Combinatorial Pharmacodynamics of Ceftolozane-Tazobactam against Genotypically Defined β-Lactamase-Producing *Escherichia coli*: Insights into the Pharmacokinetics/Pharmacodynamics of β-Lactam–β-Lactamase Inhibitor Combinations

Rachel L. Soon,a,b Justin R. Lenhard,a,b Zackery P. Bulman,a,b Patricia N. Holden,a,b Pamela Kelchlin,a Judith N. Steenbergen,a,* Lawrence V. Friedrich,c Alan Forrest,a,b Patricia N. Holden,a,b Patricia Kelchlin,a Judith N. Steenbergen,a,* Brian T. Tsuji,a,b

Laboratory for Antimicrobial Pharmacodynamics, New York State Center of Excellence in Bioinformatics & Life Sciences,a and School of Pharmacy and Pharmaceutical Sciences,b University at Buffalo, Buffalo, New York, USA; Merck & Co., Lexington, Massachusetts, USA;

Despite a dearth of new agents currently being developed to combat multidrug-resistant Gram-negative pathogens, the combination of ceftolozane and tazobactam was recently approved by the Food and Drug Administration to treat complicated intra-abdominal and urinary tract infections. To characterize the activity of the combination product, time-kill studies were conducted against 4 strains of *Escherichia coli* that differed in the type of β-lactamase they expressed. The four investigational strains included 2805 (no β-lactamase), 2890 (AmpC β-lactamase), 2842 (CMY-10 β-lactamase), and 2807 (CTX-M-15 β-lactamase), with MICs to ceftolozane of 0.25, 4, 8, and >128 mg/liter with no tazobactam, and MICs of 0.25, 1, 4, and 8 mg/liter with 4 mg/liter tazobactam, respectively. All four strains were exposed to a 6 by 5 array of ceftolozane (0, 1, 4, 16, 64, and 256 mg/liter) and tazobactam (0, 1, 4, 16, and 64 mg/liter) over 48 h using starting inocula of $10^6$ and $10^8$ CFU/ml. While ceftolozane-tazobactam achieved bactericidal activity against all 4 strains, the concentrations of ceftolozane and tazobactam required for a ≥3-log reduction varied between the two starting inocula and the 4 strains. At both inocula, the Hill plots ($R > 0.882$) of ceftolozane revealed significantly higher 50% effective concentrations (EC$_{50}$) at tazobactam concentrations of ≤4 mg/liter than those at concentrations of ≥16 mg/liter ($P < 0.01$). Moreover, the EC$_{50}$ at $10^6$ CFU/ml were 2.81 to 66.5 times greater than the EC$_{50}$ at $10^9$ CFU/ml (median, 10.7-fold increase; $P = 0.002$). These promising results indicate that ceftolozane-tazobactam achieves bactericidal activity against a wide range of β-lactam–producing *E. coli* strains.

The rising prevalence of multidrug-resistant (MDR) Gram-negative organisms has forced urgent efforts to expand the therapeutic armamentarium against these problematic pathogens. Recent endeavors to develop new cephalosporin compounds exhibiting promising antipseudomonal activity led to the discovery of ceftolozane (ceftolozane, previously designated CXA-101 or FR264205) (1). This novel agent has been credited with enhanced stability to popular chromosomally mediated cephalosporin resistance mechanisms (including hyperexpression of AmpC β-lactamase enzymes and efflux pumps) (2), with a low propensity for cross-resistance to the two cephalosporin and the 4 strains. At both inocula, 

The resulting ceftolozane-tazobactam combination was found to display remarkable activity against a range of MDR Gram-negative species (7) and was subsequently approved by the Food and Drug Administration to treat complicated intra-abdominal and urinary tract infections in adults (9).

Although the activity of ceftolozane-tazobactam against *E. coli* has been characterized in prior *in vitro* studies (10–12), a systematic analysis of the combination’s performance across strains producing different β-lactamases has yet to be investigated at multiple levels of bacterial burden. Therefore, integrating antimicrobial pharmacokinetics (PK) and pharmacodynamics (PD) to effectively evaluate the bacteriologic response to ceftolozane-tazobactam is warranted (13). Our objective was to utilize time-kill studies to characterize the bacterial killing effects of ceftolozane...
and tazobactam alone and in combination against different β-lactamase-producing *E. coli* strains.

