



A Novel Chlorinated Biocide Against Bacterial and Dermatophyte Infections Evaluated Using a Porcine Wound Infection Model.

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Abstract

Introduction:

Staphylococcus aureus and *Pseudomonas aeruginosa* are the most frequent pathogens isolated from wound infections which can delay wound healing and lead to chronic wounds.¹ *Trichophyton rubrum* is responsible for most dermatophyte infections worldwide with reported antifungal resistance rising², and as such there is a need for novel antimicrobial that can effectively reduce diverse microbial infections.

Methods:

A porcine model was used, and three animals were assigned to either: Methicillin-Resistant *S. aureus* USA300 (MRSA USA300), *P. aeruginosa* ATCC 27312 (PA 27312), or *T. rubrum* ATCC 28188 (TR28188). Forty-five 10 mm full thickness (infected with MRSA USA300 or PA 27312) or deep partial thickness wounds (infected with TR28188) were created on each animal. After biofilm formation³, baseline wounds were recovered for microbiological analysis, then stabilized chlorinated biocide (via liquid, hydrogel, or combination of both), vehicle and untreated controls were applied and covered with polyurethane film dressing. Betadine povidone-iodine solution and polyhexanide-betaine gel were used for MRSA USA300 and PA 27312 and clotrimazole and miconazole were used for TR28188 as positive controls. Wounds were recovered for microbiological analysis on Days 3 and 7. Histological analysis was performed on the TR28188 samples.

Results:

The greatest reduction of MRSA USA300 was observed for wounds treated with chlorinated biocide liquid/hydrogel combination, with a greater than 2 Log CFU/g (99%) reduction compared to untreated control on Day 7. Against PA 27312, polyhexanide-betaine gel exhibited the greatest reduction at Day 3, followed by chlorinated biocide hydrogel yielding 97% and 96% reductions, respectively compared to untreated control, while on day 7, chlorinated biocide combination treated wounds exhibited the lowest PA 27312 counts. Chlorinated biocide combination had the greatest efficacy against TR28188 on Days 3 and 7 compared to untreated control (97% and 98% reduction, respectively). Chlorinated biocide did not exhibit any detriment to wound healing at both time points.

Discussion:

The novel chlorinated biocide evaluated in this study demonstrated considerable efficacy against the frequently isolated bacterial pathogens *S. aureus* and *P. aeruginosa* as well as the dermatophyte *T. rubrum*. The broad antimicrobial efficacy observed for this therapy makes it a promising alternative to current antiseptics or antimicrobials.

Introduction

Pathogens in wounds delay wound healing and can lead to chronic wounds.⁴ Antiseptics, antibiotics and antifungals may be used for treating infected wound however they are susceptible to resistance.^{5,6} Active chlorine is used endogenously by granulocytes and monocytes has shown potential for use as a broad antimicrobial.⁷ In this study, we evaluated a novel stabilized chlorinated biocide based on this mechanism against wound infections by notable pathogens using a porcine model.

References

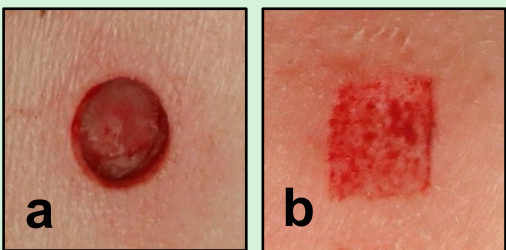
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1. Experimental Animals:

- Three (3) female specific-pathogen-free (SPF) swine were used in this study due to the anatomical similarities between human and porcine skin.⁸

