Objective: The objective of this study was to assess the efficacy of humanized cefazolin tissue concentrations against methicillin-susceptible Staphylococcus aureus (MSSA) and Enterobacteriaceae in an in vitro pharmacodynamic model.

Methods: Nine clinical isolates [five MSSA (cefazolin MIC range 0.5 – 2.0 mg/L), two Escherichia coli (cefazolin MICs 1.0 and 2.0 mg/L) and two Klebsiella pneumoniae (cefazolin MICs of 1.0 and 2.0 mg/L)] were evaluated with a starting inoculum (0 h) of 10^6 cfu/mL. Time–kill curves were built and the area under the bacterial killing and regrowth curve (AUBC) was calculated.

Results: The starting inoculum had a mean ± SD of 6.3 ± 0.28 log10 cfu/mL. Cefazolin human simulated targets for peak, trough and half-life were 13.0 mg/L, 2.6 mg/L and 2.6 h, respectively. Control isolates grew to 8.5 ± 0.2 log10 cfu/mL. Against MSSA, cefazolin achieved a reduction from 0 h of 2.18 ± 0.67 and 2.38 ± 1.24 log10 cfu/mL, at 4 and 24 h, respectively. Cefazolin achieved a reduction in bacterial density of 2.34 ± 0.35 and 2.68 ± 0.99 log10 cfu/mL at 4 and 24 h, respectively, when tested against Enterobacteriaceae. No significant difference was observed when comparing AUBC based on MIC values. The rate of initial bacterial reduction of Enterobacteriaceae was rapid, with a decrease of >3 log10 cfu/mL by 4 h, while MSSA exhibited a gradual reduction in bacterial density over one dosing interval.

Conclusions: The observed antibacterial effects of cefazolin support its continued utility against susceptible S. aureus, E. coli and K. pneumoniae in skin and skin structure infections.

Keywords: Staphylococcus aureus, in vitro, Enterobacteriaceae, antibiotic exposures, lower limb infections
Materials and methods

Bacterial strains

Nine clinical isolates (five MSSA isolates, two E. coli isolates and two K. pneumoniae isolates) were selected for use in this study. The MIC for all organisms was determined a minimum of two times by the broth microdilution method in accordance with the CLSI. In addition to cefazolin, MICs of ceftaroline, ceftriaxone, daptomycin, oxacillin, tigecycline and vancomycin for MSSA were also determined. MICs of cefazolin, cefepime, ceftaroline, ceftriaxone, ciprofloxacin, piperacillin/tazobactam and tigecycline for Enterobacteriaceae were determined.

Antibiotics

Cefazolin (Apotex Corp., Weston, FL, USA; lot 101D026; expiry date, March 2015) was obtained from the Department of Pharmacy at Hartford Hospital.

Simulated drug exposures

Tissue concentrations for cefazolin were based on patient pharmacokinetic data generated by Bhalodi et al. In brief, an in vivo microdialysis technique was used to measure interstitial concentrations of cefazolin in seven patients, each with a chronic lower limb infection. This technique provided continuous measurements over a dosing interval, describing the antimicrobial exposures in these patients with compromised lower limb vasculature. These human data were simulated in an IVPD model with a peak tissue concentration of 13 mg/L and a half-life of 2.6 h, resulting in a tissue trough concentration of 2.6 mg/L.

In vitro pharmacodynamic model

A one-compartment in vitro model was used for all experiments. Each experiment consisted of three independent models (two experimental treatment models and one growth control model), which ran concurrently for each isolate. To achieve uniform temperatures of 37°C, the models were stationed in a temperature-modulated circulating water bath. All experiments were performed with a starting (0 h) inoculum of 10⁶ cfu/mL. Inoculum preparation included making a bacterial suspension of 10⁶ cfu/mL from subcultures of the test isolate (incubated at 37°C for 24 h) with 0.9% normal saline for injection. Three millilitres of inoculum were injected into each 300 mL model to achieve the starting inoculum of 10⁶ cfu/mL. Cation-adjusted Mueller–Hinton broth (CAMHB) (Becton, Dickinson and Company, Sparks, MD, USA) was used as the bacterial growth medium for all experiments. Once inoculated, the bacteria were allowed to enter the log phase of growth over 30 min. Cefazolin was then administered as a bolus into the model (0 h) to simulate a steady-state trough of free drug concentration achieved in human lower limb tissue after intravenous doses of 1 g of cefazolin every 8 h. Following this initial bolus, cefazolin was infused at the beginning of each dosing interval, using a peristaltic pump (Masterflex L/S Model 7524-10; Cole-Parmer, USA), into the model at a pre-determined rate. Each experiment was conducted over 24 h and performed in duplicate to assure reproducibility.

