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Research Article

Evaluation of the Elution of Polyhexamethylene Biguanide from Two Wound Dressing Materials

Katie Stone*, Dean Hendrickson, Helen Sims, Luke Wittenburg and Doreene R Hyatt

College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado

***Corresponding Author:** Katie Stone, College of Veterinary Medicine and Biomedical Sciences, Colorado State University, Fort Collins, Colorado.

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Abstract

Topical antibiotics and antimicrobials play a vital role in healing and combating wound infections, with topical antiseptics being detrimental to wound healing progress due to their cytotoxic effects and retardation of fibroblasts [1]. Polyhexamethylene biguanide (PHMB), a polymer with efficacy against a wide variety of gram positive and gram negative bacteria [2], is showing promising effects in wound healing. According to Chindera K., *et al*. [3] PHMB kills bacteria selectively over host cells and acquired resistance to PHMB has not been reported and that it is unlikely to succumb to resistance. The manufacturer states that PHMB infused dressings have been used to resist bacterial colonization, reduce bacterial penetration into wounds, limit cross-contamination of other bacteria and maintain balance of native skin flora. Given these two benefits of PHMB, the use of it in wounds would prove beneficial from a resistance standpoint as well as its selectivity for bacteria over regenerating host cells. In addition, PHMB works as a biological barrier against pathogenic agents and creates an environment favorable to proliferation of normal epidermis flora [4].

Given this, PHMB makes for an excellent topical antimicrobial to aid in wound healing compared to other topical antimicrobials or antiseptics. PHMB is designed to stop penetration of bacteria into a wound but in order to determine how to use PHMB best its properties of bacterial inhibition first needed to be explored. Once this is established conclusions can be made as to how PHMB should be applied to wounds and used in treatment protocols.

It was hypothesized that the PHMB would elute from the dressing into the wound environment and kill bacteria, thus preventing growth where there is contact of dressing to the wound and that PHMB will elute off of the dressings, allowing for inhibition of bacterial growth in wounds distant from the dressing.

Keywords: *Elution; Polyhexamethylene Biguanide (PHMB); Wounds; Dressing*

Introduction

Wound infections, including multi drug resistant bacteria and biofilms are common in the equine patient [5-8]. The challenges these wounds pose necessitate an improvement in how we prevent and treat wound infections. Wound dressings have long been a cornerstone of wound management [9], with sprays, ointments, or salve being some of the most common dressings used [10].

Antimicrobial resistance (AMR) is an ever-present threat to prevention and treatment of a range of infections caused by bacteria, parasites, protozoa, and viruses. By causing delayed wound healing and allowing infections to persist, treatments must be extended. Different antibiotics or antimicrobials must be used and new protocols must be formed to combat resistant infections. There are a multitude of steps that can be taken to prevent AMR infections. Rational use of antimicrobials, regulation of over-the-counter availability of antibiotics, improved hand hygiene, and improved infection prevention and control via aseptic surgical techniques are steps that can be implemented to play a role in limiting these infections. However simple these steps seem to be, treating AMR infections proves to be difficult. In order to find out what bacteria are resistant to and how to best mitigate resistance, susceptibility testing is done in order to select the antimicrobial with the lowest MIC and MIC breakpoint [11]. These steps help mitigate the effects AMR may have on bacteria and ensure judicial use of antimicrobials are used with the goal of faster wound healing.

Materials and Methods

In order to evaluate the elution behavior of PHMB, one-gram squares of Kerlix™ AMD gauze (G) and Kendall™ AMD foam (F) dressing were incubated in equine serum collected from recipient mares at Colorado State University's Equine Reproduction Laboratory. The dressings were incubated for 7 days with fluid aliquoted each day. Plain Kerlix™ gauze without PHMB (G-) and plain Kendall™ foam (F-) without PHMB were similarly incubated to serve as controls. In order to prepare the diffusion disks, 25 µL of serum was aliquoted onto sterile 6 mm filter disks starting on day 0 and continuing through day 7. Disks were made from each of the conditions: F+, G+, F-, G-, representing foam and gauze both with and without PHMB, respectively, and plain serum. These were desiccated overnight after incubation and stored. The inhibitory properties of PHMB were evaluated against 4 different bacteria: *Pseudomonas aeruginosa, Streptococcus equi* ssp. *zooepidemicus*, *Escherichia coli*, and *Staphylococcus aureus*. Serum with no dressing material or PHMB added was used as a control for antimicrobial activity of the pooled serum, accounting for degradation of the serum over time. Three isolates of each bacterial species were obtained from equine samples submitted to the CSU Veterinary Diagnostic Laboratories. Commercially available ATCC cultures of *Pseudomonas aeruginosa, Escherichia coli*, *Enterococcus faecalis* and *Staphylococcus aureus* were also used for comparison.

