A Novel Approach using Biofilm Time Kill Curves to Assess Bactericidal Activity of Daptomycin Combinations against Biofilm Producing *Enterococcus faecium* (*Efm*) and *Enterococcus faecalis* (*Efc*)

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Introduction

- Enterococci are one of the leading causes of hospital-acquired infections including medical device infections (MDIs)[1]
- Combination therapy typically employed against MDIs due to biofilm formation[2-5]
- Resistance to antimicrobials is common and increasing including vancomycin resistance[1]
- Synergistic activity has been noted with daptomycin (DAP) combinations including beta-lactams, fosfomycin (FOF), rifampin (RIF)
- Minimal data available to support these combinations against biofilm-producing enterococci

Methods

Bacterial Strains

- 4 vancomycin resistant enterococci (VRE) strains were evaluated
- 2 clinically derived, isogenic strains of *E. faecium* (R7206 and R7207)
- 2 clinical *E. faecalis* strains (R6981 and R7808)

Antimicrobials

- DAP (Cubist Pharmaceuticals, Lexington, MA, USA) was commercially purchased
- Ampicillin (AMP), ceftriaxone (CRO), FOF, RIF (Sigma Chemical Co., St. Louis, MO) were commercially purchased

Media

- Mueller Hinton Broth (MHB) supplemented to 50 mg/L of Ca^{2+} was used for susceptibility testing and *in vitro* experiments
- Brain heart infusion agar (BHIA; Difco, Detroit, MI) was used for colony enumeration

Susceptibility Testing

- Minimum inhibitory concentration (MIC) and biofilm MIC (BMIC) testing were performed in duplicate as previously described[8-9]

In Vitro Biofilm Time-Kill Experiments

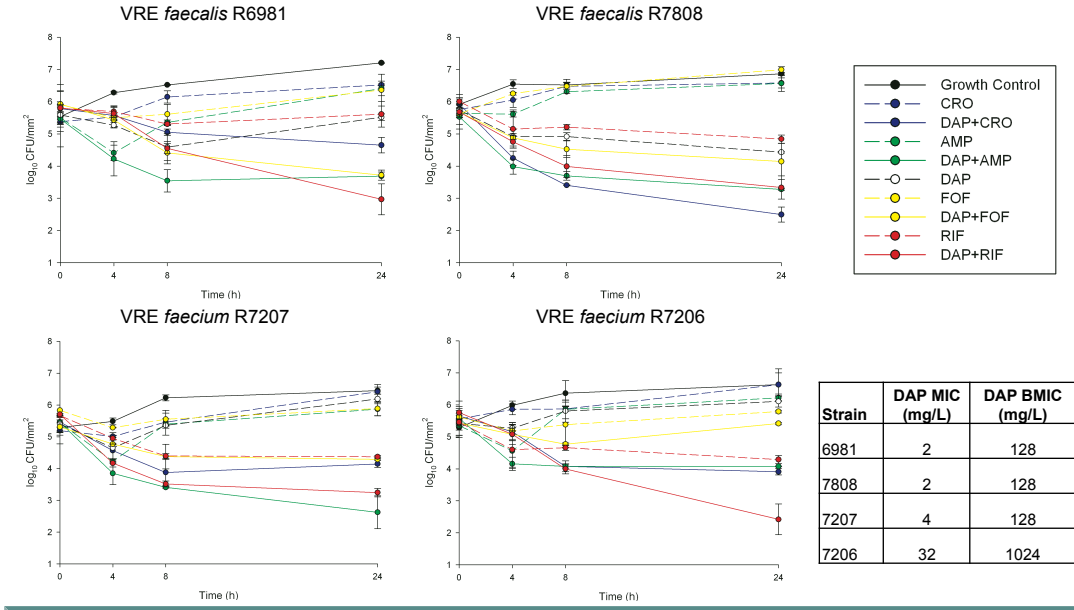
- 3mm polyurathane beads were suspended in glucosee supplemented tryptic soy broth (GSTSB)
- GSTSB was aspirated and beads placed into 24-well macrowell plates
- Target starting inoculum was ~1x10^6 CFU/cm^2
- Wells were filled with MHB and antimicrobials were added at simulations of free therapeutic drug concentrations for all agents
- Beads were removed aseptically at 0, 4, 8, and 24h and placed in normal saline
- Biofilm embedded organisms were recovered via alternating cycles of vortexing and sonication
- Recovered biofilm cells were plated on BHIA for 24h at 35°C
- Monotherapy simulations included:
  - AMP (2g), DAP (12mg/kg), CRO (2g), FOF (2g), RIF (450mg)
- Combination simulations included:
  - DAP+AMP, DAP+CRO, DAP+FOF, DAP+RIF

Experiment Definitions

- Therapeutic enhancement: ≥ 2-$\log_{10}$CFU/cm^2 reduction over the most active agent alone
- Bactericidal: ≥ 3-$\log_{10}$CFU/cm^2 reduction from the starting inoculum

This research was conducted without external financial support

Results



Strain	DAP MIC (mg/L)	DAP BMIC (mg/L)
6981	2	128
7808	2	128
7207	4	128
7206	32	1024

Conclusions

- Against VRE *faecalis*, all drug combinations displayed therapeutic enhancement
- Against VRE *faecium*, DAP+AMP, DAP+CRO displayed enhancement against both strains and DAP+RIF in one strain
- Beta-lactam + DAP combinations appear to be most effective against biofilm-producing VRE despite high DAP BMICs
- This methodology appears successful in evaluating the potential for combination therapy against biofilm-producing VRE

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