Bacterial density over time was determined by sampling from each model at pre-determined timepoints (0, 1, 2, 4, 6, 8, 10, 12, 16, 18, 20 and 24 h) and serial dilution in normal saline. Aliquots of diluted sample were plated for quantitative culture utilizing tryptose soy agar plates (100 mm diameter) with 5% sheep blood. Colony counts were read after 16–20 h of incubation at 37°C. The lower limit of detection for bacterial density was 1.7 log₁₀ cfu/mL. To evaluate the antibiotic activity of the regimen of 1 g of cefazolin every 8 h, bacterial density was measured by the change in log₁₀ cfu/mL from 0 h at 4 and 24 h. Bactericidal activity was defined as a reduction in bacterial density of at least 3 log₁₀ cfu/mL. Time–kill curves were constructed and the area under the bacterial killing and regrowth curve (AUBC) was calculated to assess antibiotic efficacy.

Antibiotic concentration and exposure determinations

Samples of CAMHB broth taken from each of the treatment models were assayed for cefazolin at 0, 2, 4, 6, 8, 10, 16, 18 and 24 h. All samples were immediately stored at –80°C until analysis. Cefazolin was analysed by a validated HPLC method at the Center for Anti-Infective Research and Development, as described previously. For the assay, the lower and upper detection limits were 0.5 and 30 mg/L, respectively. The interday coefficient of variation (CV) for the cefazolin check samples of 0.8 and 20 mg/mL was 5.9% and 5.1%, respectively (n = 22), while the intraday CV was 2.1% and 3.1% (n = 10), respectively. The cefazolin assay was linear over a concentration range of 0.5–30 mg/L. Based on achieved concentrations in each model, the peak and trough concentrations were reported as those observed by analysis and the half-life was calculated as 0.693/k, where k is the estimated elimination rate constant calculated as the natural log (ln) of the measured peak/trough divided by its change in time. The AUBC over each 24 h period (log₁₀ cfu/h/mL) was calculated using a model-specific growth control for comparison.

Statistical analysis

Differences in AUBC at 24 h within MSSA and Enterobacteriaceae, based on MIC values, were assessed by one-way ANOVA or by the t-test where applicable. In addition, mean bacterial reductions at 4 and 24 h were compared between MSSA and Enterobacteriaceae using the t-test. An a priori P value < 0.05 was considered statistically significant.

Results

MIC determination

The MICs of cefazolin for MSSA F 2-13, MSSA F 10-29, MSSA F 44-14, MSSA F 19-13 and MSSA F 18-8 were 0.5, 0.5, 1, 1 and 2 mg/L, respectively. The MICs of cefazolin for the Enterobacteriaceae isolates E. coli AZ 18-25, E. coli AZ 6-17, K. pneumoniae JJ 7-10 and K. pneumoniae JJ 7-33 were 1, 1, 2, 1 and 2 mg/L, respectively. Phenotypic profiles of all isolates used are shown in Table 1.

Pharmacokinetic analysis

Pharmacokinetic parameters (peak, trough and half-life) observed in the models were 13.68 ± 2.66 mg/L, 3.34 ± 1.03 mg/L and 3.02 ± 0.08 h, respectively. A comparison between observed patient pharmacokinetics and observed model pharmacokinetics can be seen in Figure 1. These resulted in a mean cefazolin %T > MIC of 100% for all MSSA and Enterobacteriaceae isolates, as would be expected in the clinical setting when 1 g of cefazolin every 8 h is administered.

Antibacterial activity

MSSA isolates

The average bacterial density of the starting inoculum was 6.42 ± 0.25 log₁₀ cfu/mL. Control isolates grew to 8.44 ± 0.19 log₁₀ cfu/mL over 24 h. Figure 2 summarizes the time–kill curves for cefazolin against all MSSA isolates. The mean changes in bacterial density at 4 and 24 h against the five MSSA isolates...
are provided in Table 2. MSSA isolates exhibited a significantly slower progression of bacterial reduction by 4 h (P < 0.0001) when compared with the rapid reductions observed with Enterobacteriaceae. No significant differences in AUBC reductions were seen, regardless of MIC (P > 0.05). The AUBCs over 24 h for cefazolin are also summarized in Table 2.