### MATERIALS AND METHODS

#### Bacterial strains.

The four isogenic strains of *E. coli* employed for this study were engineered by Merck & Co. to differentially express a single β-lactamase; these included (i) 2805 (wild type, no β-lactamase), (ii) 2890 (AmpC β-lactamase), (iii) 2842 (CMY-10 β-lactamase), and (iv) 2807 (CTX-M-15 β-lactamase) (Table 1). β-Lactamase expression was modulated by assembly of the enzyme open reading frame per published GenBank sequences (strain 2890, *Pseudomonas aeruginosa* AmpC and *ampR* [5’ region], GenBank accession no. X54719.1; strain 2842, *E. coli* K989298 ESBL precursor [bla*CMY-10*], GenBank no. AF381617.1; strain 2807, *E. coli* strain 405/06 plasmid pKC405 β-lactamase CTX-M-15 [blaCTX-M-15], and insertion sequence IS26 TnPa, Genbank no. GQ274993.1), insertion into a pBR322 cloning vector (GenBank no. J0749), and replacement of the *blaTEM-1* open reading frame in the β-lactamase-deficient *E. coli* DH10B parent strain. The native *blaTEM-1* promoter was included in the modified pBR322 plasmid to regulate the expression of the desired β-lactamase. Prior to experiments, subcultures of 2805 was performed on tryptic soy agar (TSA) with 5% sheep blood (BD Biosciences, Franklin Lakes, NJ), while 2809, 2842, and 2807 were subcultured in the presence of 25 μg/ml tetracycline to maintain the selection of plasmids.

#### Antibiotics, susceptibility testing, and medium.

Analytical-grade cefotizone and tazobactam powders were obtained from Merck & Co. (Kenilworth, NJ). Fresh stock solutions were prepared immediately prior to experiments in Milli-Q water. Standard broth microdilution methods were adopted from the Clinical and Laboratory Standards Institute (CLSI) (14) for the determination of MICs. The MIC experiments were performed in quadruplicate; while time-kill experiments were performed in duplicate; all studies were conducted using cation-adjusted Mueller-Hinton broth (CaMHB, at 25.0 mg/liter CaCl$_2$ and 12.5 mg/liter MgCl$_2$; Difco, Detroit, MI).

#### Time-kill experiments.

Time-kill experiments were performed for cefotizone and tazobactam alone and in combination, using previously described methods (15). Briefly, fresh bacterial colonies from overnight growth were added to CaMHB to provide a bacterial suspension, which was diluted with CaMHB to achieve the desired starting inoculum of $10^8$ or $10^9$ CFU/ml in a 50-ml Falcon tube. A 6 by 5 array of cefotizone (0, 1, 4, 16, 64, and 256 mg/liter) and tazobactam (0, 1, 4, 16, and 64 mg/liter) concentrations was tested as monotherapy and in combination over a period of 48 h. Thus, 30 treatment regimens were examined in total against each strain at both starting inocula. The choice of studied concentrations was based on clinically achievable targets discerned from free-drug PK profiles in healthy volunteers (16).

Samples were withdrawn at 1, 2, 4, 8, 24, 26, 28, 32, and 48 h after dosing. Colony counts were performed by plating 50-μl aliquots of each diluted sample onto tryptic soy ager (TSA) plates containing 5% sheep blood (Becton Dickinson, Franklin Lakes, NJ) using an automated spiral dispenser (Whitley automatic spiral plater; Don Whitley Scientific Limited, West Yorkshire, England). Plates were incubated at 37°C for 24 h, and viable bacterial counts were determined (log$_{10}$ CFU/ml) using a laser bacteria colony counter (ProtoCOL version 2.05.02; Symbiosis, Cambridge, England). Bactericidal activity (99.9% kill) was associated with a ≥3.0-log$_{10}$ CFU/ml decrease in bacterial density compared to the initial inoculum at any time. Experiments were conducted over 48 h (as opposed to the traditional 24-h standard) to provide insight into the pharmacodynamics of each combination beyond 24 h, and also to better discriminate between the killing activities of the different cefotizone and tazobactam concentrations. As the degradation of cefotizone and tazobactam at 35°C was of concern, concentrations of cefotizone and tazobactam were measured in the absence of bacteria over 48 h to calculate the rate of degradation for each agent.

