Pharmacokinetics-Pharmacodynamics of Tazobactam in Combination with Cefepime in an In Vitro Infection Model

Running title: PK-PD of Tazobactam

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ABSTRACT (word count = 250)

We previously demonstrated that for tazobactam administered in combination with ceftolozane, the pharmacokinetic-pharmacodynamic (PK-PD) index that best described tazobactam efficacy was the percentage of the dosing interval that tazobactam concentrations were above a threshold (%T>threshold). Using data from studies of Enterobacteriaceae-producing ESBL, a relationship between tazobactam %T>threshold and reduction in log$_{10}$ CFU from baseline, for which tazobactam threshold concentration was the product of the isolate’s ceftolozane-tazobactam MIC value and 0.5, was identified. However, since the kinetics of cephalosporin hydrolysis vary among ESBLs and compounds, it is likely that the translational relationship to derive the tazobactam threshold concentration varies among enzymes and compounds. Using a one-compartment in vitro infection model, the PK-PD of tazobactam administered in combination with cefepime was characterized and a translational relationship across ESBL-producing Enterobacteriaceae was developed. Four clinical isolates, two Escherichia coli and two Klebsiella pneumoniae, known to produce CTX-M-15 β-lactamase enzymes and displaying cefepime MIC values of 2 to 4 mg/L in the presence of 4 mg/L tazobactam, were evaluated. Tazobactam threshold concentrations from 0.0625-1 times the tazobactam-potentiated cefepime MIC value were considered. The threshold that best described the relationship between tazobactam %T>threshold and change in log$_{10}$ CFU from baseline was the product of 0.125 and the cefepime-tazobactam MIC ($R^2=0.813$). The magnitude of %T>threshold associated with net bacterial stasis and a 1-log$_{10}$ CFU/mL
reduction from baseline at 24 hours was 21.9 and 52.8%, respectively. These
data will be useful to support the identification of tazobactam dosing regimens in
combination with cefepime for evaluation in future clinical studies.
INTRODUCTION

Due to the increasing prevalence of β-lactamase-producing Enterobacteriaceae, there is renewed interest in β-lactam-β-lactamase inhibitor combinations. This interest encompasses combinations involving new cephalosporins or inhibitors and new combinations of old agents. Tazobactam is a penicillanic acid sulfone β-lactamase inhibitor that has been used in combination with piperacillin for over two decades [1]. Tazobactam was approved by the United States Food and Drug Administration for clinical use with the anti-pseudomonal cephalosporin ceftolozane in 2014 [2]. Cefepime-tazobactam combinations, with an 8:1 ratio, are available from multiple manufacturers in India, but are not licensed elsewhere [3].

Tazobactam extends the spectrum of ceftolozane activity to include many extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Like ceftolozane, cefepime is unstable in the presence of many ESBL enzymes but has greater inherent stability to Enterobacterial AmpC enzymes [4]. Administering tazobactam in combination with cefepime increases the agent’s spectrum of activity to include many ESBL-producing Enterobacteriaceae [5, 6].

Recently, we demonstrated that the percentage of the dosing interval that tazobactam concentrations remained above a threshold (%T>threshold) was the pharmacokinetic-pharmacodynamic (PK-PD) measure best associated with efficacy for tazobactam when administered in combination with ceftolozane [7].
The tazobactam threshold concentration that allowed co-modeling of Enterobacteriaceae producing various ESBL enzymes was the product of the individual isolate's ceftolozane-tazobactam MIC value and 0.5 [8]. However, since the kinetics of β-lactam hydrolysis, maximum reaction velocity ($V_{\text{max}}$) and Michaelis-Menten Constant ($K_m$), varies among compounds and enzymes [9], it is likely that the tazobactam threshold concentration and translational relationships will vary among cephalosporins. Another source of potential variation is the permeation rates of the cephalosporin relative to the inhibitor, as cefepime is said to rapidly permeate cellular membranes [10].