Enterobacteriaceae isolates

The average bacterial density of the starting inoculum was 6.08 ± 0.21 log_{10} cfu/mL. Control isolates grew to 8.74 ± 0.07 log_{10} cfu/mL over 24 h in the models. Figure 3 summarizes the time–kill curves for cefazolin against all Enterobacteriaceae isolates. The mean changes in bacterial density at 4 and 24 h against the four Enterobacteriaceae isolates are provided in Table 2. When comparing 24 h bacterial reductions observed with MSSA and Enterobacteriaceae, no statistically significant difference was found (P > 0.05). As with MSSA, Enterobacteriaceae showed no significant difference in AUBC, regardless of MIC (P > 0.05). The AUBCs over 24 h for cefazolin are also summarized in Table 2.

Discussion

With today’s focus on emerging pathogens with various resistances to many mainstay regimens, it is imperative that clinicians optimize antimicrobials pharmacodynamically whenever

<table>
<thead>
<tr>
<th>Isolate</th>
<th>CFZ</th>
<th>OXA</th>
<th>TZP</th>
<th>FEP</th>
<th>CRO</th>
<th>CPT</th>
<th>VAN</th>
<th>CIP</th>
<th>DAP</th>
<th>TGC</th>
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<td>ND</td>
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<td>0.25</td>
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</tr>
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<td>ND</td>
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<td>0.25</td>
<td>0.125</td>
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<td>0.5</td>
<td>0.25</td>
</tr>
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<td>ND</td>
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<td>1</td>
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<td>0.125</td>
</tr>
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<td>2</td>
<td>&lt;0.06</td>
<td>&lt;0.25</td>
<td>&lt;0.06</td>
<td>ND</td>
<td>&lt;0.015</td>
<td>ND</td>
<td>0.125</td>
</tr>
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<td>2</td>
<td>&lt;0.06</td>
<td>&lt;0.25</td>
<td>&lt;0.06</td>
<td>ND</td>
<td>0.03</td>
<td>ND</td>
<td>0.5</td>
</tr>
<tr>
<td>E. coli AZ 6-17</td>
<td>2</td>
<td>ND</td>
<td>2</td>
<td>&lt;0.06</td>
<td>&lt;0.25</td>
<td>&lt;0.06</td>
<td>ND</td>
<td>&lt;0.015</td>
<td>ND</td>
<td>0.125</td>
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<tr>
<td>K. pneumoniae JJ 7-33</td>
<td>2</td>
<td>ND</td>
<td>1</td>
<td>&lt;0.06</td>
<td>&lt;0.25</td>
<td>&lt;0.06</td>
<td>ND</td>
<td>0.03</td>
<td>ND</td>
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</tr>
</tbody>
</table>

CFZ, cefazolin; OXA, oxacillin; TZP, piperacillin/tazobactam; FEP, cefepime; CRO, ceftriaxone; CPT, ceftaroline; VAN, vancomycin; CIP, ciprofloxacin; DAP, daptomycin; TGC, tigecycline; ND, not determined.

This table shows the reported modal MIC (mg/L) unless otherwise noted.

*Median.
In this study, five MSSA clinical isolates with cefazolin MICs in the range of 0.5–2 mg/L were used. It is important to note that these isolates are on the upper end of the MIC distribution, with MIC₅₀ and MIC₉₀ being 0.5 and 1 mg/L, respectively. The %T>MIC of 100 was achieved for all isolates, exceeding the required pharmacodynamic target of 30%–40% of the dosing interval. As expected, on average cefazolin exhibited bactericidal activity against MSSA. The changes in bacterial density at 4 and 24 h were observed in order to depict the rate of kill for both MSSA and Enterobacteriaceae. A slow progression of killing was observed across all MSSA models regardless of MIC, indicated by less than half of its overall bacterial density reductions occurring at 4 h. However, after 24 h of simulating a regimen consisting of 1 g of cefazolin every 8 h, the mean bacterial density was reduced by >3 log₁₀ cfu/mL. As the only MSSA isolate with an MIC of 2 mg/L, F 18-8 exhibited a slight rebound in growth, resulting in a 24 h bacterial reduction of ~1 log₁₀ cfu/mL less than the mean stated above. These simulated cefazolin tissue concentrations seem to provide more than adequate antimicrobial exposures for all the tested MSSA isolates. It would be expected that patients being treated with monotherapy of 1 g of cefazolin every 8 h, under these pharmacokinetic and vasculature conditions, would reach pharmacodynamic targets against MSSA.

