In vitro activity of daptomycin and vancomycin lock solutions on staphylococcal biofilms in a central venous catheter model

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Abstract
Background. Catheter lock solutions are used for prevention and management of catheter-related bloodstream infections. We investigated the activity of daptomycin and vancomycin lock solutions against Staphylococcus aureus and Staphylococcus epidermidis in an in vitro central venous catheter (CVC) model.

Methods. Biofilm-producing reference strains of S. aureus and S. epidermidis were evaluated. After 24 h of bacterial growth in a CVC model, daptomycin and vancomycin bactericidal activity were separately evaluated as a lock solution using 0.5, 1 and 35 mg/ml. Calcium carbonate (50 mg/l) was added to all lock solutions containing daptomycin. Each CVC was drained, flushed and sonicated at 72 h to assess CFU/ml.

Results. After 72 h of exposure in the catheter lock solutions, daptomycin and vancomycin at 0.5, 1 and 5 mg/ml demonstrated bactericidal activity (>3.0 log10 CFU/ml) against S. aureus and S. epidermidis (P ≤ 0.001). Heparin lock solution alone produced a non-significant reduction in S. aureus and S. epidermidis (1.92 ± 0.07 and 1.65 ± 0.03 log10 CFU/ml, respectively). Daptomycin 5 mg/ml lock solution +/− heparin eradicated (limit of detection 2.0 log10 CFU/ml) S. epidermidis at 72 h as did the vancomycin 5 mg/ml plus heparin. S. aureus was only eradicated from the daptomycin 5 mg/ml catheter lock-solution.

Conclusions. Our CVC model demonstrated that 72 h of exposure to 5 mg/ml lock solutions of daptomycin (plus calcium), +/− heparin or 5 mg/ml of vancomycin plus heparin demonstrate promise in treating catheter infections with biofilm-producing S. epidermidis. Similarly, 5 mg/ml of daptomycin (plus calcium) as a lock solution shows great promise in treating S. aureus catheter infections.

Keywords: biofilms; catheter lock solutions; daptomycin; Staphylococcus aureus; Staphylococcus epidermidis; vancomycin

Introduction

Biofilms are complex bacterial communities embedded in a self-producing slime. This slime is made up of bacteria in stationary phase, hydrophilic polysaccharides (glycocalyx) and minerals such as calcium which are essential to the structural integrity of the biofilm [1–4]. It is advantageous for bacteria to form stable communities of protection rather than live as free planktonic cells [5]. This protection is critical for the pathogenicity of most bacteria, including Staphylococcus aureus and S. epidermidis.

Staphylococcus aureus and S. epidermidis infections are a major problem in hospital settings, especially among patients with indwelling devices [6]. Most serious infections such as endocarditis, osteomyelitis and catheter-related infections are caused by biofilm-producing strains [7–9]. Once these bacteria colonize patients, damaged tissue and the integument surrounding indwelling devices become an ideal target site for bacterial access, development of biofilm and ensuing infection [10]. For many patients who develop catheter-related bloodstream infections (CRBSI), the device must be removed, thus, causing loss of vascular access, additional invasive procedures and added health care cost [11]. One of the biggest challenges in health care and specified goal of the Centers of Diseases and Control is the prevention of CRBSI [12].

Once staphylococcal biofilms form, they are difficult to eradicate. A number of agents have been evaluated for the prevention and disruption of biofilms, but few have proven successful [13–17]. Anti-staphylococcal agents such as linezolid and vancomycin have been
evaluated in many *in vitro* biofilm models [13,16,18]. Study results consistently demonstrate suppression of bacterial growth in a biofilm, but neither agent significantly eradicates bacterial colonization. These agents, like others, do not demonstrate significant activity in established biofilms due to either a lack of penetration, drug inactivation or due to the state of bacterial cell division within the biofilm [7,19,20]. Promising agents must penetrate into the biofilm extracellular layer and have bactericidal activity irrespective of the bacteria’s physiological state. They must also prevent further biofilm formation. Lipopeptides, such as daptomycin, display rapid bactericidal activity against staphylococci, irrespective of bacterial growth phase [21,22]. This compound also demonstrates the ability to successfully penetrate into congealed bacterial masses, such as the vegetations formed in endocardial infections [22]. Also, daptomycin is approved by the US Food and Drug Administration for the treatment of methicillin-susceptible and methicillin-resistant *S. aureus* (MSSA and MRSA, respectively) bacteremia, including right-sided endocarditis. This new indication may increase the utility of this drug for the treatment of bloodstream infections, thus allowing for combination lock therapy and intravenous therapy for blood stream infections.