Using a one-compartment in vitro infection model, the objectives of this study were two-fold. The first objective was to confirm that $%T>\text{threshold}$ described the PK-PD of tazobactam when administered with cefepime. The second objective was to identify a tazobactam threshold concentration that would allow co-modeling of isolates and thereby, identify candidate tazobactam dosing regimens for future combination with cefepime.
METHODS

Bacteria, antimicrobial, and \( \beta \)-lactamase inhibitor. Cefepime and tazobactam were obtained from Toronto Research Chemicals (Toronto, Canada) and ACS Dobfar (Tribiano MI, Italy), respectively.

The challenge panel of four clinical Enterobacteriaceae isolates with CTX-M-15 ESBLs was obtained from JMI laboratories (North Liberty, Iowa, USA). The panel was comprised of two \textit{Klebsiella pneumoniae} and two \textit{Escherichia coli} isolates chosen based upon cefepime MIC value when assayed with tazobactam (4 mg/L).

Media and \textit{in vitro} susceptibility studies. Susceptibility studies were conducted in accordance with Clinical and Laboratory Standards Institute guidelines [11] using cation-adjusted Mueller-Hinton broth (BD laboratories, Franklin Lakes, New Jersey, USA) in a broth micro-dilution method. Isolate susceptibility to cefepime was determined alone and in combination with a fixed tazobactam concentration (4 mg/L). All susceptibility studies were conducted in triplicate over a 2-day period.

Whole genome sequencing, epidemiology typing and resistance genes. Total genomic DNA, extracted using QIAmp genomic DNA kit (Qiagen, Hilden, Germany), was used to prepare paired end True-Seq library and cluster generation. Samples were sequenced using Illumina\textsuperscript{\textregistered} MiSeq instrument.
Sequences were aligned into multiple contigs using Lasergene NGen Denovo assembly protocol (DNASTar, Madison, WI). The ResFinder web server (www.genomicepidemiology.org) was used to identify acquired antimicrobial resistance genes in the respective assembled genomes, using a threshold of 98.0% identity. Assembled genomes were also utilized for determining the multilocus sequence typing (MLST) and analyzing the sequences of protein membrane genes (OmpC, OmpF and OmpK37), \textit{ampC} (in \textit{E. coli}) and \textit{acrA}. DNA and protein analysis was performed using the Lasergene® software package (DNASTar; Madison, Wisconsin). Amino acid sequences obtained were compared to those of \textit{E. coli} ATCC 25922 and \textit{K. pneumoniae} ATCC 13833. One additional clinical isolate of each species was also used as control strains and both isolates exhibited a cefepime-tazobactam MIC results of 1 mg/L.

\textbf{Determination of transcription levels of the intrinsic AmpC (\textit{E. coli} only), AcrA efflux pump and outer membrane protein genes.} The transcription levels of \textit{ampC}, \textit{acrA}, and \textit{ompC}, \textit{ompF} and \textit{ompK37} were determined by comparing the transcription levels of these selected targets to those from control isolates (cefepime-tazobactam MIC, 1 mg/L). Total genomic RNA samples were extracted from each isolate using the RNeasy Mini Kit in a fully-automated robotic workstation (Quiacube; Qiagen, Valencia, CA) and residual DNA was eliminated with RNase-free DNase (Promega, Madison, WI). Sample quality and quantification of the genomic RNA were assessed using the Agilent 21000
Bioanalyzer utilizing the RNA 6000 Nano Kit according to manufacturer instructions (Agilent, Santa Clara, CA). Reverse-transcription PCR was performed in triplicate using QuantiTect SYBR Green RT-PCR Kit (Qiagen, Germantown, MD) in the StepOne Plus instrument (Lie Technologies, Foster City, CA).