Additional isolates were tested to represent commonly involved Gram-negative pathogens in ABSSSIs. Four clinical isolates of Enterobacteriaceae (two E. coli isolates and two K. pneumoniae isolates) with cefazolin MICs of 1 mg/L and at the susceptible breakpoint of 2 mg/L were tested. As with MSSA, all Enterobacteriaceae isolates had a %T>MIC that exceeded current pharmacodynamic targets. It is important to note that, when treating Gram-negative pathogens with cefazolin, the %T>MIC is required to be higher (60%–70%) than when treating Gram-positive pathogens (30%–40%). Bacterial reductions were observed with all isolates. No differences in bacterial reductions were seen between isolates when comparing MICs. All Gram-negative isolates exhibited a rapid rate of killing that was characterized by reductions of >3 log₁₀ cfu/mL in bacterial

Figure 3. Mean bacterial densities over 24 h for Enterobacteriaceae isolates. Data are plotted as the means of the multiple models for the treatments and the mean of all corresponding growth control isolates. The lower limit of detection (broken line) was 1.7 log₁₀ cfu/mL.

Table 2. Mean change in bacterial density at 4 and 24 h and AUBC from 0 to 24 h for cefazolin against all isolates

<table>
<thead>
<tr>
<th>Isolate</th>
<th>MIC (mg/L)</th>
<th>Change in bacterial density (log₁₀ cfu/mL) from 0 h</th>
<th>AUBC (log₁₀ cfu·h/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>4 h</td>
<td>24 h</td>
</tr>
<tr>
<td>MSSA F 2-13</td>
<td>0.5</td>
<td>−2.07</td>
<td>−4.24</td>
</tr>
<tr>
<td>MSSA F 10-29</td>
<td>0.5</td>
<td>−0.71</td>
<td>−4.28</td>
</tr>
<tr>
<td>MSSA F 44-14</td>
<td>1</td>
<td>−0.95</td>
<td>−3.03</td>
</tr>
<tr>
<td>MSSA F 19-13</td>
<td>1</td>
<td>−0.01</td>
<td>−4.66</td>
</tr>
<tr>
<td>MSSA F 18-8</td>
<td>2</td>
<td>−1.51</td>
<td>−2.64</td>
</tr>
<tr>
<td>Mean±SDc</td>
<td></td>
<td>−1.18±0.67</td>
<td>−3.58±1.24</td>
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<tr>
<td>E. coli AZ 18-25</td>
<td>1</td>
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<td>K. pneumoniae JJ 7-10</td>
<td>1</td>
<td>−3.17</td>
<td>−2.84</td>
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<td>E. coli AZ 6-17</td>
<td>2</td>
<td>−3.97</td>
<td>−3.85</td>
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<td>K. pneumoniae JJ 7-33</td>
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<td>Mean±SDc</td>
<td></td>
<td>−3.45±0.35</td>
<td>−2.68±0.99</td>
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</table>

aValues for each isolate are the means of two to five independent time–kill experiments.
bData are presented as mean±SD of all time–kill curves (two to five per isolate). Negative numbers indicate reductions in cfu from 0 h.

dMean stated above. These simulated cefazolin tissue concentrations seem to provide more than adequate antimicrobial exposures for all the tested MSSA isolates. It would be expected that patients being treated with monotherapy of 1 g of cefazolin every 8 h, under these pharmacokinetic and vasculature conditions, would reach pharmacodynamic targets against MSSA.

Possible. Cefazolin is commonly used to treat ABSSSIs, caused by MSSA. Antibiotics can vary in their ability to achieve effective concentrations in foot lesions due to specific drug pharmacokinetics and patients’ vascular permeabilities. As new tissue concentration data are generated, a correlation between concentrations at the site of infection and efficacy against clinical isolates should be assessed. Here we set out to assess the antibacterial effects of human simulated tissue concentrations following 1 g of cefazolin every 8 h against both MSSA and Enterobacteriaceae.

