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The Efficacy of a Nitric Oxide-Releasing formulation on a Nasal isolate of Methicillin Resistant *Staphylococcus aureus* in Porcine Wound Model

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Abstract:

Introduction: The colonization of Staphylococcus aureus (SA) acquired in nosocomial infections may contribute to acute and chronic infections. As a commensal microorganism with the ability to form a biofilm, SA can dwell on the skin, nostrils, throat, perineum, and axillae of healthy humans. Nitric oxide (NO) is an endogenously produced gaseous molecule with already demonstrated broad-spectrum antimicrobial activity against several groups of microorganisms. Hydrogels have become a commonly used delivery system and in this study, NO was incorporated into a hydrogel to demonstrate the efficacy to reduce a nasal isolate of methicillin-resistant *Staphylococcus aureus* (MRSA) in porcine wound model.

Methods: Methicillin-Resistant *Staphylococcus aureus* MRSA BAA1686 isolated from nasal infection was used in a porcine wound infection model. Deep partial-thickness wounds (10mm x 7mm x 0.5mm) were made on three animals using a specialized electrokeratome. All wounds were inoculated and then covered with polyurethane film dressings for biofilm formation. After 48 hours, three wounds were recovered from each animal for baseline enumeration. The remaining wounds were randomly assigned to six treatment groups and treated once daily. The treatment groups are as follows: NO topical ointments concentrations of 0.3, 0.9 and 1.8%, Vehicle Ointment, Mupirocin 2% (positive control), and Untreated Control. Microbiological recoveries were conducted on day 4 and 7.

Results: The greatest efficacy observed from the NO formulations against MRSA BAA1686 was the 1.8% concentration. This agent was able to reduce more than 99% of bacterial counts when compared to Baseline, Vehicle Ointment, and Untreated Control wounds on both assessment days. Mupirocin 2% was the overall best treatment against MRSA BAA1686 on both assessment days, with a significant reduction ($p \leq 0.05$) of 4.70±0.13 Log CFU/mL from day 4 to day 7.

Discussion: Overall, the positive control Mupirocin 2% was the most effective in eliminating MRSA BAA1686 throughout the study. This experiment demonstrated a downward trend from the highest concentration of NO topical ointment formulations to the lowest concentrations on both assessment days (0.3% - 1.8%). Out of all NO topical ointments, the highest concentration (1.8%) was the most effective with the potential to be an alternative treatment against a MRSA nasal strain biofilm.

Introduction:

The spread of MRSA has become an endemic globally in many health care facilities, being identified as the most common cause of skin and soft tissue infections.[1] Nasal colonization is a predecessor to infections in multiple cases, prevention and proper treatment remedies can decrease the risk of infections by discovering them with nasal screenings.[2,3] NO as a novel agent has shown beneficial results in healing infected wounds, as NO can cause bacterial cell death by damaging cell membranes, proteins and DNA.[4] An *in-vivo* study using NO-releasing hydrogel formulations proved its antimicrobial efficiency predominantly against MRSA within polymicrobial infected wounds.[5] The NO formulation of NVN4428 similar to the one used for this study has shown its proficiency against various strains of SA in a previous *in-vitro* study.[6] The purpose of this study was to evaluate various concentrations of a NO topical gel with hydrogel to examine its ability to reduce the bacterial load in MRSA BAA1686 inoculated wounds on a porcine model.

References

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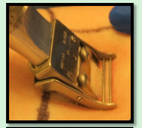
Materials and Methods:

1. Experimental Animals:

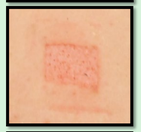
Three (3) swine were used as our experimental animal due to the morphological, physiological, and biochemical similarities between porcine skin and human skin.[7]

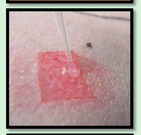
2. Wounding Technique:

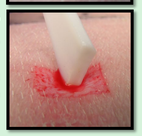


A specialized electrokeratome was used to create fifty-one (51) deep partial thickness wounds (measuring 10mm x 7mm x 0.5mm deep) on the paravertebral and thoracic area of each animal.