2. Wounding Technique:

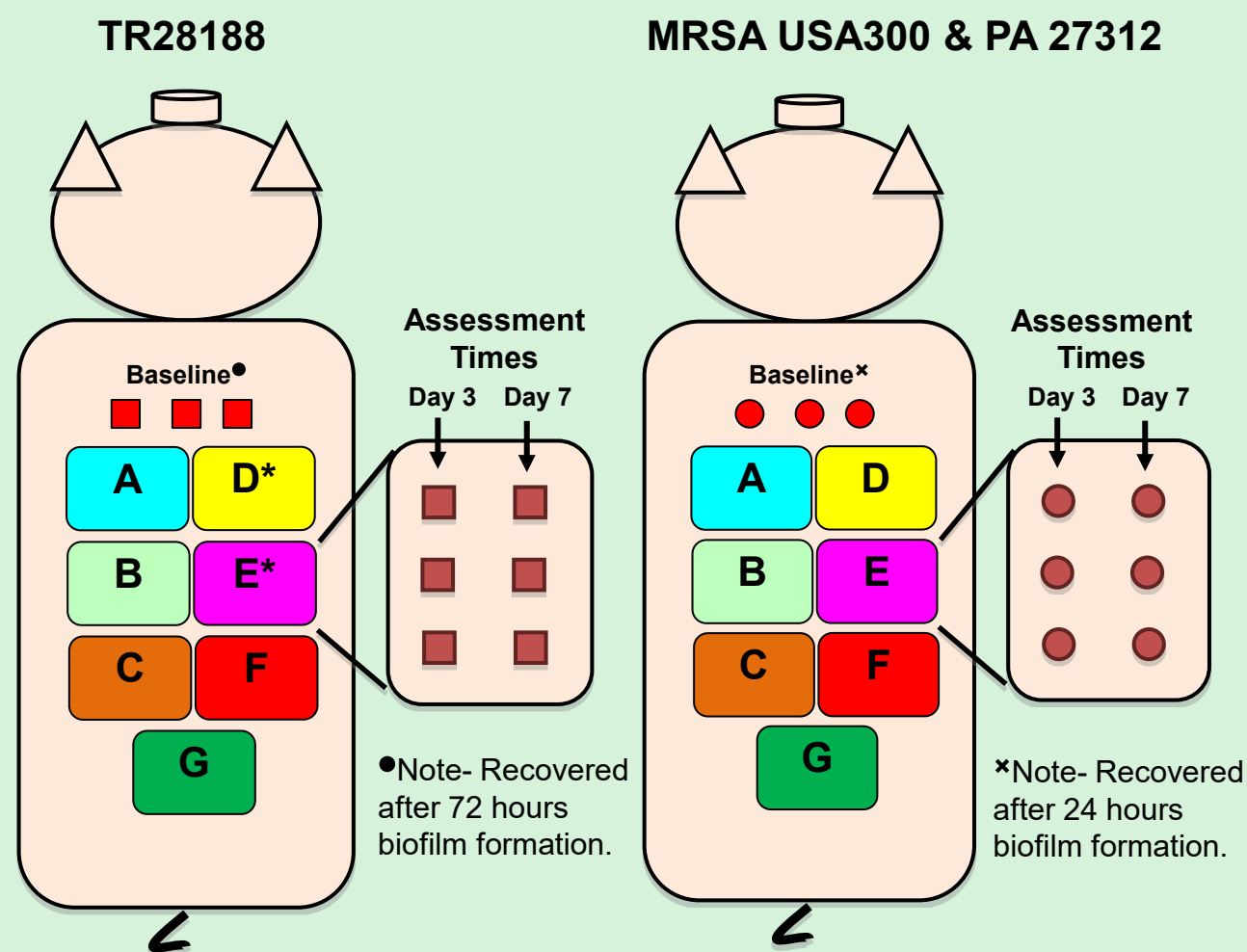
- Forty-Five (45) wounds were created on each animal, either 10 mm full thickness wounds (a) or deep partial thickness wounds (10 mm x 7 mm x 0.5 mm deep) (b)



3. Inoculation

- After wound creation, the wounds were inoculated with 25 µL MRSA USA300 or PA 27312 or 100 µL of TR28188.
- Wounds were then covered with polyurethane film dressing to allow for biofilm formation (24 hours for MRSA USA300 and PA 27312, 72 hours for TR28188).

4. Experimental Design



Treatment Groups

- A. Avantamine Liquid
- B. Avantamine Hydrogel
- C. Avantamine Liquid + Avantamine Hydrogel
- D*. Clotrimazole or D. Betadine Povidone-Iodine Solution
- E*. Miconazole or E. Prontosan Gel
- F. Sterile PBS + Unchlorinated Vehicle Control
- G. Untreated Tegaderm Control

