

TESTING OF ANTIMICROBIAL EFFICACY OF WOUND DRESSING BY IN VITRO ELUTION MODEL

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PURPOSE

The purpose of this study was to evaluate the efficacy of antimicrobial treated gauze dressing (Kerlix® AMD™) in an elution model in vitro.

MATERIALS

Test Articles

Sterile Kerlix AMD (#3331):	0.2% PHMB
Sterile positive control (Kerlix):	2.0% PHMB
Sterile negative control:	untreated Kerlix gauze

Challenge Organisms

<i>Staphylococcus aureus</i>	ATCC 25923
<i>Staphylococcus epidermidis</i>	ATCC 49134
<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

METHODS

Preparation of media/reagents/plates

- 0.9 % Sodium Chloride solution in 9 ml aliquots was prepared and autoclaved per specifications.
- Tryptic Soy Broth (TSB) was prepared following manufacturer's directions, pipetted into 10 ml aliquots and autoclaved per specifications.
- 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 10^5 dilution. The second row was labeled with the same information, and with 10^4 dilution rather than 10^5 . The third row and the fourth row were labeled the same way, with 10^3 and 10^2 as dilutions, 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 hours and the third row was labeled 48 hours. Each tube had corresponding duplicate sterile petri dishes that were also labeled accordingly.
- A small portion of the bacteria was aseptically transferred to sterile TSA and incubated overnight to initiate bacterial growth. The following day, a TSA plate was streaked with the bacteria growing in the broth and incubated overnight to obtain growing colonies.

Preparation of Test Article

$\frac{3}{4}$ -inch circular disks were aseptically cut from each test sample including positive and negative controls.

Challenge Assay

- A few of the growing colonies were harvested from the streaked plate and placed in 10ml of saline and a 10^8 dilution was prepared.
- 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 inoculated with 100 μ l of 10^8 bacterial dilution to make the 10^6 dilution. The tube was then vortexed and two 1000 μ l aliquots were taken from it. One aliquot was delivered to the corresponding petri dish. The second aliquot was delivered to the tube labeled 0 hour, 10^5 dilution sample #1. This tube was then vortexed and two 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. This steps were repeated until tube labeled 0 hour, 10^2 dilution, samples #1 was prepared. 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 microliter of bacterium and placed in the 25^o C incubator.
- All of the above steps were performed for the experimental test samples including positive and negative control samples.
- At 24 hours, inoculated 24 hour tubes were removed from the incubator and serial dilutions were performed and plated as described previously for the 0 hour time interval.
- At 48 hours, inoculated 48 hour tubes were removed from the incubator and 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 testing was performed in two phases. The results of the first phase are illustrated in Table 1 for 0, 24 and 48-hour time points. Both actual counts and respective log reduction values are illustrated. The log reduction values are calculated as the Log (final counts/ 10^6). In the first phase (Table 1) only the test sample (Kerlix AMD) was tested. Phase 2 studies included the test sample, positive and negative controls as well as additional test organisms.

TABLE 1: 0/24/48 h results at 10^6 inoculation levels for challenge organisms

ORGANISM	INCUBATION TIME (HOUR)	Counts, CFU/ml	Log Reduction
<i>S. aureus</i>	0	8.9×10^1	4.1
	24	0	6.0
	48	0	6.0
<i>S. epidermidis</i>	0	1.35×10^2	3.9
	24	0	6.0
	48	0	6.0
<i>P. aeruginosa</i>	0	0	6.0
	24	0	6.0
	48	0	6.0
<i>E. coli</i>	0	5.0×10^0	5.3
	24	0	6.0
	48	0	6.0
<i>C. albicans</i>	0	1.41×10^4	1.8
	24	1.02×10^4	2.0
	48	6.95×10^3	2.2

TABLE 2: 0/24/48 h results at 10^6 inoculation levels for challenge organisms

ORGANISM	INCUBATION TIME (HOUR)	Counts, CFU/ml			Log Reduction		
		CONTROL		TEST SAMPLE	CONTROL		TEST SAMPLE
		(-)	(+)		(-)	(+)	
<i>S. coagulase</i>	0	1.10×10^5	0	4.43×10^2	1.0	6.0	3.4
	24	7.97×10^2	0	0	3.1	6.0	6.0
	48	0	0	0	6.0	6.0	6.0
<i>P. mirabilis</i>	0	5.47×10^5	0	8.63×10^4	0.3	6.0	1.1
	24	2.23×10^5	0	0	0.7	6.0	6.0
	48	-	0	0	-	6.0	6.0
<i>S. marcescens</i>	0	4.67×10^5	0	2.32×10^5	0.3	6.0	0.6
	24	2.39×10^5	0	0	0.6	6.0	6.0
	48	1.10×10^6	0	0	0.0	6.0	6.0
<i>E. cloacae</i>	0	4.30×10^5	0	0	0.4	6.0	6.0
	24	5.97×10^4	0	0	1.2	6.0	6.0
	48	2.33×10^5	0	0	0.6	6.0	6.0
<i>K. pneumoniae</i>	0	8.33×10^4	0	4.13×10^2	1.1	6.0	3.4
	24	7.37×10^4	0	0	1.1	6.0	6.0
	48	6.73×10^4	0	0	1.2	6.0	6.0
<i>E. faecalis</i>	0	1.89×10^6	43	9.40×10^5	0.0	4.4	0.0
	24	1.18×10^6	0	5.80×10^2	0.0	6.0	6.0
	48	6.30×10^5	0	0	0.2	6.0	6.0
<i>A. anitratus</i> (aka <i>A. baumannii</i>)	0	7.97×10^4	0	7.33×10^4	1.1	6.0	1.1
	24	1.36×10^5	0	0	0.9	6.0	6.0
	48	1.44×10^6	0	0	0.2	6.0	6.0

DISCUSSION

In vitro elution model is a test in which the test sample is in direct contact with inoculated suspension of planktonic test organisms. Upon contact with the surrounding solution, the antimicrobial from the test disk is released into the medium. The above results indicate a range of 0 to 6-log reduction of bacteria upon immediate introduction of the test articles. Results also indicate that Kerlix AMD gauze (0.2% PHMB) is as effective as the positive control (2.0% PHMB) and both exhibit a 6-log reduction (>99.9% killing) in bacterial counts at 24 and 48 hours. The negative control demonstrated no antimicrobial effect (mostly <1 or 0 log reduction) as expected except for *S. coagulase* that exhibited a 6-log reduction after 48-hour incubation. The cause of this is unknown.

CONCLUSION

Under the conditions of this study, Kerlix AMD exhibited significant antimicrobial efficacy against all test organisms.

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