

Evaluation of in-vitro disk diffusion and pathogen recovery methods to assess antimicrobial wound dressings

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BACKGROUND

Cutaneous wounds are prone to bacterial colonization and/or infection. Topical antimicrobial, silver (Ag) impregnated agents and other antimicrobial wound dressings are used in a variety of clinical settings. Absorbent wound dressings with and without Ag-based agents are also commonly used. While there are no widely accepted guidelines to determine the antimicrobial efficacy of such agents, we developed and evaluated an in-vitro method to test the efficacy of topical wound dressings.

OBJECTIVES

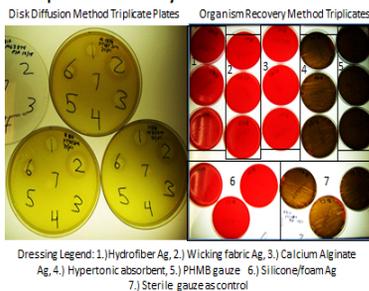
•No direct clinical correlation and outcome studies have been published to date for assessment and predictability of such in-vitro testing results for wound care products. To help address these concerns some researchers have paired the disk diffusion test with log reduction and time killing tests to further assess the bactericidal properties of the compounds in the dressings. This test has supported the data from the disk diffusion tests but it is an indirect comparison of the two tests and may not accurately reflect what occurs in vivo.

•The purpose of this study is to directly test the ability to evaluate the bactericidal properties of various wound care materials against a variety of gram-positive and gram-negative microorganisms. Further, we investigate the ability to recover these organisms from the wound dressings after the dressings (disks) were in place on the agar plates for 48h and results from these disk diffusion tests were obtained.

Material and Methods

In-vitro susceptibility testing was performed for 81 pathogens including 16 *A. baumannii* (ACB), 16 *P. aeruginosa* (PSA), and 19 MRSA. Organisms, previously isolated from wounds in burn center patients were used. Organisms were tested against the following topical wound dressings: Hydrofiber Ag, Fabric Ag, Calcium alginate Ag Hypertonic saline ribbon, Polyhexamethylene biguanide (PHMB) gauze, Foam/Silicone Ag, using a disk-diffusion method (DDM). Using the disk diffusion principle (CLSI M02-A10), Mueller-Hinton agar (MH) plates were inoculated from 0.5 McFarland stock solutions of the organisms. Wound dressings were purchased from the respective manufacturers and cut under sterile conditions in such way to obtain 7mm squares that were placed on the inoculated MH agar. A 7mm disk of sterile gauze was included as a negative control. MH plates were incubated in triplicate for 48 hours. Zone diameters for inhibition of bacterial growth were obtained at 24 and 48 hours. The dressings were then transferred to 5mL of sterile saline, vigorously shaken to recover any viable pathogens from the dressings, and 100µL of the saline was cultured for 24 hours with the amount of growth recorded for comparison with the inhibition data. Results were evaluated for consistency, validity, and correlation between the zone of inhibition and pathogen recovery data.

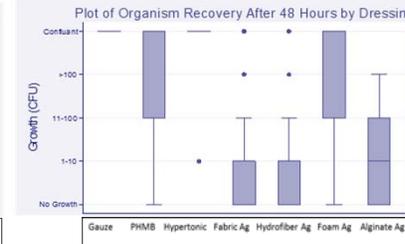
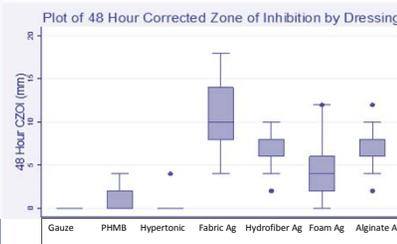
Reproducibility of test methods



RESULTS

Table 1: Mean and Standard Deviation of the Corrected Zone of Inhibition (CZOI) in millimeters (mm)