One-compartment *in vitro* infection model and sample processing. The one-compartment *in vitro* infection model utilized in these studies has been described previously [12]. Briefly, the model consists of a central infection compartment containing growth medium, the challenge isolate and magnetic stir bars to ensure the homogeneity of drug concentrations and ensure even dispersion of bacteria within the compartment. This central infection compartment was attached to a stir plate and the entire unit was placed within a temperature and humidity controlled incubator set at 35°C. Drug-free growth medium was pumped into the central infection compartment via a computer-controlled peristaltic pump while growth medium was simultaneously removed through an exit port and captured in a waste container. The challenge isolates were aseptically inoculated into the central infection compartment, and the peristaltic rate of diffusion was set at a flow rate that allowed for the simulation of human concentration-time profiles for the drug(s) under study. The test compounds were infused via computer controlled syringe pumps, allowing simulation of the desired half-lives, dosing frequencies, and concentrations. Samples for CFU determination and drug concentration assay were collected from the central infection compartment using...
a sterile syringe and needle through a rubber septum at pre-determined time-points. 

The initial inoculum was 1.0 x 10^6 CFU/mL of the challenge isolate, prepared from a culture grown overnight on trypticase soy agar supplemented with 5% lysed sheep blood (BD Laboratories, Franklin Lakes, New Jersey). Isolates were taken from these overnight cultures and grown to mid-logarithmic phase in a flask of Mueller-Hinton broth set in a shaking water bath at 35°C and 125 rotations per minute. The bacterial concentration was determined by optical density referenced against previously-confirmed growth curves for each challenge isolate.

After inoculation into the one-compartment in vitro infection model, bacteria were exposed to changing concentrations of cefepime and tazobactam simulating human half-lives of 2 hours for cefepime [13] and 1 hour for tazobactam [14].

One milliliter samples were collected for CFU determination at 0, 2, 4, 6, 8, 12, and 24 hours. Each sample was centrifuged, washed, and re-suspended with sterile normal saline twice to prevent drug carryover and was then cultured on trypticase soy agar enriched with 5% sheep blood, as well as Mueller-Hinton agar infused with cefepime at four times the potentiated MIC value and tazobactam at a fixed concentration of 4 mg/L. Plated samples were incubated at 35°C for 24 hours and colonies were counted for enumeration of bacterial density. A few colonies were collected from the drug-containing agar plates to...
survey for any decrease in sensitivity to cefepime-tazobactam. One milliliter
samples were collected from the growth compartment for drug assay at 1, 3, 5, 7,
9, 11, 13, and 24 hours, sterile-filtered, then immediately frozen at -80°C until
assayed for drug concentration.

Mutation frequency studies. The mutation frequency to drug resistance was
estimated by plating 4 mL of log-phase growth suspension containing an average
concentration of 6.17 x 10^8 CFU/ml onto agar containing four times the baseline
cefepime MIC value with a fixed 4 mg/L of tazobactam. The bacterial density in
the suspension was determined by quantitative culture, and the ratio of colonies
on the drug-containing plates to that of the starting inoculum provided an
estimate of the drug resistance frequency within a total population. The assay
was performed in duplicate and a subset of isolates from each trial were taken
from the drug-containing plates and re-tested by standard MIC methodology to
confirm decreased susceptibility.