#### Pharmacodynamic analyses.

The bacterial killing effect (E) of monotherapy combinations was quantified as the log ratio change in bacterial density (CFU/ml) at 48 h versus preantibiotic exposure at 0 h (see equation 1) (17). Point-based analyses were performed on plots of E versus cefotizone concentrations. Taking into consideration the general mechanism of action of β-lactam–β-lactam inhibitor combinations (whereby inhibitors are understood to bind to inactivating β-lactamase enzymes, thus allowing β-lactam agents to exert their action), we attributed the majority of the killing activity exerted by the cefotizone-tazobactam combination to the effect of cefotizone. Consequently, using nonlinear regression, concentration-effect relationships were fit to Hill-type models for each strain at each fixed tazobactam concentration, according to equation 2, where $E_m$ is the measured effect in the absence of cefotizone, $E_{max}$ is the maximal effect, C is the concentration of cefotizone, $EC_{50}$ is the concentration at which there is a 50% maximal effect, and $H$ is the Hill constant. Statistical analyses of $E_{max}$ and $EC_{50}$ parameters were conducted to determine the effect of increasing tazobactam concentrations, using a nonparametric Friedman two-way analysis of variance (ANOVA) with pairwise comparisons ($P < 0.05$). Differences in parameter estimates at $10^8$ versus $10^9$ CFU/ml were determined using hypothesis testing ($P < 0.05$, F-test). All PD analyses and statistical evaluations were performed using Systat (version 13.0.05; Systat Software, IL).

#### RESULTS

### MICs and degradation rates.

The cefotizone MICs of each strain without tazobactam and in combination with 4 mg/liter tazobactam are presented in Table 1. In the absence of tazobactam, the wild-type non-β-lactamase-producing strain 2805 exhibited the lowest MIC, at 0.25 mg/liter. Strains 2890 and 2842 producing class C β-lactamases exhibited MICs of 4 and 8 mg/liter, respectively, while high resistance to cefotizone was displayed by the non-β-lactamase-producing strain 2807, with an MIC of 128 mg/liter. Strains 2890 and 2842 producing class A β-lactamase exhibited MICs of 0.5 and 1 mg/liter, respectively, while high resistance to cefotizone was displayed by the non-β-lactamase-producing strain 2807, with an MIC of 128 mg/liter.

### Time-kill experiments.

Time-kill profiles illustrating the change in bacterial density of the four *E. coli* strains following exposure to cefotizone alone and in combination with tazobactam are presented according to fixed tazobactam concentrations at both $10^6$ and $10^8$ CFU/ml in Fig. 1 and 2, respectively.

#### Strain 2805 (no β-lactamase).

For the susceptible strain 2805 at $10^6$ CFU/ml, complete bactericidal activity reaching undetectable levels was achieved within 4 h. The majority of killing was achieved in the first 2 h, with a gradual decline over the subsequent 8 h. The minimal bactericidal concentration (MBC) was reached after 24 h, with no viable bacterial cells detected at 48 h. The MIC of cefotizone against strain 2805 was $10^8$ CFU/ml, with a 3-log$_{10}$ decrease in bacterial density observed at 48 h. The MBC was reached after 24 h, with no viable bacterial cells detected at 48 h. The MIC of cefotizone against strain 2805 was $10^8$ CFU/ml, with a 3-log$_{10}$ decrease in bacterial density observed at 48 h.

#### Strain 2890 (CMY-10 β-lactamase).

For the resistant strain 2890, the MIC of cefotizone was $10^8$ CFU/ml, with a 3-log$_{10}$ decrease in bacterial density observed at 48 h. The MBC was reached after 24 h, with no viable bacterial cells detected at 48 h. The MIC of cefotizone against strain 2890 was $10^8$ CFU/ml, with a 3-log$_{10}$ decrease in bacterial density observed at 48 h.

#### Strain 2842 (CMY-10 β-lactamase).

For the resistant strain 2842, the MIC of cefotizone was $10^8$ CFU/ml, with a 3-log$_{10}$ decrease in bacterial density observed at 48 h. The MBC was reached after 24 h, with no viable bacterial cells detected at 48 h. The MIC of cefotizone against strain 2842 was $10^8$ CFU/ml, with a 3-log$_{10}$ decrease in bacterial density observed at 48 h.