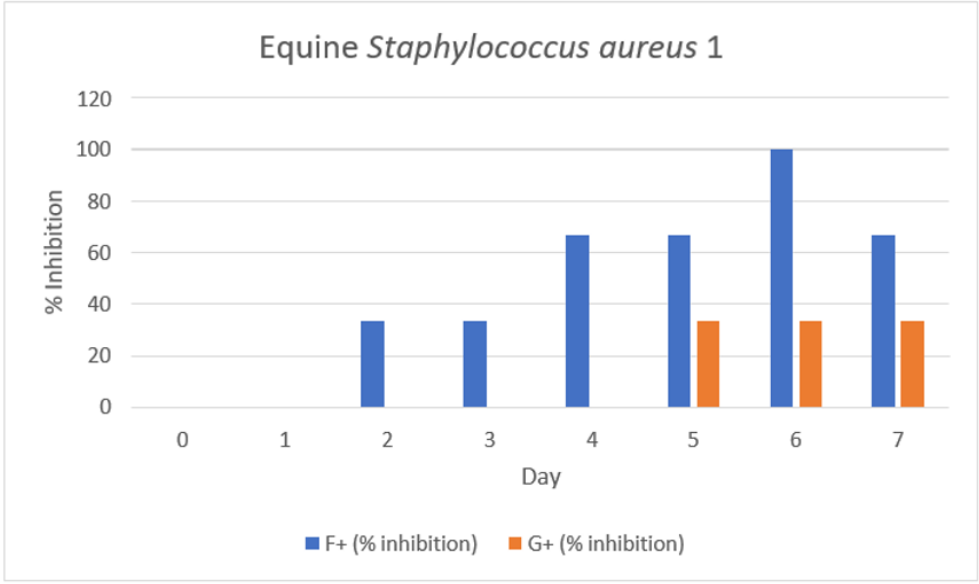
To begin the inoculation system, the bacteria were plated onto 5% sheep blood Muller- Hinton agar plates overnight at 37°C. Five isolated bacterial colonies greater than 1 mm diameter were transferred with an inoculation wand to an inoculation tube and vigorously vortexed for 10 seconds. This released the bacteria from the wand. The solution sat for five minutes and was vortexed again. The bacterial suspension was then used to inoculate the entire agar surface of Mueller-Hinton agar plates. Application of the desiccated antimicrobial-infused disks or control disks occurred after letting the excess moisture evaporate for at least three minutes, but not to exceed fifteen minutes, from the bacterial-inoculated Mueller-Hinton agar plates. Within fifteen minutes of the disks being applied, plates were inverted and placed face-down in a non-CO_2 35°C incubator for 16 - 18 hours.

To read the endpoint, plates were checked for a confluent lawn of growth, purity, and uniformly circular zones of inhibition. If the lawn was not confluent, the plate could be incubated for a maximum of 24 hours. The endpoint was taken as the zone showing no visible growth that could be detected with the unaided eye.

