The antimicrobial activity of silver-containing dressings against surface-associated microorganisms in a dual-species community

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Introduction

- Microorganisms found in hard-to-heal wounds are invariably present in a surface-attached phenotype[1]
- These surface- and self-attached microbial communities usually contain multiple species[2]
- This polymicrobial nature of wound bioburden increases virulence and tolerance to antimicrobial agents[3,4]
- We evaluated antimicrobial dressings containing silver against surfaced-associated microorganisms in a dual-species community grown in a stringent simulated wound model

STUDY OBJECTIVE

The aim of this *in vitro* study was to evaluate the effectiveness of different antimicrobial dressings against dual species communities grown in a simulated wound model

Methods

Test dressings

- Carboxymethylcellulose dressing containing ionic silver, ethylenediaminetetraacetic acid (EDTA), and benzethonium chloride (BEC) (‘**CISEB**’)
- Non-adherent polyethylene mesh with polyester core dressing containing silver oxysalts (‘**PPSO**’)
- Cellulose ethyl sulphonate fibre dressing containing ionic silver (‘**CESIS**’)
- Polyacrylate (polyabsorbent) fiber dressing with acrylic core containing silver sulphate (‘**PSS**’)
- Secondary film cover dressing

Challenge organisms

- *Klebsiella pneumoniae* (NCTC 9156)
- Community-associated Methicillin-resistant *Staphylococcus aureus* (CA-MRSA; ATTC® BAA™-1556, clone of USA300

Protocol

- Challenge organisms were grown on gauze for 24 hours in a nutritious broth and fetal bovine serum (FBS) mixture with an initial inoculum number of ~1×10^6 colony forming units (CFU)/mL in total
- Following incubation, the gauze-attached dual-species community was rinsed twice to remove planktonic bacteria and placed on the central contact plate within a simulated wound assembly (SWA), and total viable counts were performed to establish initial bacteria numbers
- The SWA consisted of a bovine leather-covered Perspex plate (simulating peri-wound skin), surrounding a central insert of a 55 mm diameter agar contact plate (simulating a moist wound bed with a reservoir of isotonic nutrients), supporting the dual-species communities
- The simulated wound area was covered with the test primary dressing (10×10 cm samples) and hydrated with 8 mL simulated wound fluid (50/50 Maximum Recovery Diluent: FBS)
- The SWA was incubated at 35±3 ºC for 6, 24, 48, and 72 hours
- At each timepoint, enumeration of surviving bacteria was performed, with n=3 for each test dressing and n=1 for the equivalent control; whereby no dressing was applied to the surface attached dual species community

Results

- **CISEB** dressing was observed to reduce the dual-species community within 24 hours with a >4 $\log_{10}$ kill observed for *K. pneumoniae* and a >6 $\log_{10}$ for MRSA from an initial challenge of ~1×10^8 CFU/mL for each separate challenge organisms present
- This kill rate was sustained for the duration of the challenge period, with **CISEB** dressing reducing the dua-species community to non-detectable levels (<30 CFU per test) by 72 hours for *K. pneumoniae* and by 48 hours for MRSA
- **PPSO** dressing demonstrated an initial reduction at 6 hours of ~2 $\log_{10}$ for both *K. pneumoniae* and MRSA. However, this dressing was unable to further reduce the population after this time, with bacterial numbers at subsequent timepoints being similar to or greater than those at 6 hours for both bacterial species
- **CESIS** dressing exhibited a slight, gradual reduction in bacterial numbers for the dual-species community over the course of the test period, reducing each challenge organism by ~2.5 $\log_{10}$ in 72 hours
- **PSS** dressing was shown to have little to no impact on the dual-species community, with levels remaining similar or greater than those recovered before dressing application, the initial growth (the T_{0hr} count)
- The no-dressing **control** demonstrated that the dual-species community remained viable throughout the test period and that species population proportionality was maintained

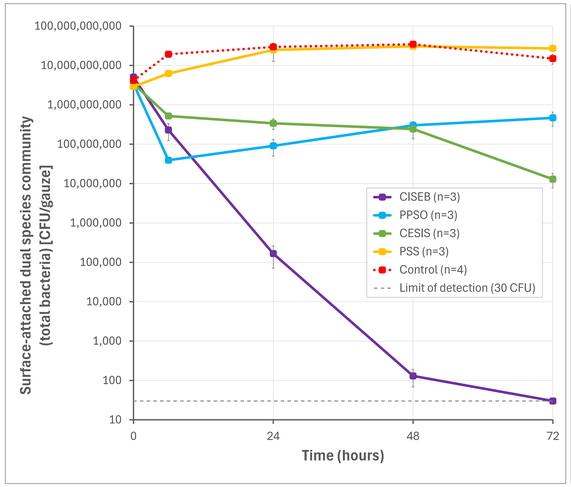

Figure 1. Antimicrobial activity of test dressings against a surface-attached dual-species community (total population) of *K. pneumoniae* and CA-MRSA over a 72-hour test period.

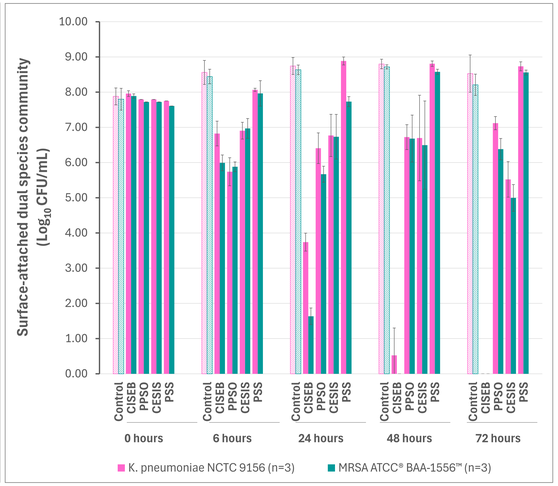

Figure 2. Antimicrobial activity of test dressings against a surface attached dual-species community (challenge organisms shown individually) of *K. pneumoniae* and CA-MRSA over a 72- hour test period. The data has been normalized by subtracting the limit of detection (30 CFU).

Discussion

- The surface-attached dual-species community considers the polymicrobial nature of microbial communities found clinically, and this study showed that not all silver-containing dressings are equally effective against these complex communities
- It is important that wound dressings contain proven additional components, that can aid in the breakdown of these surface-attached communities, as well as an antiseptic agent, to ensure the effective reduction of polymicrobial surface-attached communities.

CONCLUSION

Using a surface-attached dual-species community in a stringent *in vitro* test, it was demonstrated that not all silver-containing dressings can reduce these types of microbial communities. Only CISEB showed enhanced activity, which may be attributed to the additional agents contained within the dressing – EDTA and BEC – in addition to ionic silver

References & Footnotes

1.Malone M et al. *J Wound Care* 2017;26(1):20–25.
2.Wolcott R et al. *Clin Microbiol Infect* 2013;19(2):107–112.
3.Anju VT et al. *Antibiotics (Basel)* 2022;11(12):1731.
4.Orazi G & O'Toole GA. *J Bacteriol* 2019;202(1):e00530-19.
5.Bowler P & Parsons D. *Wound Medicine* 2016;14:6–11.

CISEB: Aquacel® Ag+ Extra™ (Convatec, UK)
PPSO: KerraContact® Ag (Crawford Healthcare, UK)
CESIS: Durafiber Ag (Smith & Nephew, UK)
PSS: UrgoClean Ag (Urgo Medical, UK)
Secondary film cover dressing: Tegaderm™ (3M Health Care, UK)

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