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5. Treatment Regimen

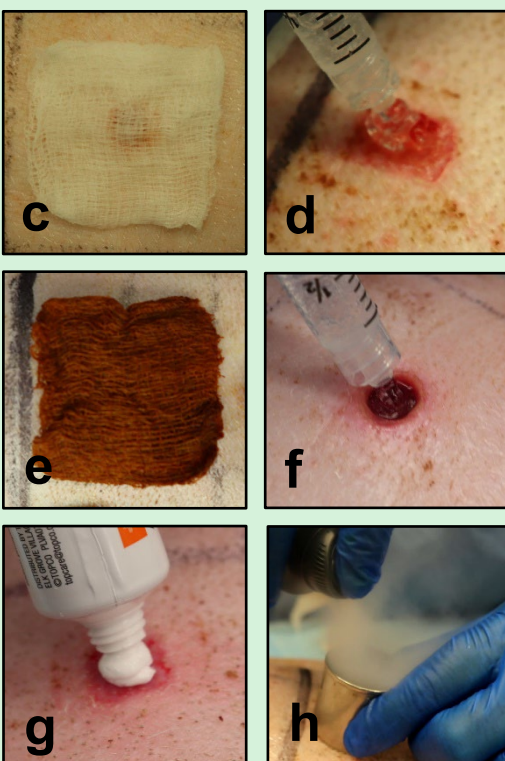
- Wounds were treated daily for the study duration covered with polyurethane film dressings.

- Liquid treatments (Avantamine liquid (c), Betadine povidone-iodine (e), and Sterile PBS) were applied with soaked gauze in place on wounds for 10 minutes.

- Avantamine hydrogel (d), Prontosan gel (f), Clotrimazole (g) and Unchlorinated Vehicle Control were applied with 200 µL of treatment.

- For the combination treatment, hydrogel was applied after liquid-soaked gauze application.

- Miconazole was sprayed as a thin layer into a sterile cylinder to apply following product directions (h).

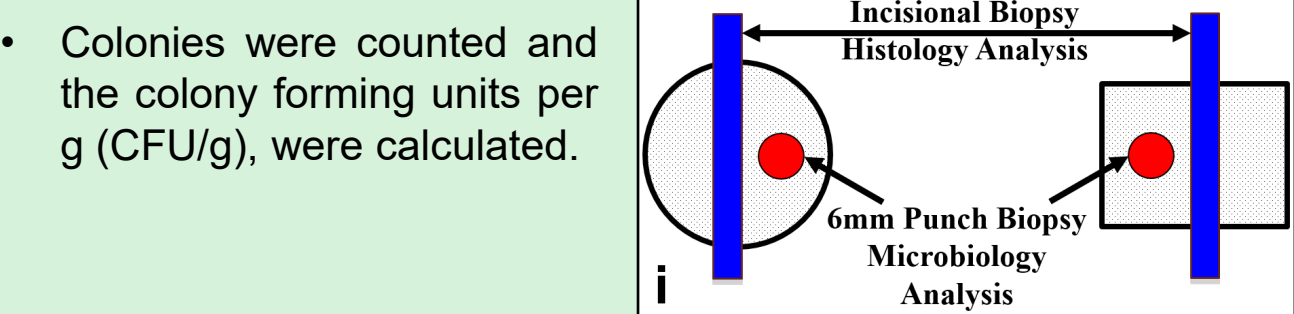


6. Microbiology Assessment

- Three wounds were assessed per treatment group on Days 3 & 7.

- 6mm punch biopsies (i) of the wound were weighed then placed in Dey Engley Neutralizing Broth and homogenized.

- Serial dilutions (j) were made and then plated onto specific media using the Spiral Plater System (k). ORSAB - MRSA (l), PA Agar with CN Supplement - PA (m) and DTM -TR (n).



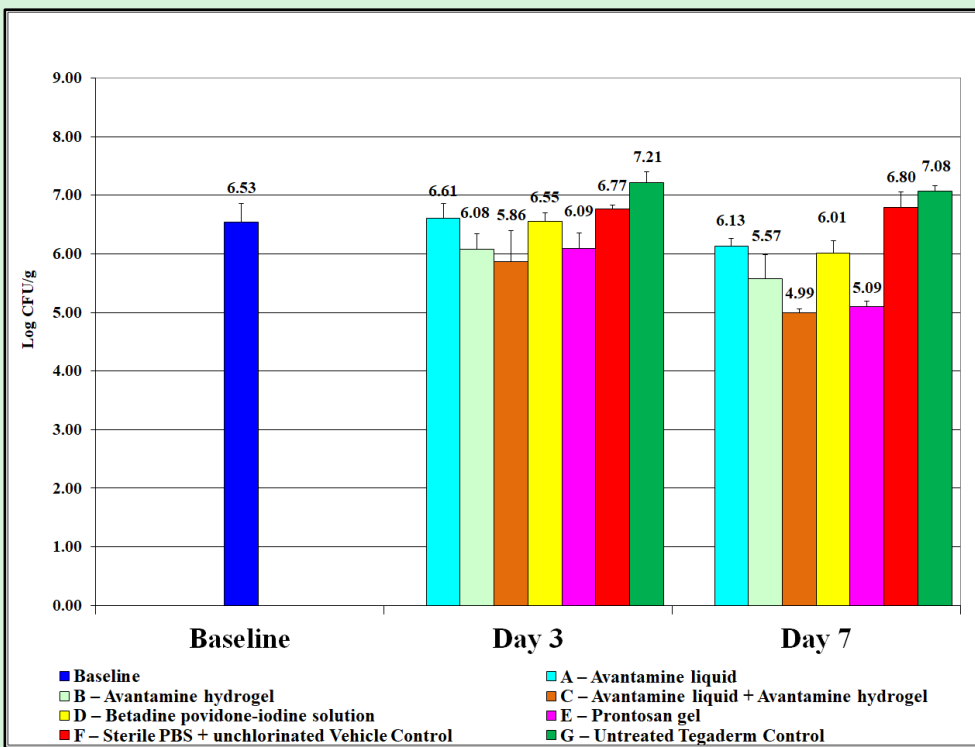
7. Histology Assessment (TR animal)