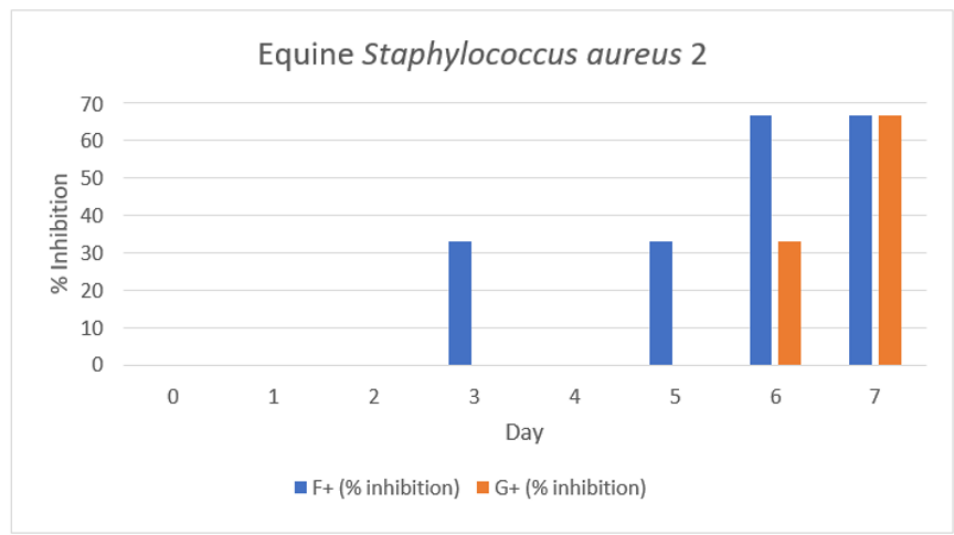
Results

Inhibition of bacterial growth occurred directly beneath disks made with serum incubated with Kerlix™ AMD gauze (G+) and Kendall™ AMD foam (F+) dressings on plates inoculated with the four *Staphylococcus aureus* isolates. This included the ATCC strain and the 3 isolates from CSU's Veterinary Diagnostic Laboratory. No inhibition occurred on the plates inoculated with the other three bacteria, nor under the control disks, specifically the F-, G-, plain serum or blank disks. For S. aureus, inhibition was first seen on day 2 beneath the F+ disks, while the first inhibition by a G+ disk wasn't observed until day 5. The average number of replicate disks causing inhibition of bacte-

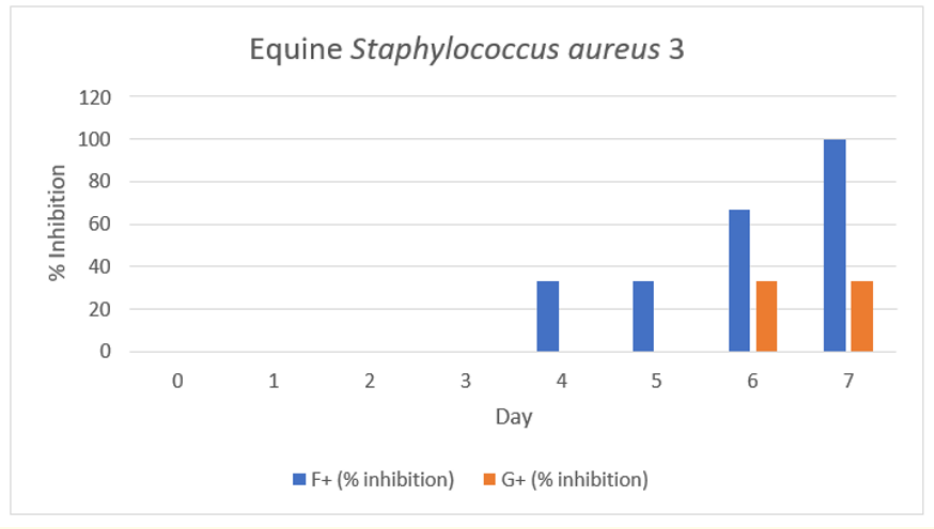
rial growth increased over time for both F+ and G+. Graph 1-4 demonstrate the percent of plates that showed inhibition over a one-week period for *S. aureus* strains. With three plates being measured, if only one plate showed inhibition it correlated to a 33.3% inhibition, 2 plates being 66.6% and all 3 plates showing inhibition equating to 100% inhibition in that given strain.