In this study, five MSSA clinical isolates with cefazolin MICs in the range of 0.5–2 mg/L were used. It is important to note that these isolates are on the upper end of the MIC distribution, with MIC₅₀ and MIC₉₀ being 0.5 and 1 mg/L, respectively. The %T>MIC of 100 was achieved for all isolates, exceeding the required pharmacodynamic target of 30%–40% of the dosing interval. As expected, on average cefazolin exhibited bactericidal activity against MSSA. The changes in bacterial density at 4 and 24 h were observed in order to depict the rate of kill for both MSSA and Enterobacteriaceae. A slow progression of killing was observed across all MSSA models regardless of MIC, indicated by less than half of its overall bacterial density reductions occurring at 4 h. However, after 24 h of simulating a regimen consisting of 1 g of cefazolin every 8 h, the mean bacterial density was reduced by >3 log₁₀ cfu/mL. As the only MSSA isolate with an MIC of 2 mg/L, F 18-8 exhibited a slight rebound in growth, resulting in a 24 h bacterial reduction of ~1 log₁₀ cfu/mL less than the mean stated above. These simulated cefazolin tissue concentrations seem to provide more than adequate antimicrobial exposures for all the tested MSSA isolates. It would be expected that patients being treated with monotherapy of 1 g of cefazolin every 8 h, under these pharmacokinetic and vasculature conditions, would reach pharmacodynamic targets against MSSA.

Additional isolates were tested to represent commonly involved Gram-negative pathogens in ABSSSIs. Four clinical isolates of Enterobacteriaceae (two E. coli isolates and two K. pneumoniae isolates) with cefazolin MICs of 1 mg/L and at the susceptible breakpoint of 2 mg/L were tested. As with MSSA, all Enterobacteriaceae isolates had a %T>MIC that exceeded current pharmacodynamic targets. It is important to note that, when treating Gram-negative pathogens with cefazolin, the %T>MIC is required to be higher (60%–70%) than when treating Gram-positive pathogens (30%–40%). Bacterial reductions were observed with all isolates. No differences in bacterial reductions were seen between isolates when comparing MICs. All Gram-negative isolates exhibited a rapid rate of killing that was characterized by reductions of >3 log₁₀ cfu/mL in bacterial
density 4 h after initiation of cefazolin. This was in contrast with the observations made with MSSA isolates, but the exact reason for this difference is unknown. Some concentration-dependent killing was observed in the models, characterized by repeated increasing and decreasing bacterial densities around the simulated troughs and peaks, respectively. Overall, this observation did not seem to grossly affect the sustained bacterial reductions at 24 h. Historically, bacterial reductions observed at 24 h have been predictive of efficacy. That being said, additional studies may be warranted to assess the activity of cefazolin over an extended period of time.

Nonetheless, these data confirm that, in a patient with intact immunity, it can be expected that 1 g of cefazolin every 8 h would be sufficient to treat a lower limb infection when exhibiting the described tissue concentrations. A study by Brill et al. evaluated 2 g of cefazolin intravenously in obese and non-obese patients undergoing laparoscopic Toupet fundoplication surgery. The authors observed decreased tissue concentrations following the administration of a one-time dose of 2 g of cefazolin. While it is hard to apply the observations in that study to our observations, given the stark differences in study design, it appears that higher doses may be needed in morbidly obese patients (BMI >40) in the setting of organisms with MICs approaching the breakpoint.

In this study, we evaluated the pharmacodynamic tissue concentration profile resulting from the administration of 1 g of cefazolin every 8 h against susceptible isolates of S. aureus, E. coli and K. pneumoniae. When simulating the tissue concentrations observed in infected patients, sustained bacterial reductions were observed over 24 h, with rapid bactericidal activity observed against Gram-negative isolates and progressive, sustained kill of S. aureus. These data support the clinical utility of the regimen of 1 g of cefazolin every 8 h in patients with lower limb infections due to susceptible staphylococci, E. coli and K. pneumoniae.

Acknowledgements
We thank Christina Sutherland for conducting cefazolin HPLC analysis. Additionally we thank Pam Tessier and Henry Christensen for their help with the in vitro experimentation.

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Transparency declarations
None to declare.

References