3. Inoculation:

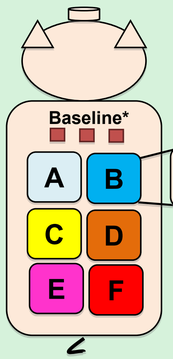






- Methicillin Resistant *Staphylococcus aureus* ATCC BBA1686 (MRSA BAA1686) clinically isolated from nasal infections was used to inoculate each wound.
- Each wound received 25µL of the MRSA BAA1686 inoculum at 10^6 CFU/mL and was spread with a Teflon spatula (10 seconds).
- Eight (8) wounds were assigned to each treatment group (6 groups total), and 3 wounds were used as a baseline.
- All wounds were then covered with a polyurethane film for 48 hours (to allow biofilm formation).

4. Experimental Design:



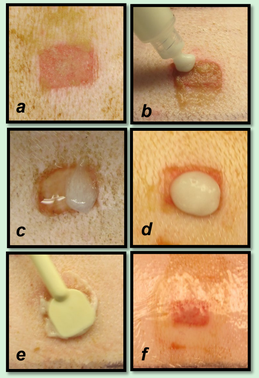
Treatment Groups

A. NVN 4428 (0.3%)
B. NVN 4428 (0.9%)
C. NVN 4428 (1.8%)
D. Vehicle Ointment
E. Positive Control (Mupirocin)
F. Untreated Control

Assessment Times

*Baseline wounds were recovered 48 hours after biofilm formation and immediately before the first treatment.

5. Treatment Regimen:

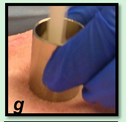


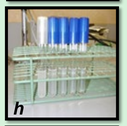
a. After 48 hours, polyurethane film was removed, baseline wounds were recovered, and all wounds were ready for treatment.
b. An example of each wound (except for Untreated Control) receiving their assigned topical treatment.
c. An example of 200mg assigned formulation gel with Hydrogel on wound.
d. An example of 200mg of Positive Control (Mupirocin) on wound.
e. Each topical treatment was spread around the wound with a sterile Teflon spatula.
f. All wounds including Untreated Control were covered with a polyurethane film dressing (Tegaderm; 3M, St. Paul, MN).

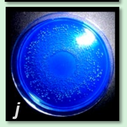
- All wounds were treated daily and Tegaderm dressings were replaced after each treatment application.

Microbiology Analysis:

6. Wound Recovery:







- Baseline wounds were recovered 48 hours after inoculation and prior to treatment application. On days 4 and 7 post treatment, four wounds per treatment group were recovered by using the scrub technique.
- One (1) mL of all-purpose neutralizer solution was pipetted into a sterile steel cylinder at the center of each wound and scrubbed with a sterile Teflon spatula for 30 seconds (photo g).
- Serial dilutions were made (photo h) and quantified using the Spiral Plater System (Spiral Biotech, Norwood, MA) which deposits a defined amount (50µL) of suspension over the surface of a rotating agar plate (photo i).
- MRSA BAA1686 was isolated on ORSAB (Oxacillin Resistance Screening Agar Base) and incubated aerobically at 37±2°C for 36-48 hours (photo j).
- The colony forming units per milliliters (CFU/mL) were calculated.