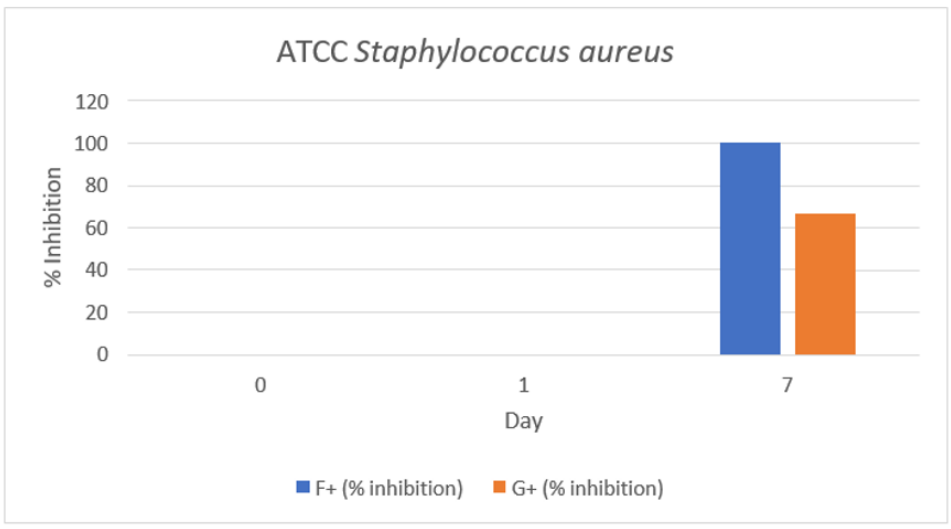
Graph 1: *Percent inhibition of equine S. aureus strain one.*



Graph 2: *Percent inhibition of equine S. aureus strain two.*

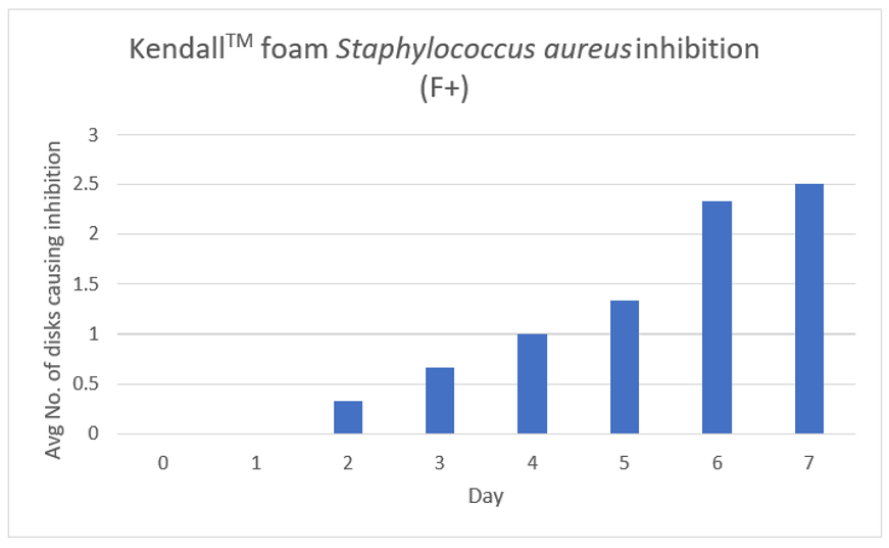


Graph 3: *Percent inhibition of equine S. aureus strain three.*

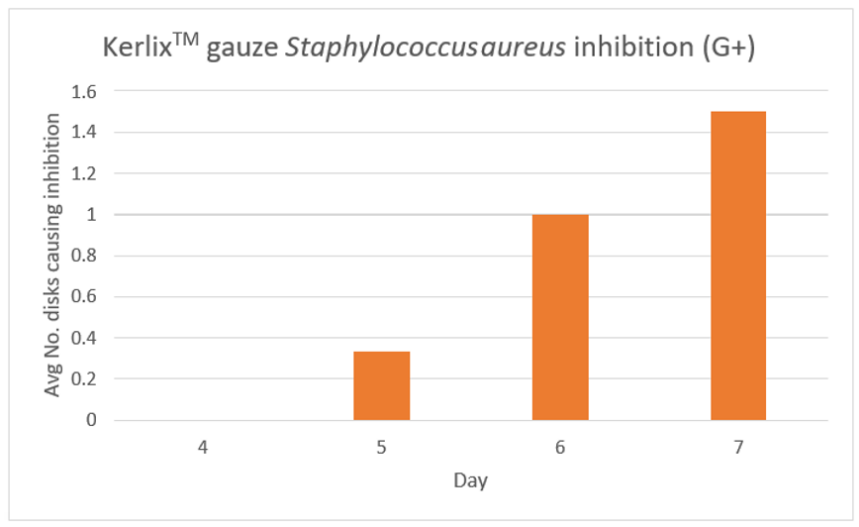


Graph 4: *Percent inhibition of ATCC S. aureus.*

The average inhibition relating to the number of disks placed in each bacterial strain are demonstrated in graph 5 and 6. In graph 5, there were three disks for each strain used. For F+, it took seven days for 2.5 disks to have an area of inhibition for *S. aureus*. For G+ in graph 6, only 1.5 disks were causing inhibition by day seven.