#### Strain 2807 (CTX-M-15 β-lactamase).

For the resistant strain 2807, the MIC of cefotizone was $10^8$ CFU/ml, with a 3-log$_{10}$ decrease in bacterial density observed at 48 h. The MBC was reached after 24 h, with no viable bacterial cells detected at 48 h. The MIC of cefotizone against strain 2807 was $10^8$ CFU/ml, with a 3-log$_{10}$ decrease in bacterial density observed at 48 h.

<table>
<thead>
<tr>
<th>Strain</th>
<th>Type</th>
<th>AmpC class (4)</th>
<th>β-Lactamase</th>
<th>Cefotizone MIC with tazobactam concn of (mg/liter):</th>
</tr>
</thead>
<tbody>
<tr>
<td>2805</td>
<td>None</td>
<td></td>
<td></td>
<td>0.25</td>
</tr>
<tr>
<td>2890</td>
<td>AmpC</td>
<td>C</td>
<td></td>
<td>4 4 1 0.5 0.5</td>
</tr>
<tr>
<td>2842</td>
<td>CMY-10</td>
<td>C</td>
<td></td>
<td>8 4 4 1 0.5</td>
</tr>
<tr>
<td>2807</td>
<td>CTX-M-15</td>
<td>A</td>
<td></td>
<td>&gt;128 &gt;128 8 2 1</td>
</tr>
</tbody>
</table>

### TABLE 1 β-Lactamase production and MICs of each strain to cefotizone in the absence and presence of tazobactam.
able limits of eradication was achieved within 24 h in response to monotherapy with 4 mg/liter ceftolozane ($-6.48 \log_{10}$ CFU/ml, Fig. 1A). No improvement in activity was gained at higher concentrations or in combination with tazobactam (Fig. 1B to E). At $10^8$ CFU/ml, bactericidal activity was attained at low tazobactam concentrations (0 to 4 mg/liter) in combination with ceftolozane at $16 \text{ mg/liter}$ ($-6.43$ to $-8.33 \log_{10}$ CFU/ml; Fig. 2A to E), or at higher tazobactam concentrations of $16 \text{ mg/liter}$ in combination with $16 \text{ mg/liter}$ ceftolozane ($-5.32$ to $-8.23 \log_{10}$ CFU/ml; Fig. 2D and E).

**Strain 2890 (AmpC).** Rapid activity was demonstrated with ceftolozane monotherapy at concentrations of 4 to 256 mg/liter against 2890 at $10^6$ CFU/ml, with a decrease of $-2.39$ to $-3.53 \log_{10}$ CFU/ml after 4 h, followed by regrowth, whereby bacterial counts mimicked those of the untreated control within 24 h (Fig. 1F). A concentration-dependent trend toward a greater level of ceftolozane killing was observed in combination with increasing tazobactam concentrations. After 48 h, sustained bactericidal activity was attained with combinations of $\geq 4 \text{ mg/liter}$ ceftolozane and $\geq 16 \text{ mg/liter}$ tazobactam ($-3.93$ to $-6.33 \log_{10}$ CFU/ml; Fig. 1I and J). At $10^8$ CFU/ml, both monotherapy and combination regimens containing $\geq 4 \text{ mg/liter}$ ceftolozane were mostly inactive (below $-0.5 \log_{10}$ CFU/ml), while bacterial counts were reduced to undetectable limits with all regimens containing ceftolozane concentrations of 256 mg/liter (Fig. 2F to J). Bactericidal activity at 48 h was noted for $64 \text{ mg/liter}$ ceftolozane in combination with $\geq 4 \text{ mg/liter}$ tazobactam ($-4.01$, $-5.65$, and $-5.18 \log_{10}$ CFU/ml, Fig. 2H to J, respectively). The arm with $16 \text{ mg/liter}$ ceftolozane was capable of achieving bactericidal activity at 48 h when in the presence of 16 and $64 \text{ mg/liter}$ tazobactam ($-3.12$ and $-4.09 \log_{10}$ CFU/ml, respectively).

