An Assessment Of The Antimicrobial Activity Of A Novel, Nitric Oxide Generating Wound Treatment System According To The Modified AATCC Test Method 100

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Introduction

Chronic wounds contain mixed species microbiota which can contribute to the recalcitrance of chronic wounds. The FDA's recommended method for antimicrobial wound dressing testing is the Modified AATCC Test Method 100. The method dictates testing should be carried out on three Gram positive bacteria, 3 Gram negative bacteria, 1 yeast and 1 mould. Testing should include a swatch of the finished product which should be conditioned to emulate clinical use. Dressings should also be tested over their product use-life and compared to a non-active version of the test dressing. The FDA acceptance criteria for this test method is ≥ 4 log reductions for all microorganisms tested. The aim of this study was to assess the antimicrobial activity of a nitric oxide wound dressing according to a test method modified from AATCC Method 100.

Method

Samples of a novel, nitric oxide generating wound treatment system were prepared aseptically and inoculated with individual suspensions of three Gram positive microorganisms, three Gram negative microorganisms, 1 yeast and 1 mould. Dressings were incubated at 37°C ± 2°C for 24 hours and 48 hours. During the 48-hour experiment, the dressings were inoculated with 500 µl simulated wound fluid after 24 hours incubation in order to mimic an exuding wound. After the incubation period, dressings were placed into 10 ml neutraliser and sonicated for 5 minutes. The resulting suspension was serially diluted, plated onto appropriate agar and incubated at the optimum temperature for each microorganism.

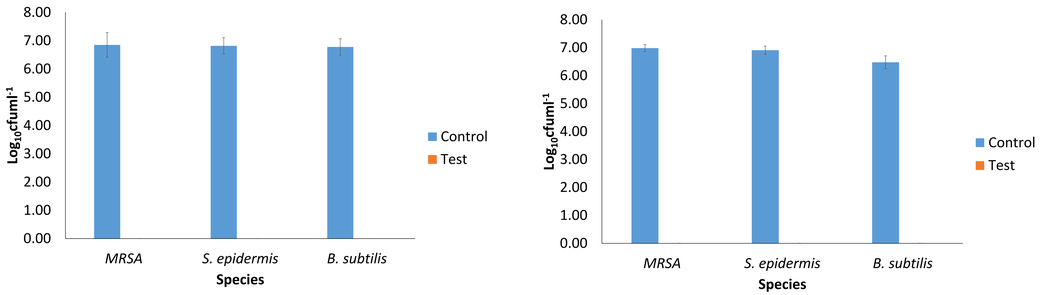

Figure 1. Quantity of viable organisms recovered from control and test dressings after 24 hours (left) and 48 hours (right) incubation with Gram positive bacteria Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Bacillus subtilis*.

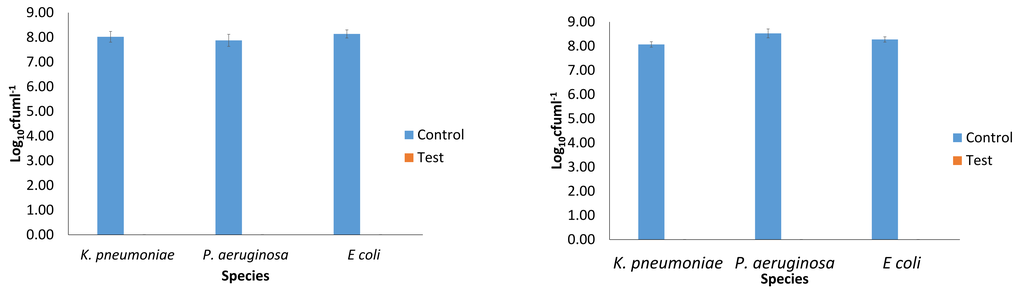

Figure 2. Quantity of viable organisms recovered from control and test dressings after 24 hours (left) and 48 hours (right) incubation with Gram negative bacteria *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Escherichia coli*.

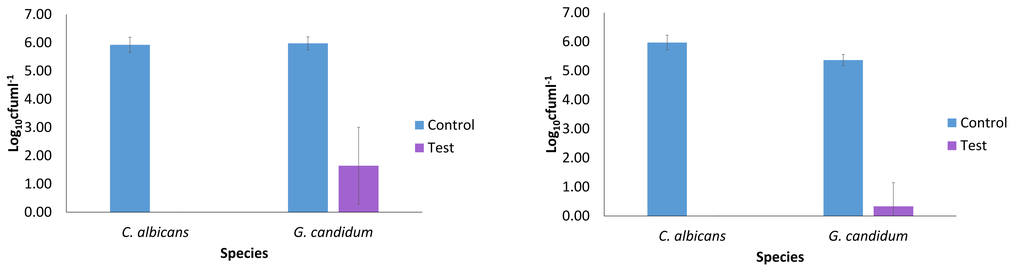

Figure 3. Quantity of viable organisms recovered from control and test dressings after 24 hours (left) and 48 hours (right) incubation with the yeast *Candida albicans* and the mould *Geotrichum candidum*.

Results

Following 24 hours and 48 hours incubation, no viable organisms were recovered from test dressings inoculated with Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Bacillus subtilis, Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Escherichia coli*. This equated to a >6 log reduction in viable bacteria compared to the control dressing. Following 24 hours and 48 hours incubation, no viable organisms were recovered from test dressings inoculated with *Candida albicans.* Minimal *Geotrichum candidum* was recovered from dressings following incubation and equated to a >4 log reduction in viable fungi compared to the control dressing.

Discussion and Conclusions

All microorganisms tested can be found in wounds. *Staphylococcus aureus and Pseudomonas aeruginosa* are widely reported as key causal bacteria. Studies of chronic venous leg ulcers by Gjødsbol et al. (2006) reported that *S. aureus and P. aeruginosa* occupied 93.5% and 52.2% of ulcers, respectively. In addition, a study of 676 patients with signs of infected wounds, reported that 191 patients (28.2%) were infected with *S. aureus*. Chronic wounds harbouring *P. aeruginosa* have also been reported as larger than wounds that did not, and the presence of *P. aeruginosa* delayed the healing of wounds.

The novel, nitric oxide generating wound treatment system was able to minimise microbial colonisation of the dressing, kill a broad spectrum of bacteria within the dressing and provide sustained antimicrobial activity in the dressing for up to 48 hours. The dressing was able to meet the FDA criteria of a >4 log reduction within the dressing required for a product to claim anti-microbial activity. The data suggests that application of the novel, nitric oxide generating wound treatment system could potentially treat infection and could have useful clinical implications. Clinical studies are required to confirm these observations.

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