Dose-ranging studies. Duplicate dose-ranging studies were conducted in order
to determine the dose-response relationship for each challenge isolate. In these
studies, a fixed cefepime dose of either a 1 or 2 g was administered either alone
or in combination with tazobactam, using an every 8 hour (q8h) schedule. The
modelled tazobactam doses ranged from 8 to 4,000 mg following the same q8h
schedule. Both cefepime and tazobactam were administered over a 1 hour
infusion.
Analytical method. All samples were assayed by liquid chromatography-tandem mass spectrometry LC/MS/MS (Waters Xevo TQ-S, Milford MA) and drug levels were quantitated using external standardization. Standard curves ranged from 0.500 to 200 mg/L for cefepime and from 0.0100 to 20.0 mg/L for tazobactam. The standard curves were linear over their respective ranges ($r^2=0.974$ and 0.988 or greater) for cefepime and tazobactam, respectively. The lower limit of quantification was 0.500 mg/L for cefepime and 0.0100 mg/L for tazobactam. The intra-assay percent coefficient of variation (%CV) for cefepime quality control samples at concentrations of 5.0, 25.0, and 100 mg/L was 15.2% or less. The intra-assay %CV for tazobactam quality control samples at concentrations of 0.05, 0.5, and 5.0 mg/L was 7.04% or less. Inter-assay %CVs for the cefepime quality control samples at concentrations of 5.00, 25.0, and 100 mg/L were 8.70% or less and 5.83 % or less for tazobactam quality control samples at 0.0500, 0.500, and 5.00 mg/L. Diluted quality control samples for tazobactam (100 mg/L) exhibited a inter-assay %CV of 5.24% or less and an intra-assay %CV of 6.19% or less in runs which required dilution of samples into the calibration curve range.

Pharmacokinetic-pharmacodynamic analysis. Data from the dose-ranging studies were evaluated using Hill-type models and non-linear least squares regression. The data were weighted using the inverse of the estimated measurement variance. Relationships between change in log$_{10}$ CFU at 24 hours
and tazobactam %T>threshold were evaluated. Tazobactam %T>threshold was identified through an iterative process in which candidate tazobactam threshold concentrations, representing the product of the tazobactam-potentiated cefepime MIC value and 1, 0.5, 0.25, 0.125, and 0.0625 for each individual isolate, were evaluated. Discrimination among tazobactam threshold concentrations was based on the evaluation of the dispersion of data along the %T>threshold axis and optimization of $r^2$ values for the relationship between change in log$_{10}$ CFU at 24 hours and tazobactam %T>threshold.
RESULTS

In vitro susceptibility testing, whole genome sequencing, epidemiology typing and determination of transcription levels of intrinsic AmpC, AcrA, and outer membrane protein levels. Table 1 shows the β-lactamase enzyme(s) identified within the challenge panel and the MIC values for cefepime and tazobactam alone and combined with 4 mg/L of tazobactam. All four isolates carried CTX-M-15, and it was the sole enzyme in *E. coli* 30854. The remaining isolates produced additional enzymes including OXA-1/30, TEM-1 and SHV-1 and -28. MIC values ≥ 256 mg/L for tazobactam and ≥ 32 mg/L for cefepime alone were recorded for all four isolates. When cefepime was studied in combination with 4 mg/L tazobactam, the MIC values of the isolate panel ranged from 2 to 4 mg/L, representing the high end of the susceptible range for cefepime against Enterobacteriaceae [11].

An array of resistance determinants were detected, especially in isolates 39930 and 25021 as shown in Table 1. Higher expression levels of OmpC (47- to 62-fold more than the control strain) were observed in both *E. coli*. Moreover, *E. coli* 30854 also expressed the intrinsic AmpC gene 17-fold more than the control strain. In addition, *K. pneumoniae* 604 and 25021 showed lower expression levels (approximately a third) of OmpK37 when compared with that of a control strain.
Sequence analysis of genes of interest demonstrated that all isolates included in the study had several alterations in the OmpK36 (OmpC analogue of *E. coli*)-encoding gene. Several alteration, deletions and insertions were noted, including an insertion in the L5 region of OmpC in both *E. coli*, and mutations and six amino acid deletions in L5 and L6, respectively in both *K. pneumoniae* isolates. Other genes investigated (*ampC, acrA, OmpF* and OmpK37) showed sequences similar to the control isolates.