- Incisional biopsies (i) were collected from the center of the wound with normal skin on both sides and placed in formalin, then embedded in paraffin wax, sectioned, and stained with Hematoxylin and Eosin (H&E).
- Histology analysis was conducted by assessing the following:
- Percentage of re-epithelization (percent of wounded area covered by newly formed epidermis).
- White cell infiltration (WCI): leukocytic infiltrates. Mean Score: 1 = absent, 2 = mild, 3 = moderate, 4 = marked, 5 = exuberant.
- Granulation tissue (percent of wound bed with granulation tissue) graded as follows: 0 = 0, 0.5 = 1-10%, 1 = 11-30%, 2 = 31-50%, 3 = 51-70%, 4 = 71-90%, 5 = 91-100%.

Results

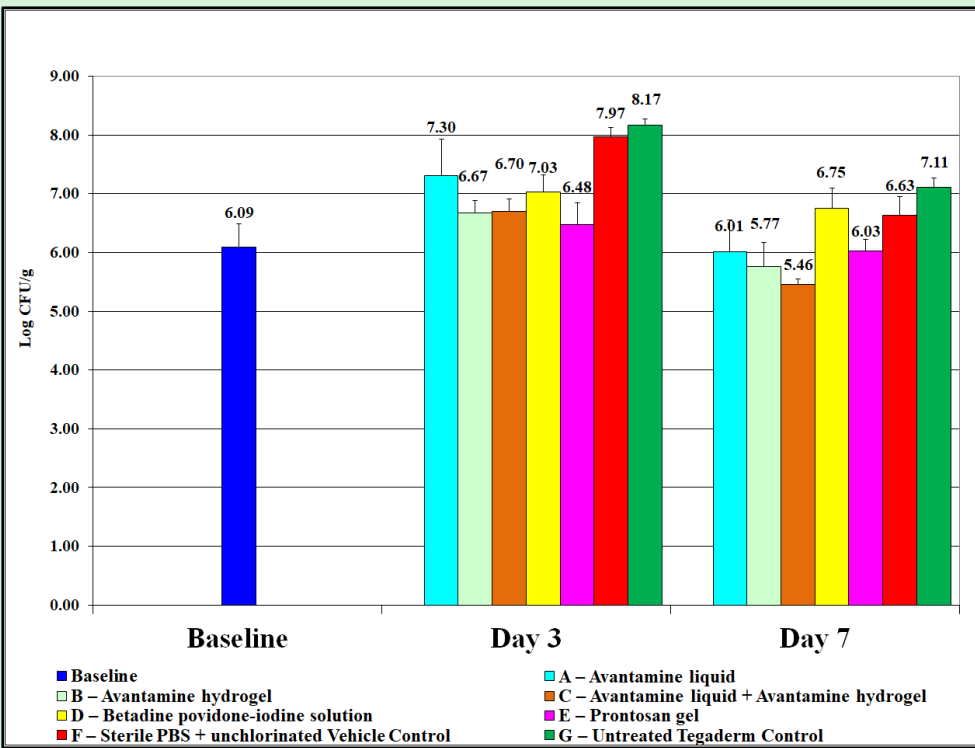
MRSA USA300 Results

- Avantamine liquid (A) and Avantamine Hydrogel (C), exhibited a 76.66% (Day 3) and 97.12% (Day 7) reduction in bacterial counts compared to baseline.
- Prontosan gel also considerably reduced MRSA burden in wounds by 96.37% compared to baseline on Day 7.
- The gel only treated wounds (Avantamine hydrogel and Prontosan gel) had greater reductions in bioburden compared to liquid only treated (Avantamine liquid and Betadine povidone-iodine) on Days 3 and 7.
- Untreated wounds exhibited an increase of 78.89% bioburden from baseline to Day 3.



