Summary
Studies reported herein evaluated the efficacy of 0.5% polyhexamethylene biguanide (PHMB)-treated foam dressings to prevent the growth of methicillin-resistant *Staphylococcus aureus* (MRSA). PHMB-containing Kendall™ AMD antimicrobial foam and a control dressing containing no PHMB (Copa™ standard foam dressing), were directly inoculated with clinical isolate of MRSA and placed on a growth medium for selected time intervals. The presence or absence of microbial growth was quantified using the plate counts and was visually assessed using scanning electron microscopy. At all time points, the Kendall™ AMD antimicrobial foam dressing significantly reduced the MRSA growth compared to control dressings.

Introduction
The Copa™ standard foam dressing is a soft foam dressing with high fluid retention. The Kendall™ AMD antimicrobial foam dressing is similar to the Copa™ standard foam dressing, but it also contains 0.5% PHMB as a broad-spectrum antimicrobial agent [1]. The PHMB functions as an agent that reduces bacterial colonization within the dressing and bacterial penetration through the dressing.

Broad-spectrum activity of various Kendall™ AMD antimicrobial dressings against gram-positive and gram-negative bacteria as well as fungi has been demonstrated in several *in vitro* studies [2-6]. In one study, these dressings visually exhibited a high degree of antimicrobial efficacy and reduced growth of MRSA and vancomycin-resistant *Enterococcus faecalis* (VRE) at 24 and 48 hours following direct inoculation on the dressing at the 10⁵ CFU/mL challenge level [4,5]. In another study, these dressings exhibited high antimicrobial activity by 3-6 log reduction of MRSA challenge and 4-5 log reduction of VRE challenge at 30 minutes and 2 hours post inoculation of the dressing [6].

The efficacy of Kendall™ AMD antimicrobial dressings has also been demonstrated in a human clinical case series [7,8]. It has been demonstrated that packing wounds with Kendall™ AMD antimicrobial gauze, to reduce growth within the dressing itself, may be beneficial in reducing the bacterial bioburden in terms of both the total amount of microorganisms and the number of species [9]. Various study results suggest that the Kendall™ AMD antimicrobial sponge can help control organisms such as MRSA and *P. aeruginosa* in institutional settings [10].

The purpose of this study was to evaluate the efficacy of Kendall™ AMD antimicrobial foam dressings to prevent MRSA growth within the dressing. Test dressings were directly inoculated with a MRSA suspension and incubated for a period of time. The bacterial growth was quantified using plate counts and imaged using SEM.

Materials

**Test Articles/Controls**
- Copa™ standard foam dressing, Lot# 817832 (Control dressing)
- Kendall™ AMD antimicrobial foam dressing, Lot# 726314 (Test dressing)

**Challenge Organism**
The MRSA strain used in this study was obtained from Dr. Mark Shirtliff, Department of Microbiology and Immunology, School of Medicine, University of Maryland-Baltimore. It was originally isolated from a bone debridement sample from a patient undergoing treatment for osteomyelitis at the University of Texas Medical Branch.

Methods

**Inoculation and Bacterial Growth**
Dressing samples were cut into 25 mm (1 inch) diameter disks, and each side of the disk was inoculated with 1 mL of 10⁶ CFU/mL of MRSA in 10% tryptic soy broth (TSB). Afterwards, the disks were placed on 50% Trypticase Soy Agar (TSA) plates and incubated at 37°C for 24, 72, or 168 hours. To ensure the dressings stayed hydrated, each dressing was occasionally re-hydrated with 1 mL sterile PBS.

**Quantification of Bacterial Growth**
At selected time points (24, 72, and 168 hours) the dressings were removed from the TSA plates and immersed in 25 mL of DE neutralizing broth and vortexed. The samples were then allowed to sit for 15 minutes, followed by an additional 1 minute of vortexing. Afterwards, 1 mL of the solution was removed, serially diluted, and plated on 100% TSA. The plates were incubated at 37°C overnight and the number of colony forming units (CFU) per dressing was calculated. Dressing samples were also evaluated immediately after the initial inoculation to represent the T=0 time point. Statistical analysis for significance was determined using two-tailed t-test assuming unequal variances with α=0.5 and p≤0.05 considered to be significant.

