus (MRSA) ATCC 33592
Phosphate buffered solution
Dey/Engley Neutralizing Broth
Tryptic Soy Agar with 0.07% Lecithin and 0.5% Polysorbate 80
Zymox [®] Otic solution - Hydrocortisone Free

Table 2. Results Percentage Reduction

Exposure Time CLR153407-3	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

and PBS were 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, 5 minutes, 1.0 mL from the inoculated test product was 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 of the dilutions was plated in duplicate. Melted tryptic soy agar with polysorbate 80 and lecithin was added as the growth medium. The plates were incubated at $32.5 \pm 2.5^\circ\text{C}$ minimum 48 hours. The same procedure was repeated for the phosphate buffered solution (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 for each interval.

The Log10 reduction is calculated as follows:

$$\text{Log}_{10}(\text{initial count}) - \text{Log}_{10}(\text{x time interval}) = \text{Log}_{10} \text{reduction}$$

RESULTS AND DISCUSSION

Minimum bactericidal concentration is defined as 3 log reductions from the initial inoculum¹. The product, elicited a >99.99% reduction at all the time intervals. This represents a >4.0 log reductions at all three testing times.

The results indicate that the Zymox[®] Otic solution has antibacterial activity against Methicillin-Resistant *Staphylococcus aureus* (MRSA) at 30 seconds, 1 minute and 5 minutes.