**Pharmacokinetics.** The targeted cefepime and tazobactam pharmacokinetic profiles were well-simulated in the *in vitro* infection model for all studied dosing regimens. **Figure 1** shows the relationship between observed and targeted drug concentrations. As evidenced by the high coefficient of determination values (cefepime, $R^2=0.967$; tazobactam, $R^2=0.991$), there was excellent precision but with a modest tendency to underpredict concentrations, as evidenced by the slope values (cefepime, 14.18%; tazobactam, 13.53%).

Drug concentrations for cefepime and tazobactam were each fit to a one-compartment model with zero-order input and first-order elimination. The pharmacokinetic data for each agent were well described by this model.

**Mutation frequency studies.** The mean densities the of drug-resistant subpopulation observed at four times the baseline cefepime-tazobactam MIC for
each challenge isolate are presented in Table 2; these frequencies ranged from $1.3 \times 10^{-7}$ to $3.5 \times 10^{-8}$ CFU.

Dose-ranging studies. Bacteria in the no-treatment control arms grew well in each case, reaching a density exceeding $1.0 \times 10^{8}$ CFU/mL by 12 hours (Figure 2A-D). The cefepime and tazobactam monotherapy control regimens behaved as expected: i.e., the tazobactam arms performed similarly to the no-treatment control arms and the cefepime arms provided some initial cell kill but with full regrowth by 24 hours.

The range of tazobactam doses used in combination with the fixed cefepime dosing regimens provided a full spectrum of drug effects for each challenge isolate. For example, the low-intensity cefepime (1 g)-tazobactam (8 mg) regimen behaved similarly to cefepime (1 g) alone while intermediate-intensity cefepime (1 g)-tazobactam (15.6 to 125 mg) dosing regimens resulted in net bacterial stasis at the 24 hour time point (Figure 2A) and the, high-intensity cefepime (1 g)-tazobactam (250-500 mg) regimens achieved slightly more than a 1 log$_{10}$ CFU/mL reduction from baseline (Figure 2A).

Drug-resistant isolates were observed for all controls, including the cefepime and tazobactam monotherapy dosing regimens. Drug-resistant isolates were also observed for three of the cefepime-tazobactam dosing regimens and typically occurred in low-intensity tazobactam regimens (8 to 250 mg) (Figure 2A-D). The
MIC values of the isolates collected from the drug-containing plates from the mutation frequency studies and dose-ranging studies were determined and are presented in Table 2.

**Pharmacokinetic-pharmacodynamic analysis.** The relationships between change in log_{10} CFU over 24h and the tazobactam %T>threshold for tazobactam threshold concentrations from 0.0625- to 1-times the cefepime-tazobactam MIC are presented in Figure 3. The coefficient of determination (r^2) and scatter of data about the fitted function across the X-axis were most optimal for the Hill functions describing the relationships between the change in log_{10} CFU/mL from baseline and the product of the cefepime-tazobactam MIC and either 0.0625 or 0.125. Emphasis was placed on a tazobactam threshold concentration of 0.125 times the cefepime-tazobactam MIC. The basis for the focus on this threshold was the modestly better scatter of data across the range of tazobactam %T>thresholds than that based on the data using the tazobactam threshold concentration of 0.0625 times the MIC, without less apparent clustering of data points at the lower (0) and upper (100) margins of the range. The %T>thresholds based on the tazobactam threshold concentration of 0.125 times the cefepime-tazobactam MIC associated with net bacterial stasis and a 1-log_{10} CFU reduction in bacterial burden at 24 hours were 21.9 and 52.8%, respectively. The parameter estimates (standard errors) for the relationship between change in log_{10} CFU and tazobactam %T>threshold were E_0 2.69 (0.22), E_{max} 12 (38.3), Hill’s constant 0.48 (0.65), and EC_{50} 277.51 (3241.81).
Discussion

The objectives of these studies were two-fold. The first was to use a one-compartment \textit{in vitro} infection model to confirm that \%T$>\text{threshold}$ described the PK-PD of tazobactam when administered in combination with cefepime. The second was to identify a tazobactam threshold concentration that would allow co-modeling across isolates, and using this, \%T$>\text{threshold}$ targets that could be used to identify candidate tazobactam dosing regimens in combination with cefepime for future study.

