

TESTING OF ANTIMICROBIAL EFFICACY OF WOUND DRESSING BY ZONE OF INHIBITION

Chirag B. Shah, Ph.D.
Manager R&D Biosciences
Kendall, a division of Tyco Healthcare Group LP
Mansfield, MA

PURPOSE

The purpose of this study was to evaluate the efficacy of 0.2% PHMB treated gauze dressing (Kerlix[®] AMD[™]) using a Zone of Inhibition assay.

MATERIALS

Test Samples

Sterile Kerlix A.M.D. (#3331):	0.2% PHMB
Sterile negative control:	Kerlix Bandage roll #6715 (untreated Kerlix)

Challenge Organisms

<i>Staphylococcus aureus</i>	ATCC 25923
<i>Pseudomonas aeruginosa</i>	ATCC 33494
<i>Escherichia coli</i>	ATCC 25922
<i>Candida albicans</i>	ATCC CL310
<i>Staphylococcus coagulase</i>	ATCC 27734
<i>Proteus Mirabilis</i>	ATCC 51286
<i>Serratia marcescens</i>	ATCC 49472
<i>Enterobacter cloacae</i>	ATCC 49141
<i>Klebsiella pneumoniae</i>	ATCC 49134

METHOD

Preparation of Test Article

¾ -inch circular disks were aseptically cut from each test sample and negative control.

Challenge Assay (Zone of inhibition)

A challenge organism from previously inoculated broth was streaked onto agar plates and incubated 24 hours. After 24 hours a small amount of bacteria was placed into 10 ml of sterile phosphate buffered saline (0.09%) solution. The resulting solution was then vortexed and matched to a 0.5 McFarland standard (10^8). One (1) ml of the 10^8 -suspension was then pipetted into 9 ml of sterile PBS solution and labeled as 10^7 . Two (2) tubes were made per bacteria.

Trypticase Soy Agar (TSA) was prepared and placed in 250 ml Pyrex lab bottles – 200 ml of agar per bottle. All bottles were then steam sterilized. 20 ml (2 inoculated tubes) of prepared 10^7 – suspension was poured into warm liquid agar. The bottle was manually shaken to ensure uniform distribution of bacteria. Using sterile disposable pipettes (10 ml volume), 20 ml inoculated agar was taken and deposited onto a sterile disposable petri dish. The plate was then manually spiraled to evenly distribute the agar on the bottom of the plate. The agar was then allowed to harden. Disks ($\frac{3}{4}$ “) were cut from Kerlix and Kerlix AMD bandage roll (all 6 plies). One Kerlix and one Kerlix AMD. disk was dropped on to the cooled agar. Three plates were prepared per organism. 400 microliters of PBS solution was deposited slowly onto each disk just to ‘wet’ each disk. Using a Q-tip, the wetted disks were gently pressed to ensure that each disk had good contact with the agar. After 18-24 hours of incubation the zone surrounding each disk was measured.

The zones were measured approximately at the three or four cardinal points of the disk. The zone of inhibition is a clear ring all around the disk. At each point, using a caliper, the edge of the disk through the clear ‘zone’ to the start of the cloudy part of the agar was measured in mm. The average of the three or four readings were recorded as the Zone of Inhibition (ZOI).

The above procedure was repeated for each challenge organism.

RESULTS

The measured zones of inhibitions for Kerlix AMD (0.2% PHMB) and Kerlix (untreated) for each test organism are illustrated in Table I. There was no zone observed for untreated Kerlix roll (negative control). Kerlix AMD exhibited varied zones of inhibition for different test organisms. The presence of zone of inhibition surrounding the test disks indicate release of PHMB and antimicrobial activity of the dressing.

TABLE I: Zone of inhibition after 24-hour incubation

ORGANISM	Zone of Inhibition, mm	
	Kerlix A.M.D.	Kerlix
<i>S. aureus</i>	2.01	0
<i>P. aeruginosa</i>	0.43	0
<i>E. coli</i>	2.10	0
<i>C. albicans</i>	0.83	0
<i>S. coagulase</i>	1.55	0
<i>P. mirabilis</i>	0.62	0
<i>S. marcescens</i>	1.37	0
<i>E. cloacae</i>	1.62	0
<i>K. pneumoniae</i>	1.97	0

DISCUSSION

Zone of inhibition testing determines bacterial susceptibility to antimicrobials based on the diffusion of the antimicrobial into surrounding agar medium. As the test disk is applied onto the inoculated surface of the test medium the wetted disks absorb water from the agar medium and the release of the antimicrobial (i.e. PHMB) is initiated. The antimicrobial migrates through the adjacent agar medium. As a result, a gradually changing gradient of the antimicrobial

concentration develops in the agar surrounding the disk. As the antimicrobial diffusion progresses, microbial multiplication also proceeds. However, no growth will appear in the area where the antimicrobial is present in inhibitory concentrations: the more susceptible the test organism, the larger the zone of inhibition.

ZOI for most of the test organisms was between 1 and 2 mm. The ZOI was less than 1 mm for *P. aeruginosa*, *C. albicans* and *P. mirabilis* indicating lower susceptibility to PHMB released from the test disk compared to other test organism.

CONCLUSION

Under the conditions of this study, the test material (Kerlix AMD gauze dressing) exhibited antimicrobial efficacy against all test organisms and this activity was different for each organism as indicated by different zones of inhibition.

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