We compare daptomycin and vancomycin activity using three different *in vitro* biofilm methodologies. The first assay evaluates each agent’s effectiveness in preventing biofilm formation in planktonic isolates. The second assay evaluates each agent’s activity in a pre-formed biofilm. The third methodology is an *in vitro* catheter model that evaluates each agent’s activity in a catheter lock solution by quantifying antimicrobial kill. A potential clinical finding from this *in vitro* work is the observation of daptomycin’s activity against biofilm-producing bacteria and daptomycin’s use as a 72 h catheter lock solution.

**Methods**

**Antimicrobials**

Daptomycin analytical powder was provided by Cubist Pharmaceuticals, Inc., (Lexington, MA, USA), vancomycin analytical powder was purchased from Sigma-Aldrich (St. Louis, MO, USA) and heparin sodium (10 000 U/ml with 0.1 mg/l benzyl alcohol as a preservative) was commercially obtained (Baxter Healthcare Corporation, USA). Henceforth, in this article, all uses and discussion of heparin will refer to heparin sodium plus benzyl alcohol. Stock solutions of each antibiotic were freshly prepared each week and kept frozen at −4°C.

**Test organisms**

Biofilm-producing reference strains of *S. aureus* and *S. epidermidis* (MSSA; ATCC 35556 and MRSE; ATCC 35984, respectively) and a non-biofilm forming *S. epidermidis* (ATCC 12228) control were evaluated [23].

**Culture media and growth conditions**

The medium for biofilm growth was Bacto Tryptic Soy Broth (TSB; Becton Dickinson, Sparks, MD, USA) plus 1% glucose and 2% NaCl. All cultures were incubated at 35°C unless otherwise indicated. TSB, supplemented with concentrations of physiological ionized calcium (50 mg/l; Ca-STSB) were used in all biofilm simulations (growth control, heparin sodium, daptomycin and vancomycin) due to the calcium-dependent antimicrobial activity of daptomycin [24], the unchanged activity of vancomycin in the presence of calcium [25] and to streamline all experiments regarding the effect of calcium on biofilm formation [1]. Calcium quantification was verified in all test broth solutions via the Veterans Affairs Medical Center (Providence, RI) in-house clinical laboratory. Colony counts were determined using Tryptic Soy Agar (TSA, Difco, Becton Dickinson Co., Sparks, MD, USA).

**Test organism culture**

For each *in vitro* assay and model, colonies from overnight growth on TSA were added to normal saline and adjusted to produce a 0.5 McFarland standard. This suspension was then diluted into Ca-STSB to achieve a starting inoculum of 6 log10CFU/ml.

**Susceptibility testing in planktonic organisms**

Traditional minimum inhibitory concentration (MIC) and minimal bactericidal concentrations (MBC) were determined in duplicate using methods previously described by The Clinical and Laboratory Standards Institute (CLSI) [24,26]. Colony counts for the verification of inoculum testing were determined using TSA. Daptomycin MIC determination was also conducted in the presence of lactated ringer’s solution, which provides calcium carbonate concentrations (60–130 mg/l) similar to our Ca-STSB.

