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Substantial Doses of Daptomycin and Rifampin Eradicate *S. epidermidis* Biofilm in an In Vitro Pharmacodynamic Model

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ABSTRACT

Background: The concentration of antibiotics at the site of action needed to eradicate biofilm is currently unknown. Studies have previously suggested that bacteria in biofilms are 1000-fold more resistant to antibiotics than free floating planktonic bacteria. We sought to describe high dose localized concentrations of antibiotics alone and in combination with rifampin in relation to bactericidal activity against a formed Staphylococcal biofilm.

Methods: We utilized a methicillin-resistant high biofilm-forming *S. epidermidis* strain RP62a (ATCC 35984™) over a 48-hour in vitro PD biofilm model. The Centers for Disease Control (CDC) Biofilm Reactor model was used with chromium cobalt materials to simulate an orthopedic device infection. The reactor was inoculated and underwent a 24-hr growth phase and 16-hr conditioning phase to form biofilm on the chromium cobalt coupon, and then a 48-hr PK-PD phase was run. We modeled a growth control of the isolate alone, systemic regimens of daptomycin, levofloxacin, vancomycin and rifampin, 1000x MIC concentrations of the antibiotics alone, and combination models of 1000x MIC antibiotics with 1000x MIC rifampin. Coupons with bacteria embedded in biofilm were sonicated, vortexed, and plated on Tryptic Soy Agar for colony counts. Plates were incubated up to 48hours to ensure small colony variants were counted. Bactericidal activity was defined as ≥ 3-log10 CFU/mL reduction from the initial inoculum.

Results: Systemic regimens of the antibiotics were unable to eradicate biofilm, with levofloxacin demonstrating the most effective kill. With high concentrations of antibiotics alone and in combination with rifampin, daptomycin and levofloxacin were the most effective antibiotics. Vancomycin models were not as successful in kill against biofilm and experiments had high standard deviations.

Conclusions: This study demonstrated that significantly higher concentrations of antibiotics are needed at the site of action to eradicate biofilm than what maximum systemic dosing can provide. Identifying these concentrations provides a foundation for localized antibiotic therapy and further studies are needed to elucidate these concentrations for a variety of antibiotics and biofilm-forming organisms.

*Abstract has been updated since submission

OBJECTIVE

- Describe high dose localized concentrations of antibiotics alone and in combination with rifampin in relation to bactericidal activity against Staphylococcal biofilm

METHODS

- This study utilized a methicillin-resistant high biofilm-forming *S. epidermidis* strain RP62a (ATCC 35984™)
- Minimum inhibitory concentrations (MIC) and minimum bactericidal concentrations (MBC) were identified according to Clinical and Laboratory Standards Institute (CLSI) criteria
- A CDC Biofilm Reactor was incorporated into a pharmacokinetic-pharmacodynamic (PKPD) model with chromium cobalt coupons to simulate an orthopedic device infection
- Each model run consisted of 3 phases: 1.) a 24-hr growth phase 2.) a 16-hr conditioning phase and 3.) a 48-hr PK-PD phase
- Antibiotics used included daptomycin, vancomycin, levofloxacin, and rifampin
- Experiments performed included systemic regimens of antibiotics, antibiotics at 1000x MIC, and antibiotics at 1000x MIC in combination with rifampin at 1000x MIC
- Coupons with bacteria embedded in biofilm were sonicated, vortexed, and plated on Tryptic Soy Agar for colony counts read at 24 hrs and extended to 48 hrs for slow growing small colony variants
- Bactericidal activity was defined as ≥ 3-log10 CFU/mL reduction from the initial inoculum.

RESULTS

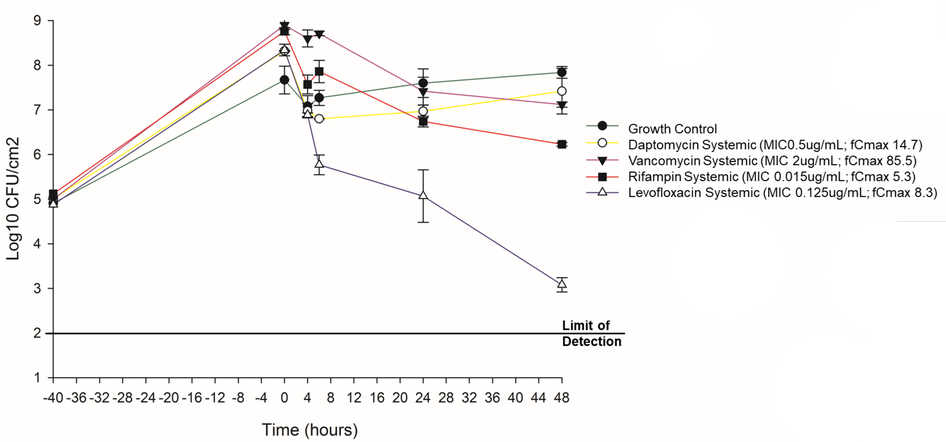

Figure 1. Systemic Regimens of Antibiotics

RESULTS

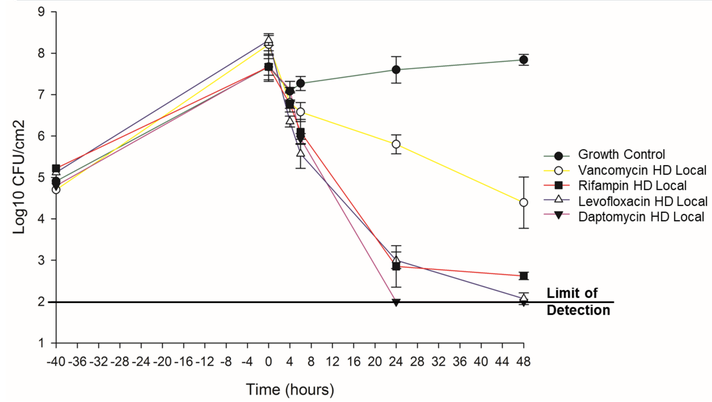

Figure 2. Antibiotic Concentrations at 1000x MIC

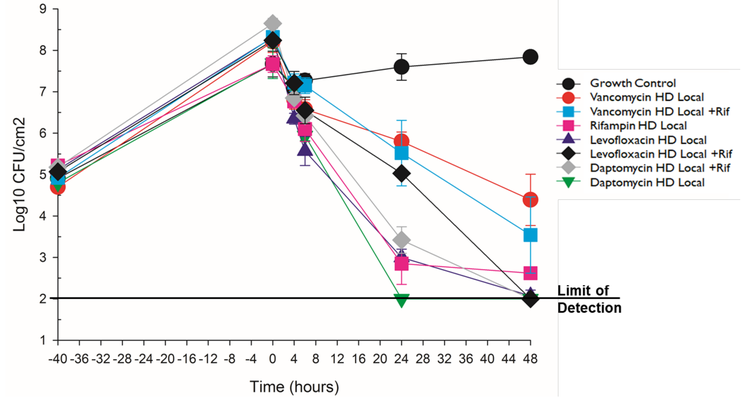

Figure 3. Antibiotic Concentrations at 1000x MIC with and without 1000x MIC Rifampin

CONCLUSIONS

- Systemic dosing of antibiotics does not provide a high enough concentration at the site of action to eradicate biofilm
- Our study demonstrated that daptomycin treatments were most effective, followed by levofloxacin. Vancomycin models were not as successful in eradicating biofilm.

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