**Introduction**

Background: Daptomycin (DAP) is a potential alternative to β-lactam therapy for invasive S. mitis infections with β-lactam resistance or β-lactam allergy; however, the ability of these strains to rapidly develop high-level and durable DAP resistance is problematic (1). Motivation: Our group has confirmed that combination of DAP-ceftazidime has enhancing activity against two representative strains of S. mitis phenotype (pencillin-resistant S. mitis-oralis 351 and penicillin-susceptible S. mitis SF100) and prevents the emergence of resistance (DAP-R) in both strains in an in vitro model of simulated endocardial vegetation (SEV) (5). The fact that ceftaroline (CRO) is a β-lactam representative of the same class of antimicrobials as ceftazidime inspired us to evaluate the DAP-CRO combination against various S. mitis-oralis organisms. Objective: Our objective was to evaluate the ability of combined DAP with β-lactam therapy to circumvent the development of high-level and durable DAP resistance (DAP-R).

Significance: With the rising occurrence of DAP resistance in S. mitis-oralis strains there is an increased urgency for development of novel combination therapeutic strategies. Our results confirm that combination of DAP-CRO enhances killing activity and reduces the emergence of DAP resistance compared to DAP alone.

**Methods**

**Bacterial strains:** Clinical human bloodstream, DAP-susceptible, S. mitis-oralis strains, 351 and SF100, were evaluated. **Media/ Antibiotics:** DAP was obtained commercially from Merck & Co., Inc. (Whitehouse Station, NJ). ceftaroline (CRO) was purchased from Henry Ford hospital (Detroit, Michigan) Mueller-Hinton broth II (MHB, Difco, Detroit, MI) with 50 mg/L of calcium and 12.5 mg/L magnesium was used for susceptibility testing. Due to the dependency of DAP on calcium for antimicrobial activity and calcium loss from the media due to calcium binding to albumin, MHB supplemented to a concentration of 75 mg/L of calcium was used in the in vitro SEV model experiments, as previously described (4). For in vitro susceptibility testing, calcium-adjusted Muller-Hinton broth supplemented with 5% lysed horse blood was used. Trypsin soy agar supplemented with 5% sheep's blood (Difco) was used for colony growth and quantification upon subculture from the SEV models.

**Susceptibility Testing:** MICs were determined in duplicate using micro-broth dilution method at 10⁻⁵ CFU/ml following the Clinical and Laboratory Standards Institute (CLSI) guidelines (5). Combinatorial MIC values for daptomycin were determined by supplementing the broth with concentrations of β-lactam antimicrobials at their 0.5x MIC to validate the ability of the CRO to reduce the DAP MIC. PK/PD models: A simulated endocardial vegetation PK/PD model (SEV) was used to design antibiotic experiments. DAP was administered once-daily via an injection port. The simulated DAP regimens with a targeted T₁₂₅ of 8 h were 8, 10 and 12 mg/kg/dl with a peak of 93.9, 123.3, 141.1 and 183.7 mg/L, respectively. The DAP regimens were tested alone and in combination with CRO 2 g (once daily). 0.5x (one-day–day–day) at corresponding peaks of 257, 64.25 µg/L. All models were performed in duplicate to ensure reproducibility. All DAP PK samples were analyzed using high performance liquid chromatography (HPLC) and CRO PK samples were analyzed with previously reported bioassay method (3).

**Results**

<table>
<thead>
<tr>
<th>Strain/ Drug</th>
<th>DAP MIC (µg/ml)</th>
<th>CRO MIC (µg/ml)</th>
<th>Drug MIC (µg/ml)</th>
<th>DAP-CRO MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF100</td>
<td>0.5</td>
<td>0.5</td>
<td>DAP</td>
<td>0.25</td>
</tr>
</tbody>
</table>

**Figure 1.** S. mitis 351, DAP-CRO 2 g (once-a-day) TTDL at 8h (6.43±0.10 g/L CRO). DAP 6-CRO 0.5 g (one-dose CRO) TTDL at 24 h (6.96±0.10 CRO). DAPalone regimens developed DAP-R at 96h (128-fold MIC increase).

**Table 2.** MIC values (from left to right) daptomycin, ceftaroline and DAP in presence of CRO

**Conclusions**

- Combinations of DAP-low dose CRO (even single dosing) demonstrated the ability to forestall the emergence of DAP-R in S. mitis-oralis strains.
- Presence of CRO in the model prevented resistance at sub-optimal CRO dosing.
- Further research in relevant in vivo models and clinically is warranted to determine the most optimized DAP-CRO dose-regimens for the prevention of emergence of DAP-R among S. mitis-oralis strains. Based on these findings other β-lactam antibiotics should be explored at sub-optimal dosing regimens in combination with DAP.

**References**


**Disclosures**

M.R is supported in part by NIDAY RO1 AI109268-01. ASB was supported in part by NIDAY RO-1AI100356. RK, SR, KS, CG, NNM, MM, CA, TTT, and PS have nothing to disclose.