Introduction

Background: Biofilms are sophisticated communities of matrix-encased and surface-attached bacteria that exhibit a distinct and specific resistant/survivor phenotype to almost all antibacterial agents, with activity reduced 10^-1000-fold. Interestingly, this augmented resistance rapidly reverts when bacteria detach from the biofilm and return to a planktonic state. However, in this in vitro pharmacokinetic and pharmacodynamic (PK/PD) model we are able to expose biofilms to shear rates that are consistent with human interface and mimic antibiotic penetration and dissolution pathways from serum antibiotic concentration in humans (1,2,3,4).

Motivation: Using biocompatible lipid drug delivery systems or liposomes can potentially enhance VAN efficacy by increasing the drug’s half life and decreasing nephrotoxicity.

Objective: The goal of this study is to establish a systematic biofilm eradication monitoring system using an array of antibiotics vs. biofilms of MRSA strains.

Significance: This research can lead to simultaneous screening and measurement of antibiotic efficiency in different strain phenotypes which ultimately results in more efficient treatment alternatives for biofilms.

Methods

Bacterial Strains: MRSA strains 494, N315 and ATCC 29213 were selected from our laboratory's strain collection for this research based on previous biofilm experiments in pharmacokinetic/pharmacodynamic models.

Susceptibility Testing: All MICs were determined in duplicate using microbroth dilution method at 1^-1 CFU/mL, following the Clinical and Laboratory Standards Institute (CLSI) guidelines (5). Combination MICs were performed in presence of half MIC or peak concentration of second antibiotic.

Time Kill Experiments: Time kill (TK) experiments were performed in MHB as growth media. The initial starting bacterial inoculum was 10^6 CFU/mL. All antimicrobials were tested at 0.5 x the MIC for each organism.

Media/ Antimicrobials: Tryptic Soy Broth (TSB), was used for coating the channels and running the experiments overnight. Vancomycin (VAN) and cefazolin (CFZ) were purchased commercially (Sigma Chemical Company, St. Louis, MO). Liposomal ceftarixin (LCFZ) and liposomal vancomycin (LVAN) were prepared in U-Bind lab.

Software: Bioflux Montage (Fluxion biosciences) was used for analyzing the biofilm area reduction.

Perfusion: 0.5 MacFarland of the organism is perfused for 5 at 2 dyne.

Figure 1. Time kill experiments for 29213 (right) and 494 (left). Both combinations of LVAN-LCFZ and CVAN-LCFZ were effective against these organisms.

Table 1. Minimum inhibitory constant (MIC) values for different treatments. 15.87-fold reduction in MIC is observed in combination of liposomal VAN-CFZ.

<table>
<thead>
<tr>
<th>Strain</th>
<th>VAN</th>
<th>CFZ</th>
<th>L-VAN</th>
<th>L-CFZ</th>
<th>L-VAN:LCFZ</th>
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<td>494</td>
<td>1</td>
<td>&gt;64</td>
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<td>0.083</td>
<td>0.125</td>
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<td>29213</td>
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<td>0.25</td>
<td>0.25</td>
<td>0.25</td>
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**Figure 2.** Comparison between biofilm area before (left) and after antibiotic addition (right) for combination of liposomal VAN-CFZ/LCFZ vs. combination of commercial VAN-CFZ in MRSA 494.

**Figure 3.** A,B,C,D. Biofilm eradication pattern after addition of each antibiotic. Maximum reduction was observed in treatment with liposomal VAN-CFZ vs. 494 as summarized in figure 2E. 43.8% improved biofilm reduction was observed while using liposomal VAN-CFZ vs. commercial VAN-CFZ. Liposomal VAN was 5.7x more than commercial VAN. Entrainment of VAN and CFZ in liposomal formulations protects them from degradation and increases their half life which leads to enhanced action of the drug at the site of infection.

**Table 1.** Comparison between different treatments. 15.87-fold reduction in MIC is observed in combination of liposomal VAN-CFZ.

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**Figure 2.** Comparison between biofilm area before (left) and after antibiotic addition (right) for combination of liposomal VAN-CFZ/LCFZ vs. combination of commercial VAN-CFZ in MRSA 494.

**Conclusions**

- Liposomal form of vancomycin-ceftarixin combination is a promising approach to biofilm associated infections.
- Further investigation is warranted to evaluate the efficacy of this formulation for other phenotypes.

**References**


**Disclosures**

MIR is supported in part by NIAID AI052869-01, RK, KS, SR, KS and ARL have nothing to disclose.