

**Antimicrobial Efficacy
by Elution of Gauze
Using Vancomycin Resistant
*Enterococcus faecalis***

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SUMMARY

The purpose of this study was to evaluate the efficacy of an antimicrobial treated gauze dressing (Kerlix[®] A.M.D. Gauze Dressing) in an elution model *in vitro*.

A challenge suspension of Vancomycin Resistant *Enterococcus faecalis* (VRE) was prepared using a spectrophotometric procedure resulting in a 1×10^8 CFU/ml dilution. A pre-cut $\frac{1}{4}$ " circular disk of the test, negative control, and positive control materials was submerged into a dilution containing approximately 1×10^6 CFU/ml suspension. Independent suspensions were made for 0, 24, and 48 hour test time points. At each time point, for each test material, dilutions were made and plated to determine the log reduction of bacteria.

Under the conditions of this study, the negative control did not show any antimicrobial effect as anticipated. The test material (Kerlix[®] A.M.D.) proved to be as effective as the positive control at all time points resulting in over a 99.9% bacterial reduction.

INTRODUCTION

A study was performed to evaluate the efficacy of an antimicrobial treated woven gauze dressing (Kerlix[®] A.M.D.) in an elution model at 0, 24, and 48 hour time intervals.

The treated article and control materials were received on December 08, 2000. Testing was initiated on December 18, 2000 and completed on December 22, 2000.

MATERIALS

The samples provided by the sponsor were identified and handled as follows:

Test Article: Material: Kerlix[®] A.M.D., Lot FG2717AG (Treated Gauze, concentration 0.2% PHMB)

Material: Kerlix[®] A.M.D., Lot KE1644AG (Negative Control-Untreated)

Material: Kerlix[®] A.M.D., Lot HE108158B3MC (Prototype, Positive Control, concentration 2.0% PHMB)

NOTE: All materials were supplied by the sponsor pre-cut for testing.

Identification: See Test Article

Storage Conditions: Room Temperature

Challenge Organism: Vancomycin Resistant *Enterococcus faecalis* (VRE), ATCC #51575

Medias/Reagents: Tryptic Soy Agar (TSA), prepared per NAMSA SOP

Tryptic Soy Agar Broth (TSB), prepared per NAMSA SOP

0.9% Sodium Chloride Tubes – 9 mL

0.5% McFarland Standard, purchased prepared.

1. Clinical isolates from ATCC (American Culture Type Collection– Rockville, MD 20852)
2. McFarland Equivalence Turbidity Standard 0.5
3. Autoclave
4. Incubator, 37°C
5. Incubator, 25°C
6. Electronic pipetters
7. Pipetter Tips, 100 microliters
8. Pipetter Tips, 1000 microliters
9. Vortex Mixer
10. Bunsen burner
11. Deionized water
12. Culture Tube Racks
13. Automatic or Digital Colony Counter
14. Sodium Chloride, U.S.P.
15. Culture Tube caps
16. Tryptic Soy Agar (soy bean casein digest agar)
17. Constant Temperature water bath
18. Autoclave
19. Disposable Petri Dishes, 100 x 15mm
20. Biohazard Autoclave Bags
21. Disposable inoculating loops

METHODS

I. Preparation: Preparation was done before the test was started.

- A 0.9% Sodium Chloride solution in 9 mL aliquots was prepared and autoclaved per validated specifications.
- Tryptic Soy Agar (TSA), was prepared following manufacturer's directions, pipetted into 20 mL aliquots, and autoclaved per validated specifications.
- $\frac{1}{4}$ inch disks from the fabric samples to be tested were received from the sponsor pre-cut; one disk per test.
- 12 Saline Tubes were placed in a culture tube rack (4 rows of 3). The first row of 3 tubes was labeled with bacteria name, sample code, date, and 10^6 dilution. The second row was labeled with the same information, and with 10^7 dilution rather than 10^6 . The third row and the fourth row were labeled the same way, with 10^8 and 10^9 as dilution factors respectively.
- 9 Saline Tubes were placed in a culture tube rack (3 rows of 3). The three rows of samples were labeled with the following information: bacteria name, sample code, date, 10^6 concentration. The first row was labeled 0 hour, the second row was labeled 24 hour and the third row was labeled 48 hour. Each tube had corresponding duplicate sterile Petri dishes that were also labeled accordingly.
- A portion of the bacteria was aseptically transferred to a sterile

brain-heart infusion broth, and incubated overnight to initiate bacterial growth.