**Prevention of biofilm formation in the presence of antibiotics**

The ability of daptomycin and vancomycin to inhibit the formation of biofilms was measured. Quantification of biofilm formation was conducted using a colorimetric microtitre plate assay [27]. Sterile flat-bottom 96-well polystyrene tissue culture plates (Costar no. 3596; Corning Inc., Corning, NY, USA) contained increasing concentrations of daptomycin (0–64 mg/l) or vancomycin (0–64 mg/l) dispensed in a 2-fold serial dilution. After overnight incubation, media and non-adherent planktonic bacteria were removed by gently washing the plate with sterile normal saline. The remaining attached bacteria were fixed by adding 99% methanol per well. Wells were emptied and dried before 200 µl of 2% crystal violet was added [28]. The dye bound to the adherent cells was resolubilized by adding 200 µl of 33% (vol/vol) glacial acetic acid to each well. Using a spectrophotometer (EL800, Bio-Tek Instruments, Inc, Winooski, VT), the optical density (OD) of each well was determined photometrically at 570 nm. Wells originally containing uninoculated medium and non-biofilm producing bacteria, ATCC 12228 were used as negative controls. The test was carried out in quadruplicate, results were averaged and standard deviations were calculated.
Antimicrobial susceptibility in established biofilms

All isolates were grown using a modified version of the Calgary Biofilm Device [19]. A final inoculum of approximately 6 log10 CFU/ml (as described above) was evaluated. A transferable solid-phase (TSP) pin lid was placed into a microtitre plate and incubated overnight in the rolling incubator (Shake N Bake Hybridization Oven, Boekel Scientific, Feasterville, PA), which allows bacterial suspension to gently flow across the pins. The TSP pin lid was then removed, rinsed in phosphate-buffered saline and placed into a new microtitre plate containing 2-fold serial dilutions of each antibiotic (64 mg/l as the highest concentration). Each plate was placed in the rolling incubator overnight. The minimum biofilm inhibitory concentration (MBIC) was read as the last well in which there was no visible growth. The minimal biofilm eradication concentration (MBEC) was defined as the minimal concentration of antibiotic required to eradicate the biofilm.

Antimicrobial activity in a catheter model

Daptomycin, vancomycin and heparin sodium activity in a catheter model was performed with multi-lumen central venous catheters (CVC) [Arrow-Howes CVC® (AK-15703-SP), Reading, PA] made up of polyurethane. We modified a previously described method [14].

Drug stability and compatibility

Previous studies have demonstrated compatibility and stability of vancomycin and heparin for up to 72 h at 37°C [29]. Daptomycin and heparin also demonstrated compatibility with no significant physical or chemical interaction [30]. Studies evaluating the compatibility of calcium together with heparin have not identified any physical or chemical interaction. Each solution was incubated over 72 h, and then evaluated for physical compatibility by particulate formation, colour change, or gas evolution.

Device inoculation and treatment

Test organisms in Ca-STSB were added to each lumen of the CVC access device. Each device was sealed and incubated overnight (24h). The next morning, sterile drug-containing syringes were inserted into each lumen of the access port. Regimen simulations included daptomycin or vancomycin alone (0.5, 1 and 5 mg/ml) or, daptomycin or vancomycin (5 mg/ml) plus heparin 5000 U/ml (plus benzyl alcohol 0.05 mg/l). Each regimen (sufficient to fill the access port) was slowly injected into each access port lumen. Catheters were clamped at the distal end as soon as the antimicrobial solution filled each lumen. This procedure was also repeated with separate CVC containing only heparin sodium solution (10000 U/ml preserved with 0.1 benzyl alcohol), and as a growth control. All combination experiments that contained heparin also contained the preservative 0.05 mg/ml benzyl alcohol. Each system was incubated at 35°C for an additional 72 h. Each organism was tested against each agent in triplicate.

Recovery of treated organisms

At 72 h, each clamped catheter system was removed from the incubator. A previously described and verified methodology was employed as follows [14]. The catheter fluid was collected by opening the clamps and collecting into a sterile test tube. A sterile needle was introduced into the open lumen of the access port and 3 ml of fresh broth was quickly flushed through the lumen and collected in a sterile test tube. To optimize the yield of visible bacteria, the flushed broth was then sonicated at 60 W for 1 min, and then vortexed for 15 s. Additionally a 3-cm cut piece of the catheter was sonicated at 60 W for 1 min. One hundred microlitres volume of the flushed sonicated broth and broth from the cut segment was transferred to TSA plates, serially diluted 10-fold and the plates were incubated overnight. The limit of detection for the flushed sonicated and vortexed cultures from the access port and lumen, and the sonicated broth of the catheter segment, was 2.0 log10 CFU/ml.