Graph 5: *Average number of disks causing inhibition per day on Kendall™ foam for S. aureus.*



Graph 6: *Average number of disks causing inhibition per day on Kerlix™ gauze for S. aureus.*

Discussion

This study showed that enough PHMB eluted off both the Kerlix™ AMD gauze and Kendall™ AMD foam dressing into equine serum to produce inhibition against both equine and ATCC *Staphylococcus aureus* isolates. These promising data suggest that the dressings may be

effective against bacterial contamination within wounds, not just where there is contact of the wound with the dressing material. There was no significant difference between the foam and gauze, however it took the foam less time to elute and cause bacterial growth inhibition and the assumption that it would correlate to elution time in a wound could be made.

While it was anticipated there would be no growth under the control disks as there were no associated antimicrobial properties, it was quite unexpected that none of the other bacteria had growth that was inhibited by PHMB infused serum. The four bacteria chosen to perform this experiment were chosen because they are all bacteria commonly isolated from equine wounds [6]. *S. aureus* was the only bacteria to show inhibition as it was the only bacteria tested that is sensitive to PHMB. The others did not have a response to PHMB infused equine serum due to insensitivity to PHMB. However, it is possible that the concentration of the PHMB in the serum was not high enough to impact these bacteria and that they might still be susceptible to direct contact with the actual dressing infused with PHMB.

ATCC strains were used for comparison to see if PHMB would influence bacteria grown under very specific conditions and compared them to bacteria that were cultured directly from equine wounds. The results showed that the PHMB infused foam and gauze had no effect on these ATCC strains, including *S. aureus*.

While PHMB has shown promising effects in equine *Staphylococcus aureus* bacterial contaminated wounds, more could be done to determine the true extent of its efficacy. In order to generate clinical bandage change recommendations further research using a more sensitive analytical technique, such as high-performance liquid chromatography (HPLC), is needed to quantify the elution behavior. However, based on these preliminary data, it can be concluded that in *S. aureus* contaminated wounds, elution of PHMB should not be relied upon as it up to seven days for PHMB to elute out of the dressing and into the serum. The foam had a faster time to elution, but with no significant difference existing between the gauze and foam, either would be appropriate. Elution is a key component to the functionality and benefits of using PHMB. By having coverage via antimicrobial elution *in situ*, it could prevent bacterial growth in the wound as it is healing, allowing for a faster recovery time and wound healing. However, at this time, it appears to be important to have direct contact of the dressing with the bacteria to provide bacterial killing [12].

Limitation of the Study

One limitation of this experiment was the way in which data was recorded. When the absence of bacterial growth beneath the disks was recorded it was strictly done in a yes or no fashion. There were no measurements performed regarding the size of inhibition under each disk, only noting whether there was or wasn't bacterial inhibition underneath the disk. Because of this, a minimum inhibitory concentration (MIC) cannot be calculated or quantified for the drug or the effects of PHMB. Further, a minimum inhibitory concentration (MIC) cannot be calculated or quantified for the drug or effects of PHMB. Looking forward, performing an experiment where PHMB is used in *S. aureus* wounds in live equine patients would be the next step in documenting specific uses and applications as well as developing a MIC with known breakpoints.

Conclusion

In conclusion, PHMB could play a role in treating wounds proven to be infected with *Staphylococcus aureus* via culture and sensitivity, as well as the possibility of other bacteria in different concentrations. Antimicrobial resistance is a great concern when treating *S. aureus* due to resistance and using PHMB to elute into wounds via infused wound dressings would be a way to mitigate resistance factors when finding an appropriate treatment to infected wounds. Without a measured MIC and only observable inhibited bacterial growth no claims can be made regarding breakpoints or true susceptibility. However, it can be applied to the timing of bandage dressing changes with the knowledge that it inhibits *S. aureus in situ*.

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