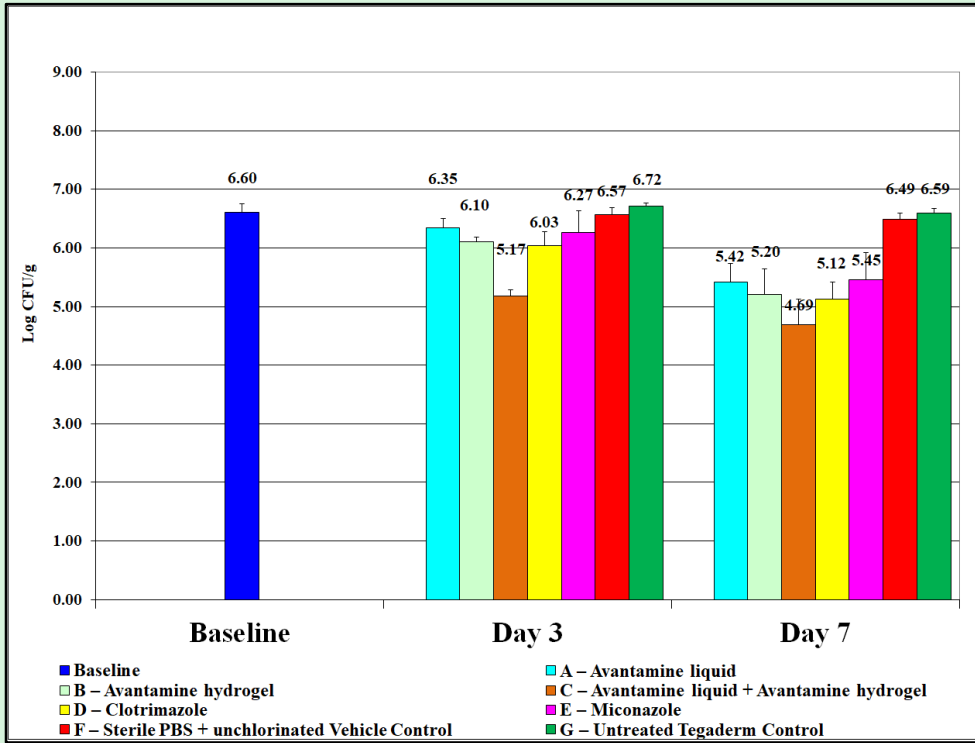
PA 27312 Results

- Although no counts were reduced below baseline on Day 3, PA 27312 was reduced most on day 3 by Prontosan gel with a 97.96% reduction compared to Untreated Tegaderm Control.
- On Day 7, the combined Avantamine Liquid and Hydrogel had 76.57 and 97.78% of reduction compared to Baseline and Untreated control, respectively.
- Betadine povidone-iodine was the least effective treatment aside from controls at day 7, having an increase in pathogen burden of 23.95% from baseline.
- The wounds treated with Avantamine liquid had bioburden 92.14% lower than Untreated Control on Day 7.



TR28188 Results

- TR28188 was reduced most in wounds treated with Avantamine liquid and Avantamine hydrogel combination. This 98.76% (or ~2 Log CFU/g) reduction compared to baseline on Day 7 was the greatest extent of reduction in this study across all organisms.
- The reductions for Avantamine liquid alone or Avntamine hydrogel alone were comparable to miconazole or clotrimazole, respectively.
- Clotrimazole treated wounds had lower TR28188 counts than wounds treated with miconazole at both assessment times (6.03±0.24 and 5.12±0.30 compared to 6.68±0.37 and 5.95±0.44 Log CFU/g for Day 3 and 7, respectively).



Histology Results

- All wounds were 100% re-epithelialized by the end of study.
- WCI scores for all treatment groups were equal to or less than untreated control with Miconazole having the lowest score.
- Levels of granulation tissue were similar on day 3 with all treatments and slightly higher with Avantamine Liquid and Clotrimazole treated wounds.

Conclusions

- The novel chlorinated biocide evaluated in this study demonstrated broad antimicrobial efficacy and reduced microbial burden to a greater extent than other tested antimicrobials, without impeding wound healing.
- The combination treatment of Avantamine liquid and Avantamine hydrogel had the best reductions by the end of the study duration for all micro-organisms tested and is a promising therapeutic for wound infections.