Data analysis

All statistical analyses were performed using SPSS statistical software (release 14; SPSS, Inc. Chicago, Ill.). After 72 h of exposure to each compound, the biofilm formation was quantified as described above, bacteria were counted and time to 99.9% kill (bactericidal activity defined as ≥3 log10CFU/ml) was compared between groups using one-way ANOVA followed by Tukey’s post-hoc test for multiple comparisons. A P-value of ≤0.05 indicates statistical significance.

Resistance

Development of resistance was evaluated at the 72-h time point for the S. aureus and S. epidermidis isolates. One hundred-μl samples taken from the catheter models were plated on TSA containing 2 mg/l of daptomycin or vancomycin to assess for the development of resistance. Plates were examined for growth after 24 and 48 h of incubation at 35°C.

Results

Susceptibility testing in planktonic organisms

MIC and MBC susceptibility results are reported in Table 1. Both S. aureus and S. epidermidis isolates were susceptible to daptomycin and vancomycin with an MIC typical of what is seen in large susceptibility studies. In the presence of lactated ringer’s solution, daptomycin MICs were the same or one dilution lower than the standard MIC results (≤0.5 mg/l).

Prevention of biofilm formation in the presence of antibiotics

The ability of daptomycin and vancomycin to inhibit the formation of biofilms was measured using a colorimetric microtitre plate assay. The results of the assay are displayed in Figure 1. The biofilms produced by S. epidermidis and S. aureus declined with increasing
concentrations of daptomycin and vancomycin. At daptomycin concentrations \( \geq 1 \text{ mg/l} \) or concentrations that exceeded the MICs, no formed biofilms were observed. However, at concentration at or below the MIC (\( \leq 0.5 \text{ mg/l} \)), daptomycin decreased biofilm formation in \textit{S. epidermidis} by 45–55\% from the positive control (no drug). Daptomycin had no effect on \textit{S. aureus} biofilm at concentrations less than the MIC (\( \leq 0.5 \text{ mg/l} \)). Vancomycin inhibited biofilm mass at concentrations \( \geq 4 \text{ mg/l} \) and \( \geq 1 \text{ mg/l} \) for \textit{S. epidermidis} and \textit{S. aureus}, respectively. The non-biofilm forming \textit{S. epidermidis} negative control isolate did not produce a biofilm (\( \text{OD}_{570} = 0.01 \pm 0.023 \)).

\textbf{Antimicrobial susceptibility in established biofilms}

All isolates and drug activity were evaluated with a modified version of the Calgary Biofilm Device that uses a TSP pin lid [19]. Results are displayed in Table 1.

<table>
<thead>
<tr>
<th>Drug</th>
<th>\textit{S. epidermidis} (ATCC35984)</th>
<th>\textit{S. aureus} (ATCC35556)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Planktonic organisms</td>
<td>Presence of biofilm</td>
</tr>
<tr>
<td></td>
<td>MIC (mg/l)</td>
<td>MBC (mg/l)</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

MBIC, minimum biofilm inhibitory concentration; MBEC, minimum biofilm eradication concentration.
where they are compared against planktonic growing bacteria. Antimicrobial activity in an established 24 h biofilm was increased by 3–4 dilutions for daptomycin (0.5–4 mg/l for *S. epidermidis* and 0.25–4 mg/l for *S. aureus*) and 1–2 dilutions for vancomycin (2–4 mg/l for *S. epidermidis* and 1–4 mg/l for *S. aureus*). MICs for the non-biofilm forming *S. epidermidis* were 0.5 mg/l and remained 0.5 mg/l after testing in the pin-lid biofilm assay. The MBIC and the MBEC for daptomycin was typically 4–6 dilutions higher then the MIC, whereas the MBEC for vancomycin was 2–4 dilutions higher than the MIC (*S. epidermidis* and *S. aureus*, respectively).