We confirmed that the PK-PD index associated with efficacy for tazobactam against Enterobacteriaceae was \%T$>\text{threshold}$ when tazobactam administered in combination with cefepime. These findings are consistent with those for tazobactam paired with ceftolozane \cite{3}. The two tazobactam threshold concentrations that allowed the entire challenge panel to be co-modeled with the most optimal fit of the model to the data were those based on the product of the cefepime-tazobactam MIC and either 0.0625 or 0.125. For reasons described above, 0.125 x cefepime-tazobactam MIC was considered the more optimal threshold. This multiple is significantly lower than the 0.5 x MIC previously identified for ceftolozane-tazobactam \cite{8}. Moreover, the \%T$>\text{threshold}$ tazobactam concentration associated with net bacterial stasis and a $1-\log_{10}$ CFU reduction from baseline were lower for cefepime-tazobactam (net bacterial stasis, 21.9; $1-\log_{10}$ CFU reduction from baseline, 52.8) than for ceftolozane-tazobactam (net bacterial stasis, 65.9; $1-\log_{10}$ CFU reduction from baseline, 77.3) \cite{8}. The
tazobactam doses administered q8h that correspond to the % T > MIC\(^*0.125\) required to achieve net bacterial stasis and a 1-\(\log_{10}\) CFU reduction from baseline were 31.25 to 62.5 mg and 125 to 250 mg, respectively, for isolates with potentiated MICs of 2 and 4 mg/L. It is worthwhile to note that one isolate, *K. pneumoniae* 604, was common to the studies described herein and those previously-conducted for ceftolozane-tazobactam [8].

When the results of both sets of evaluations are considered, these data imply that a lower tazobactam exposure was required for a given level of drug effect when tazobactam was combined with cefepime rather than ceftolozane. Possible explanations, which are not mutually exclusive, are that cefepime and ceftolozane may differ in the following ways: (i) in their lability to the ESBLs represented; (ii) in their affinity for these enzymes, which determines the extent to which they may outcompete the inhibitor for enzyme binding; and (iii) in their relative acylation and deacylation rates, which may determine the extent that the enzyme is held in a form invulnerable to attack by tazobactam or relative permeation rate into the bacterial periplasm.

There are two limitations of the studies described herein that deserve comment. The first limitation is that the one-compartment *in vitro* infection model utilized for these studies does not account for the effect of an immune system and is conducted using Mueller-Hinton broth media that optimizes bacterial growth. The impact of the former is that the magnitude of the %T>threshold for tazobactam
may be overestimated. The second limitation is that the duration of the studies carried out was 24 hours. This duration of the study was insufficient to evaluate the effect of intensity and duration of therapy of each tazobactam dosing regimen on the amplification of pre-existing drug-resistant bacterial subpopulations. The impact of the limited duration of the experiment is that the magnitude of the %T>threshold for tazobactam may in fact be underestimated. Additional studies utilizing immunocompetent and immunosuppressed animal infection and hollow-fiber in vitro infection models will be needed to address these limitations.

In conclusion, we confirmed that the PK-PD index associated with tazobactam efficacy when administered in combination with cefepime was %T>threshold. This finding was consistent with the PK-PD index associated with tazobactam efficacy when administered in combination with ceftolozane. Through this evaluation, we also identified a tazobactam threshold concentration, which was the product of the cefepime-tazobactam potentiated MIC and 0.125. The use of this threshold allowed for data from the entire challenge panel to be co-modeled with the most optimal fit of the model to the data. These data will be useful to identify candidate tazobactam dosing regimens to be administered in combination with cefepime for future study.
ACKNOWLEDGEMENTS

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D.T. is currently an employee and a shareholder of GlaxoSmithKline. S.T. was an employee at the time the research was conducted.