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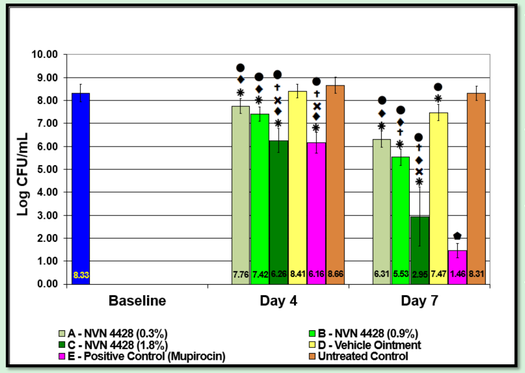
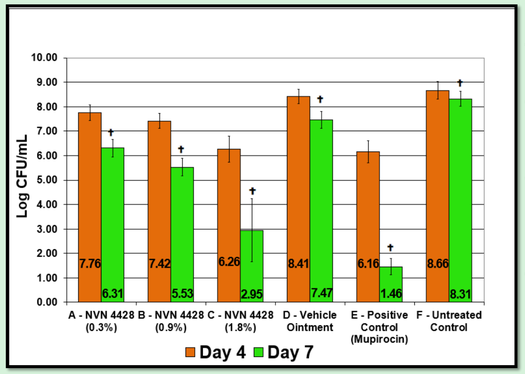
Results:



- NVN 4428 (1.8%) had significant reductions ($p \leq 0.05$) of 99.15% (2.07 ± 0.14 Log CFU/mL) when compared to Baseline and 99.61% (2.41 ± 0.17 Log CFU/mL) when compared to Untreated Control on Day 4.
- NVN 4428 (1.8%) significantly ($p \leq 0.05$) reduced the bacterial count by 2.16 ± 0.23 Log CFU/mL (99.30%) when compared to Vehicle Ointment.
- NVN 4428 (0.9%) compared to Untreated Control displayed a significant ($p \leq 0.05$) reduction of 1.25 ± 0.05 Log CFU/mL (94.33%).
- Mupirocin had significant reductions ($p \leq 0.05$) of 99.32% and 99.69% (2.17 ± 0.06 and 2.50 ± 0.09 Log CFU/mL) when compared to Baseline and Untreated Control, respectively.
- By Day 7, NVN 4428 (1.8%) exhibited significant ($p \leq 0.05$) bacterial reductions of 99.99% (over 5.30 Log CFU/mL) when compared to Baseline and Untreated Control wounds, respectively.
- NVN 4428 (1.8%), NVN 4428 (0.9%), and NVN 4428 (0.3%) each significantly ($p \leq 0.05$) reduced 99.99%, 98.84% and 93.01% of bacteria when compared to Vehicle Ointment, respectively.
- Both NVN 4428 (0.9%) and NVN 4428 (0.3%) exhibited significant ($p \leq 0.05$) bacterial reductions of ≤ 99% when compared to Baseline and Untreated Control, respectively.
- Positive Control (Mupirocin) exposed significant ($p \leq 0.05$) reductions over 6.85 Log CFU/mL (99.99%) compared to Baseline and Untreated Control, respectively.



- All treatment groups displayed significant reductions ($p \leq 0.05$) from one assessment day to the other.
- Between assessment days, NVN 4428 (1.8%) had a significant ($p \leq 0.05$) bacterial reduction of 3.31 ± 0.76 Log CFU/mL (99.95%).
- NVN 4428 (0.9%) and NVN 4428 (0.3%) demonstrated significant ($p \leq 0.05$) differences of 1.88 ± 0.04 Log CFU/mL and 1.45 ± 0.04 Log CFU/mL (98.69% and 96.42%), respectively.
- Positive Control (Mupirocin) had a significant difference of 4.70 ± 0.13 Log CFU/mL (99.99%) from Day 4 to Day 7.

Conclusions

- NVN 4428 (1.8%) performed just as good as Positive Control (Mupirocin), proving itself as an effective treatment and revealing its potential to eradicate MRSA BAA1686.
- All treatments displayed a significant ($p \leq 0.05$) declining trend of MRSA BAA1686 between assessment days with the highest NO concentration and positive control expressing the greatest reduction in MRSA counts.
- Additional studies to evaluate the antimicrobial/healing therapies on other pathogens are warranted.