**Antimicrobial activity in a catheter model**

Results obtained after a 72 h incubation of a lock solution containing daptomycin or vancomycin was evaluated quantitatively. These results are depicted in Table 2 and Figure 2. Daptomycin (plus calcium) concentrations of 0.5, 1 and 5 mg/ml resulted in bactericidal activity (>3.0 log10 CFU/ml) against *S. epidermidis* and *S. aureus* after 72 h (*P* ≤ 0.001).

Additionally, daptomycin (plus calcium) 5 mg/ml alone, or in the presence of preservative-containing heparin, eradicated *S. epidermidis* from the model at 72 h. *S. aureus* was eliminated from the 5 mg/ml daptomycin (plus calcium) lock solution with a 5.02 log10 CFU/ml decrease from initial inoculum. Vancomycin alone, at 0.5, 1 and 5 mg/ml, resulted in bactericidal activity against both *S. epidermidis* and *S. aureus* (*P* ≤ 0.001). At 5 mg/ml of vancomycin, in addition to heparin, *S. epidermidis* was eradicated from the model. Heparin lock solution alone produced a 1.92 ± 0.07 and 1.65 ± 0.03 log10 CFU/ml reduction against *S. aureus* and *S. epidermidis*, respectively. This reduction was not significant. Our limit of detection for bacterial eradication was 2.0 log10 CFU/ml.

**Drug stability and compatibility**

Each of the three combinations, vancomycin and heparin; daptomycin calcium; and daptomycin calcium and heparin were evaluated after 72 h of incubation at 35°C. All mixtures demonstrated physical

### Table 2. Activity of daptomycin and vancomycin (mg/ml) in an antibiotic lock model at 72 h after catheters were flushed, cut and sonicated

<table>
<thead>
<tr>
<th>Inoculum</th>
<th>Growth (log10 CFU/ml) at 72 h</th>
<th>Drug (mg/ml)</th>
<th>Vancomycin</th>
<th>Vancomycin plus heparin</th>
<th>Daptomycin</th>
<th>Daptomycin plus heparin</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Growth control</td>
<td>Heparin 10 000 U/ml</td>
<td>Daptomycin</td>
<td>plus heparin</td>
<td>plus heparin</td>
<td>Daptomycin</td>
</tr>
<tr>
<td><em>S. epidermidis</em>; ATCC 35984</td>
<td>7.13 ± 0.05</td>
<td>7.58 ± 0.10</td>
<td>5.48 ± 0.02*</td>
<td>0.5</td>
<td>3.79 ± 0.13*</td>
<td>2.89 ± 0.71*</td>
</tr>
<tr>
<td></td>
<td>7.51 ± 0.05</td>
<td>7.34 ± 0.14</td>
<td>5</td>
<td>2.89 ± 0.70*</td>
<td>No Growth*</td>
<td>3.97 ± 0.39*</td>
</tr>
<tr>
<td></td>
<td>7.92 ± 0.28</td>
<td>7.79 ± 0.39</td>
<td>5</td>
<td>2.89 ± 0.70*</td>
<td>No Growth*</td>
<td>3.85 ± 0.21*</td>
</tr>
<tr>
<td><em>S. aureus</em>; ATCC 35556</td>
<td>7.02 ± 0.08</td>
<td>7.92 ± 0.28</td>
<td>5.11 ± 0.49*</td>
<td>0.5</td>
<td>3.85 ± 0.31*</td>
<td>2.99 ± 1.02*</td>
</tr>
<tr>
<td></td>
<td>7.79 ± 0.39</td>
<td>7.79 ± 0.38</td>
<td>5</td>
<td>2.9 ± 0.71*</td>
<td>No Growth*</td>
<td>2.99 ± 1.02*</td>
</tr>
</tbody>
</table>

No growth; limit of detection, 2.0 log10 CFU/ml.

*10 000 U/ml heparin sodium plus 0.1 mg/l benzyl alcohol as a preservative.

*5000 U/ml heparin sodium (0.05 mg/l benzyl alcohol as a preservative).

*Daptomycin plus 50 mg/l of calcium carbonate.

*P* ≤ 0.001 when compared with inoculum at 0 h.

