Eradication of an established Staphylococcus aureus biofilm with synergistic combination of an anti-biofilm and an antibiotic agent

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Abstract

Background & Aim:

Biofilms are intrinsically resistant communities surrounded by a protective extracellular polymeric substance (EPS) causing high recurrent infections.1,2 We aim to combine biofilm destabilizing agents, cellulase, and ascorbic acid with vancomycin or daptomycin in a novel strategy to eradicate established Staphylococcus Aureus biofilms.1,3

Four unique MRSA biofilms of varying strengths/stabilities were grown in tryptic soy broth with 1% dextrose, 12.5mg/mL magnesium, 25mg/mL calcium, and 6 log10CFU/mL bacterial inoculum. Biofilms were grown 20hrs in 96-well tissue culture treated polystyrene plates then gently rinsed with sterile water before 24hr lock treatment with anti-biofilm agents or antibiotics, monotherapy or in combination. Subsequently, plates were rinsed and dried overnight to fix biofilms to the well surface. Biofilms were stained with 0.1% crystal violet (CV) for 15 minutes and then rinsed before glacial acetic acid (33%) was added to resolubilize the remaining CV. BioTek plate reader at 570nm read the optical density of the remaining CV. Eradication was defined as readings of =0.09 optical density.

Methods:

Results:

Cellulase minimum biofilm eradication concentration (MBEC) was 1.5-25mg/mL, while ascorbic-acid, vancomycin and daptomycin MBECs were greater than tested concentrations. Daptomycin with 2.5% cellulase lock caused an 76% biofilm reduction. Pre-locking with 2.5% cellulase lock for 4 hours before 5mg/mL daptornycin lock caused a 86% biofilm reduction. Ascorbic acid and vancomycin did not surpass cellulase or daptomycin therapies.

Anti-biofilm pre-locking before antibiotic was more effective than exposing both agents simultaneously.

Conclusion: Daptomycin (5mg/mL) with 2.5% cellulase was the most effective biofilm eradication. Vancomycin (5mg/mL) with

2.5% ascorbic acid was the least effective

Clinical **Implications:**