	Silver Containing Agents			Control		Non-Silver Agents	
	Fabric Ag	Hydrofiber Ag	Foam Ag	Ce Alginate Ag	Gauze	PHMB gauze	Hypertonic dressing
All Organisms (N=81)	10.7±3.2	6.3±2.1	4.2±3.1	7.0±1.9	0	0.7±1.2	0.0±0.4
Gram Positive (N=34)	12.2±3.0	7.0±2.1	4.1±3.6	7.9±1.6	0	1.5±1.4	0.1±0.7
Gram Negative (N=47)	9.6±2.8	5.8±1.9	4.2±2.7	6.3±1.3	0	0.1±0.4	0
ACB (N=16)	8.6±2.0	5.3±1.6	2.5±2.5	6.0±1.3	0	0	0
CNS (N=5)	15.2±3.0	9.2±1.1	8.4±3.3	8.8±1.8	0	3.2±1.8	0
E. coli (N=5)	8.8±1.1	4.8±1.1	6.8±2.3	5.2±1.8	0	0.4±0.9	0
Enterococcus (N=5)	8.8±1.1	3.2±1.1	2.0±2.0	5.6±0.9	0	0	0
KPLN (N=5)	6.8±2.3	4.0±1.4	4.4±2.2	5.2±1.1	0	0	0
MRSA (N=19)	12.1±2.9	7.3±1.4	3.4±3.5	8.5±0.9	0	1.4±1.2	0
MSSA (N=5)	12.8±1.1	7.6±0.9	4.8±1.8	6.8±1.1	0	2.00	0
PSA (N=16)	11.6±3.2	6.6±2.0	4.8±2.2	7.1±2.5	0	0	0
Serratia spp (N=5)	9.6±0.9	7.2±1.1	4.2±3.1	7.2±1.1	0	0.4±0.9	0



- 5/7 dressings showed inhibition of bacterial growth over the 48 hour test period.
- Silver permeated dressings had consistently wider and statistically significant zones of inhibition compared to the non-silver dressings.
- Statistical ranking of the performance of the dressings showed that the fabric Ag had the greatest CZOI values, followed by Alginate Ag, Hydrofiber Ag, Foam Ag and PHMB gauze. Hypertonic dressing did not perform statistically differently from the negative control in either CZOI or organism recovery.
- After removal of the dressings (post 48h incubation of initial agar plates), significantly fewer organisms were recovered from the three silver-impregnated dressings: Fabric Ag, Hydrofiber Ag and Alginate Ag
- Statistical ranking of the performance of the dressings showed that Fabric Ag had the lowest amount of recovered organisms after 48 hours followed by Hydrofiber Ag, Alginate Ag, Foam Ag and PHMB.
- There was a strong correlation between the CZOI and the ability to recover viable pathogens from each of the test dressings after a 48 hour exposure to the test organisms.
- The appearance of any zone of inhibition around the dressing was associated with a reduced recovery of organisms from the test dressings in all cases except for Foam Ag.
- A zone of inhibition of greater than 2mm is predictive of there being little or no recoverable organisms from the test dressings other than Foam Ag.

Summary

- The results from our study support other researchers' findings that the disk diffusion method is an appropriate method to assess the ability of the silver impregnated wound dressings to inhibit the growth bacterial organisms.
- The addition of testing the ability to recover these organisms from wound dressings after 48h of incubation was more informative in assessing the overall performance of the wound dressings.
- Our findings demonstrated that there was a strong correlation between the CZOI data and the recovery of organisms from the dressings.
- However, there was variability in the inhibition of growth around specific silver-containing wound dressings and bactericidal activity of these dressings. This observation was particularly evident for Foam Ag in which test organisms were readily recovered from dressing that had a corresponding large CZOI.
- Additional studies are necessary to further determine the correlation of clinical performance and in-vitro data for these wound dressings. Studies assessing the diffusion characteristics of these wound care agents and their effects on microbial biofilms are also needed to better assess the efficacy of wound care dressings and correlate clinical and in-vitro test results.

Selected References

1. Acar A, et al. 2011. Comparison of silver-coated dressing (Acticoat®), chlorhexidine acetate 0.5% (Bactigrass®) and mupirocin for topical antifungal effect in *Candida albicans*-contaminated full-thickness rat burn wounds. *Burns* 37: 882-885
2. Basterzi Y, et al. 2010. In-vitro Comparison of Antimicrobial Efficacy of Various Wound Dressing Materials. *Wounds* 22 (7): 165-170
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Wound Dressings Evaluated

Silver hydrofiber = Convatec Aquacel Ag
Silver fabric = Milliken Tritec Ag (was AFM Ag)
Silver calcium alginate = Hollister Restore Ag,
Hypertonic saline dressing = Mesalt Absorbent Ribbon
Silver polyurethane foam = Molnlycke Mepilex Ag
Polyhexamethylene biguanide (PHMB) = Curly AMD