### Fig. 2. Activity of daptomycin (plus calcium carbonate 50 mg/l) or vancomycin at initial, 0 h inoculum and after a 72 h catheter lock solution of 0, 0.5, 1 and 5 mg/ml on biofilm-producing a) *S. epidermidis* (ATCC 35984) and b) *S. aureus* (ATCC 35556). Solid horizontal black bar indicates the 2.0 log10 CFU/ml limit of detection.
compatibility. There was no visible haze, particulate formation, colour change, or gas evolution at 72 h.

Detection of resistance

No resistance or increase in MIC was detected in either the vancomycin or daptomycin models.

Discussion

It is estimated that there are 2400–20,000 annual deaths in the United States’ intensive care unit’s (ICU) that are associated with central venous CRBSIs. The hospital cost attributable to these CRBSIs ranges from $296 million to $2.3 billion dollars annually [31]. Hospitalized patients and patients who receive haemodialysis are at greatest risk of developing a CRBSI caused by S. epidermidis and S. aureus [6]. When a CRBSI is suspected and pathogen identification is confirmed, systemic antimicrobial therapy should be initiated and antimicrobial lock therapy must be considered if the line cannot be removed. Recently, daptomycin was approved by the US Food and Drug Administration for the treatment of MSSA and MRSA, respectively, bacteraemia, including right-sided endocarditis. This new indication may increase the utility of this drug for the treatment of bloodstream infections, thus allowing for combination lock therapy and intravenous therapy for CRBSIs. Identifying catheter lock solutions that can inhibit, eradicate or prevent staphylococcal biofilm, would be a welcome addition to the management of CRBSIs.

Previous studies have evaluated the efficacy of vancomycin and daptomycin in experimental foreign body infections and in catheter lock systems. When evaluating the literature on catheter lock solutions, major concerns are the vast difference between methodologies, dwell time of the lock solution, drug concentrations used and the impact of preservative-containing solutions such as heparin. In addition, some investigators did not make note of the biofilm-producing capabilities of the clinical isolates tested. It was our intent to simulate a clinically meaningful setting, namely the management of haemodialysis intravascular catheter infections. We selected this in vitro modelling system by using drug concentrations and lock times that are commonly used in this clinical setting.

Vaudaux et al. [32] evaluated the activity of daptomycin and vancomycin in a rat model of subcutaneously implanted tissue cages infected with a clinical S. aureus isolate obtained from a patient with catheter-related sepsis. Staphylococcus aureus biofilm formation was not measured. After 7 days of therapy with daptomycin or vancomycin, mean bacterial counts (CFU/ml) significantly decreased compared with implanted tissue cages of untreated animals, but there was no significant difference between the daptomycin and vancomycin groups. Lee et al. [33] investigated several antimicrobial agents to determine an optimal concentration and duration for antibiotic lock solutions to effectively treat catheter-related infections caused by biofilm-producing S. epidermidis and S. aureus. Antimicrobial doses of 1, 5 and 10 mg/ml were evaluated with dwell times of 1–14 days. An antibiotic lock with vancomycin (5 mg/ml, refreshed every 48 h) cleared S. epidermidis and S. aureus from the in vitro model after a 5-day dwell time. These findings are consistent with our results.

Sherertz et al. [34] evaluated vancomycin and several other agents against S. aureus in silicone Hickman catheter segments. Biofilm formation was not noted. Physiologically attainable vancomycin concentrations of 20 mg/l and 3 mg/ml were evaluated. Vancomycin did not demonstrate significant kill after a 24 h dwell time.

A study by Giacometti et al. [35] investigated vancomycin in a staphylococcal CVC infection rat model. The MIC and MBC of vancomycin and other agents against the adherent bacteria were at least 4-fold higher than those against freely growing cells. We demonstrated similar results with daptomycin and vancomycin. In these experiments, a vancomycin lock solution (1.024 mg/ml) was allowed to dwell in the catheters 1 h a day for 7 days. There was no significant reduction of bacterial concentration within the catheter model system during this time. The results of these two studies varied from our results; however, this may reflect our use of a prolonged dwell time and higher drug concentrations.