REFERENCES


Table 1. Susceptibility testing results, identified β-lactamase enzymes, and transcription levels of outer membrane proteins and AmpC expression for the Enterobacteriaceae panel

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Identified β-lactamase enzyme(s)</th>
<th>Omp transcription level&lt;sup&gt;a&lt;/sup&gt;</th>
<th>AmpC transcription level&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Microbroth MIC values&lt;sup&gt;b&lt;/sup&gt; (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>E. coli 30854</td>
<td>CTX-M-15</td>
<td>OmpC (47)</td>
<td>AmpC (17)</td>
<td>512 256 2</td>
</tr>
<tr>
<td>E. coli 39930</td>
<td>CTX-M-15, TEM-1, OXA-1/30, SHV-28</td>
<td>OmpC (62)</td>
<td>AmpC (1)</td>
<td>256 128 4</td>
</tr>
<tr>
<td>K. pneumoniae 25021</td>
<td>CTX-M-15, TEM-1, OXA-2</td>
<td>OmpK (-33)</td>
<td>ND</td>
<td>512 32 4</td>
</tr>
<tr>
<td>K. pneumoniae 604</td>
<td>CTX-M-15, OXA-1/30, SHV-1</td>
<td>OmpK (-33)</td>
<td>ND</td>
<td>512 &gt;512 4</td>
</tr>
</tbody>
</table>

<sup>a</sup> Represented as fold increases from that of control isolates which had tazobactam-potentiated cefepime MIC values of 1 mg/L using a fixed 4 mg/L concentration of tazobactam.

<sup>b</sup> All MIC values shown represent modal values.

Omp=Outer membrane protein; FEP=Cefepime; TAZ=Tazobactam; ND = Not Determined
Table 2. Mutation frequencies and MIC values for mutants collected from the drug-containing plates utilized in the dose-ranging efficacy and mutation frequency studies.

<table>
<thead>
<tr>
<th>Isolate</th>
<th>Baseline cefepime-tazobactam MIC</th>
<th>Geometric mean of cefepime-tazobactam mutation frequency</th>
<th>Cefepime/ tazobactam MIC for isolates taken from drug-containing plate</th>
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</thead>
<tbody>
<tr>
<td>E. coli 30854</td>
<td>2</td>
<td>1.3 x 10⁻⁷</td>
<td>8 to 32</td>
</tr>
<tr>
<td>E. coli 39930</td>
<td>4</td>
<td>9.4 x 10⁻⁷</td>
<td>32 to 64</td>
</tr>
<tr>
<td>K. pneumoniae 25021</td>
<td>4</td>
<td>1.8 x 10⁻⁷</td>
<td>16 to 128</td>
</tr>
<tr>
<td>K. pneumoniae 604</td>
<td>4</td>
<td>3.5 x 10⁻⁸</td>
<td>8</td>
</tr>
</tbody>
</table>

a. All MIC values shown, as mg/L, represent modal values determined using broth microdilution and tazobactam at a fixed 4 mg/L concentration.
Figure 1. The relationships between the observed and targeted PK profiles simulated over the 24 hour in vitro experiment for cefepime (A) and tazobactam (B)
Figure 2. Averaged dose-ranging study results for the four Enterobacteriaceae isolates examined (A. E. coli 30854, B. E. coli 39930, C. K. pneumoniae 25021, D. K. pneumoniae 604). The data series with black outlines represent regimens found to contain a drug-resistant sub-population on or before the 24-hour time point. FEP=Cefepime, TAZ=tazobactam.
Figure 3. Relationship between change in log$_{10}$ CFU after 24 hours of exposure and tazobactam %T$>$threshold based on data from a one-compartment in vitro infection model. The tazobactam threshold concentrations evaluated represent the product of each individual isolates cefepime-tazobactam MIC and either 1, 0.5, 0.25, or 0.125. Different colors represent each of the four different isolates examined.