II. Procedure: Challenge Assay

- From the broth, the bacteria was measured spectrophotometrically, and a 10^8 dilution of the particular bacterium was made.
- At 0 hour, A Test sample disk was placed into the tube labeled Sample #1, 0 hour tube from the rack of 9 tubes. This tube was then inoculated with 100 μ l of 10^8 bacterial dilution to make the 10^6 dilution. Immediately after the tube was vortexed, 2 - 1000 μ l aliquots were taken from it. One aliquot was pipetted to the corresponding Petri dish. The second aliquot was delivered to the tube labeled 0 hour, 10^2 dilution, sample #1. Immediately after the tube was vortexed, 2 - 1000 μ l aliquots were taken from it. One aliquot was pipetted to the corresponding Petri dish. The second aliquot was delivered to the tube labeled 0 hour, 10^4 dilution, sample #1. Immediately after the tube was vortexed, 2 - 1000 μ l aliquots were taken from it. One aliquot was pipetted to the corresponding Petri dish. The second aliquot was delivered to the tube labeled 0 hour, 10^6 dilution, sample #1. Immediately after the tube was vortexed, a 1000 μ l aliquot was taken from it and delivered to the corresponding Petri dish.
- All the above steps were repeated for samples #2 and #3.
- One disk was then placed in each of the 6 remaining tubes from the original rack of 9 tubes. Each of these 6 tubes was then inoculated with 100 μ l of bacterium and placed in the 25°C incubator.
- All of the above steps were performed for the experimental test samples, the positive control samples, and the negative control samples.
- At 24 hours, the inoculated 24 hour tubes were removed from the incubator and the serial dilutions were performed and plated as described previously for the 0 hour time interval.
- At 48 hours, the inoculated 48 hour tubes were removed from the incubator and the serial dilutions were performed and plated as described previously for the 0 hour time interval.
- After 24 hours of incubation, the inoculated dishes for the 0, 24, and 48 hour time intervals were counted and calculations were performed to determine the antimicrobial efficacy of the test samples.

RESULTS

The results for the 0, 24, 48 hour time points are noted in TABLES 1, 2, and 3 respectively. The submerged gauze articles demonstrate the rate of efficacy of the amount of antimicrobial eluted from the gauze. Results indicate no reduction of bacteria upon immediate introduction of any of the test articles (Time 0). Results do indicate Kerlix™ A.M.D. gauze to be similarly effective as the positive control reducing the bacteria count at both 24 and 48 hours. Kerlix™ A.M.D. resulted in a reduction of bacteria of greater than 99.9%. The negative control had no antimicrobial effect as expected.

TABLE 1

RESULTS "0" Time
Vancomycin Resistant *Enterococcus faecalis*

Sample ID	Final Cfu's/ml	Average CFU per group	Average Log value
1 A	1.8×10^6		
1 B	2.1×10^6	2.1×10^6	6.3
1 C	2.3×10^6		
2 A	2.2×10^6		
2 B	2.2×10^6	2.3×10^6	6.3
2 C	2.5×10^6		
3 A	2.0×10^5		
3 B	8.0×10^4	1.3×10^5	5.1
3 C	9.6×10^4		

Key:

- 1 (A-C) = Kerlix™ A.M.D. -- Product Code 3331, Lot Number FG2717AG (Treated Gauze, concentration 0.2% PHMB)
 2 (A-C) = Kerlix™ -- Product Code 6715, Lot Number KE1644AG (Negative Control – Untreated)
 3 (A-C) = Kerlix™ -- Lot Number HE108158B3MC (Prototype Positive Control, concentration 2.0% PHMB)

TABLE 2

RESULTS "24 Hours"
Vancomycin Resistant *Enterococcus faecalis*

Sample ID	Final Cfu's/ml	Average CFU per group	Average % Reduction	Average Log Reduction
1 A	1.2×10^3			
1 B	5.9×10^2	1.1×10^3	99.95	3.2
1 C	1.6×10^3			
2 A	3.5×10^6			
2 B	3.5×10^6	3.5×10^6	NR	NR
2 C	3.5×10^6			
3 A	19			
3 B	9	11	99.99	4
3 C	6			

NR = No Reduction

TABLE 3

RESULTS "48 Hours"

Vancomycin Resistant *Enterococcus faecalis*

Sample ID	Final CfU's/ml	Average CFU per group	Average % Reduction	Average Log Reduction
1 A	6.5x10 ²			
1 B	3.7x10 ²	1.6x10 ³	99.92	3.1
1 C	5.7x10 ²			
2 A	5.1x10 ⁶			
2 B	4.7x10 ⁶	5.0x10 ⁶	NR	NR
2 C	5.3x10 ⁶			
3 A	6			
3 B	2	3	99.99	4.6
3 C	1			

NR = No Reduction

CONCLUSION

Under the conditions of this study, the test material (Kerlix® A.M.D. Gauze Dressing) reduced the bacteria population in solution on an average by 3.2 log and 3.1 log at the 24 and 48 time point respectively. The response produced was similar to the positive control material. As expected the negative control did not show any antimicrobial effect.

RECORD STORAGE

All raw data pertaining to this study and a copy of the final report are to be retained in designated NAMSAs archive files.

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