**Scanning Electron Microscopy**
At selected time points (24, 72, and 168 hours) the dressings were removed from the TSA plates and immersed in 25 mL of DE neutralizing broth and vortexed. The samples were then allowed to sit for 15 minutes, followed by an additional 1 minute of vortexing. Afterwards, 1 mL of the solution was removed, serially diluted, and plated on 100% TSA. The plates were incubated at 37°C overnight and the number of colony forming units (CFU) per dressing was calculated. Dressing samples were also evaluated immediately after the initial inoculation to represent the T=0 time point. Statistical analysis for significance was determined using two-tailed t-test assuming unequal variances with α=0.5 and p≤0.05 considered to be significant.
Results
Quantification of Bacterial Growth
Antimicrobial efficacy of the Kendall™ AMD antimicrobial foam dressings was evident as quickly as the T=0 time point. There was approximately $7.17\pm0.02 \log \text{CFU/dressing}$ for the Copa™ standard foam dressing and $4.99\pm0.25 \log \text{CFU/dressing}$ for the Kendall™ AMD antimicrobial foam (p=0.004, Figure 1). This difference reflected a $2.18\pm0.14 \log$ reduction in MRSA levels with the Kendall™ AMD antimicrobial foam dressing (Figure 2). By 24 hours, the Kendall™ AMD antimicrobial foam dressing displayed a $8.87\pm0.62 \log$ reduction in MRSA growth. The log reductions at the 72 and 168 time points were $9.37\pm0.03$ and $8.88\pm0.08$, respectively.

As Figure 1 illustrates, the differences in the log counts are highly statistically significant, indicating the Kendall™ AMD antimicrobial foam dressings are dramatically more effective in reducing colony counts than the Copa™ standard foam dressing. This is represented visually in the SEM images below.

Scanning Electron Microscopy
Antimicrobial efficacy of Kendall™ AMD antimicrobial foam dressings against MRSA is also illustrated in the following SEM images (Figures 3-6). These images were collected after 0, 24, 72, and 168 hours of incubation. As the incubation period increased, MRSA growth on the Copa™ standard foam dressing was evident. There appeared to be more cocci at the later time points, and the cocci appeared to be imbedded in a connective matrix. However, no MRSA growth was evident on the Kendall™ AMD antimicrobial foam dressings. No cocci were found at the 72 and 168 hour time points. There were individual spherical-shaped objects found on both types of dressings including on dressing samples not inoculated with MRSA. These objects varied in diameter, and the texture of the objects is similar to the texture of the dressing as opposed to the smooth texture of MRSA cocci. This rough surface appearance is mostly an artifact of dressing preparation (dehydration and sputter coating) for SEM imaging.
Figure 3: SEM images of dressings at T=0; Copa™ standard foam dressing (left) and Kendall™ AMD antimicrobial foam dressing (right). Images were taken at 3000x magnification. A few cocci are present on both dressings. The images also depict the rough surface texture of the dressings.

Figure 4: SEM images of dressings at T=24; Copa™ standard foam dressing (left) and Kendall™ AMD antimicrobial foam dressing (right). Images were taken at 3000x magnification. Bacterial micro colonies are present on the Copa™ surface. Kendall™ AMD antimicrobial foam shows no evidence of bacteria, only the surface texture artifact.

Figure 5: SEM images of dressings at T=72; Copa™ standard foam dressing (left) and Kendall™ AMD antimicrobial foam dressing (right). Images were taken at 3000x magnification. Bacteria on Copa™ surface appear to be imbedded in a connective matrix. No bacteria were found on the Kendall™ AMD antimicrobial surface, only the surface texture artifact is shown.
Discussion

Traditional foam wound dressings may provide an environment for microbes to grow unchallenged, which could lead to an increase in the risk of cross contamination and increase wound bioburden. However, antimicrobial foam dressings may prevent or reduce microbial growth in dressings, potentially promoting a better environment for wound healing. The studies reported herein involved the direct inoculation of dressings, and bacterial growth was quantitated via plate counts and SEM. The Kendall™ AMD antimicrobial foam dressings exhibited significant reduction in MRSA growth compared to the control, Copa™ standard foam dressing.

Conclusion

PHMB-treated Kendall™ AMD antimicrobial foam dressing exhibited antimicrobial activity against MRSA in terms of resisting bacterial colonization within the dressing. This attribute may reduce the risks of contamination of the wound via wound dressings and may aid in managing a better wound healing environment.

REFERENCES

1. Intrinsic activity of Cosmocil CQ – Technical brief on PHMB, Avecia Biocides Inc.
7. Patricia Mertz et al, "The Effect of an Antimicrobial Gauze Dressing Impregnated with 0.2% Polyhexamethylene Biguanide (PHMB) as a Barrier to prevent Pseudomonas aeruginosa Wound Invasion", 9-01.
8. Angela L. Reitama, M.D. and George T. Rodheaver, Ph.D., "Effectiveness of a New Antimicrobial Gauze Dressing as a Bacterial Barrier", 6-01.
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10. Glenda J. Motta, RN and Donna Trigilia, APRN, "Impact of an Antimicrobial Drain Sponge Dressing Upon Specific Bacterial Isolates at Tracheostomy Sites", 5-03.

Figure 6: SEM images of dressings at T=168; Copa™ standard foam dressing (left) and Kendall™ AMD antimicrobial foam dressing (right). Images were taken at 3000 magnification. Large bacterial colonies are present on the Copa™ surface. Kendall™ AMD antimicrobial surface texture is free of bacteria.