In our study, three different methodologies were evaluated to further understand the ability of daptomycin and vancomycin to prevent, inhibit or eradicate staphylococcal biofilms. The first method evaluated the prevention of biofilm mass. We observed inhibition of S. epidermidis and S. aureus biofilm mass with daptomycin concentrations of ≥1 mg/l whereas, vancomycin inhibited the formation of biofilm mass at concentrations of ≥1 and ≥4 mg/l (S. aureus and S. epidermidis, respectively). It is noteworthy to mention that these concentrations exceed the CLSI recommended breakpoints for susceptibility. However, since breakpoints are discriminatory concentrations used to predict susceptibility at drug levels obtained with systemic therapy, extrapolating MIC breakpoints to antimicrobial use in catheter-lock solutions is difficult.

The second methodology explored antimicrobial susceptibility in an established biofilm and results were compared against planktonic growing bacteria. Antimicrobial activity in an established 24 h biofilm was increased by 3–4 dilutions for daptomycin and 1–2 dilutions for vancomycin when compared with the MICs of planktonic growing bacteria.

Finally, in an in vitro catheter model, the activity of daptomycin and vancomycin alone and at concentrations of 5 mg/ml, in the presence of preservative-containing heparin sodium was studied against biofilm-producing S. epidermidis and S. aureus in a 72 h lock solution. At a concentration of 5 mg/ml, daptomycin (plus calcium) with or without heparin eradicated S. epidermidis from the model (limit of
solution (60–130 mg/l calcium carbonate) to may be reconstituted in lactated ringer’s (mg/ml), typically exceed MIC concentrations (mg/l) patients who appear to be failing antibiotic lock isolates should be carefully monitored, especially in Daptomycin and vancomycin lock solutions in a central venous catheter model 2245 S. aureus the causative pathogen is we tested should be extrapolated with caution if solutions with the concentrations and dwell times S. aureus S. epidermidis heparin alone (data not published). It is currently unknown why vancomycin’s activity on S. epidermidis was enhanced in the presence of heparin, but daptomycin’s activity against S. aureus was not. However, the enhancement of activity between daptomycin alone and daptomycin plus heparin was not significant (difference of 1.26 log10 CFU/ml) and therefore these noted differences maybe negligible.

We conclude that 0.5, 1 and 5 mg/ml of daptomycin (plus calcium) and vancomycin lock solutions demonstrate bactericidal activity against the biofilm-producing S. aureus and S. epidermidis isolates evaluated in our model. Daptomycin 5 mg/ml lock solution alone or in combination with heparin was able to eradicate biofilm-forming S. epidermidis from our catheter model. Staphylococcus aureus was eliminated from the catheter by using 5 mg/ml of daptomycin. Catheter lock solutions containing 5 mg/ml of either daptomycin (with or without preservative containing heparin), or vancomycin with preservative-containing heparin and dwell times of 72 h, show promise for treating biofilm-producing catheter infections caused by S. epidermidis and S. aureus. Use of antibiotic lock solutions with the concentrations and dwell times we tested should be extrapolated with caution if the causative pathogen is S. aureus and eradication is the goal of such therapy. Both daptomycin and vancomycin resistance has been described with S. aureus and S. epidermidis and susceptibility of isolates should be carefully monitored, especially in patients who appear to be failing antibiotic lock therapy with either of these two agents [37–41]. However the concentrations used in lock therapy (mg/ml), typically exceed MIC concentrations (mg/l) by 1000-fold.

For pharmacists to prepare a lock solution, daptomycin may be reconstituted in lactated ringer’s solution (60–130 mg/l calcium carbonate) to approximate calcium concentrations used in our in vitro daptomycin studies.

Our results are consistent with previous clinical studies and other in vitro and animal models. A limitation of this study is the use of single S. aureus and S. epidermidis reference isolates and extrapolation to clinical settings should be done with caution. In addition, we cannot conclude that our results will hold true with antibiotic lock durations less than or greater than 72 h.

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Conflict of interest statement. This work was funded by an unrestricted investigator initiated grant from Cubist Pharmaceuticals, Lexington, MA.

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