**Strain 2842 (CMY-10).** Concentration-dependent enhancement of ceftolozane activity in combination with tazobactam was similarly observed for strain 2842 at $10^6$ CFU/ml (Fig. 1K to O). Accordingly, at $\leq 4 \text{ mg/liter}$ tazobactam, activity was negligible in combination with 1 mg/liter ceftolozane, while bactericidal activity was noted within 8 h of exposure to $4 \text{ mg/liter}$ ceftolozane ($-2.94$ to $-3.23 \log_{10}$ CFU/ml) and was followed by rapid regrowth by 24 h (Fig. 1K to M). However, bacteria were driven below detectable limits by all monotherapy and combination regimens containing $\geq 16 \text{ mg/liter}$ ceftolozane ($-6.18 \log_{10}$ CFU/ml; Fig. 2K to N). At the highest tazobactam concentration of $64 \text{ mg/liter}$, bactericidal activity was achieved with $\geq 16 \text{ mg/liter}$ ceftolozane ($-4.74$ to $-5.39 \log_{10}$ CFU/ml; Fig. 2O).

**Strain 2807 (CTX-M-15).** Strain 2807 was resistant to all ceftolozane monotherapy regimens at both inocula, with bacterial counts mirroring the control strain after 48 h. Interestingly, tazobactam maintained the ability to enhance activity in a concentration-dependent manner (Fig. 1 and 2P to T, $10^6$ and $10^8$ CFU/ml,

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**FIG 1** Change in bacterial burdens of strains 2805 (A to E), 2890 (F to J), 2842 (K to O), and 2807 (P to T) at a low inoculum ($10^6$ CFU/ml) over 48 h, following treatment with ceftolozane (0 to 256 mg/liter) at fixed tazobactam concentrations (0 to 64 mg/liter). The colored curves on each graph represent different ceftolozane concentrations, including the untreated growth control (black), tazobactam alone (gray), 1 mg/liter (red), 4 mg/liter (blue), 16 mg/liter (pink), 64 mg/liter (green), and 256 mg/liter (purple).
respective). Against both inocula, ceftolozane concentrations of 64 mg/liter achieved bactericidal activity at 48 h at a tazobactam concentration of 16 mg/liter (Fig. 1S and 2S, a to r above). In the presence of 64 mg/liter tazobactam, ceftolozane concentrations of 4 mg/liter achieved bactericidal activity at 48 h against the 10^6 CFU/ml inoculum (Fig. 1T, at or above 3.97 log_{10} CFU/ml), whereas ceftolozane concentrations of 16 mg/liter were required for bactericidal activity against the 10^8 CFU/ml inoculum (Fig. 2T, at or above 3.66 log_{10} CFU/ml).

**PK/PD analyses.** PK/PD analyses of time-kill data were performed to determine the target concentrations of ceftolozane and tazobactam associated with optimal activity. PD relationships were fit to a sigmoidal Hill-type function (equation 2), from which PD parameters were estimated (R^2 = 0.882, Table 2). The ceftolozane-tazobactam PD relationship was primarily defined by E_{max} and EC_{50} parameters, which provide a measure of the maximal capacity of efficacy and half-maximal potency, respectively. Across all strains at both inocula, no significant trends were noted in E_{max} values with increasing tazobactam concentrations (P = 0.2, ANOVA). However, in the presence of tazobactam, a clear tendency toward lower EC_{50} values was demonstrated for all strains at both inocula (Table 2). Overall, pairwise comparisons revealed that all EC_{50} differences at tazobactam concentrations of 0 to 4 mg/liter versus 16 and 64 mg/liter were statistically significant (P < 0.01, ANOVA). The changes in EC_{50} as tazobactam concentrations increased from 0 to 4 mg/liter were not statistically significant, nor was the difference in EC_{50} at tazobactam concentrations of 16 versus 64 mg/liter (P = 0.1, ANOVA). Hypothesis testing revealed that the EC_{50}s at 10^8 CFU/ml were 2.81 to 66.5 times greater than those at 10^6 CFU/ml (median, 10.7-fold increase; P = 0.002, F-test); however, no significant differences in E_{max} were observed between the two inocula (P = 0.531, F-test).

**DISCUSSION**

The introduction of ceftolozane-tazobactam has attracted much interest, with in vitro data providing evidence that this combination may present a promising addition to the therapeutic armamentarium against a range of Gram-negative pathogens, including β-lactamase-expressing strains (1–3, 5–8, 18–20). It is well recognized that appropriate antimicrobial dosing strategies necessitate a solid understanding of PK/PD principles to understand the relationship between the concentration-time profile and antimicrobial activity, allowing the design of regimens that maximize bacterial eradication and minimize the development of resistance. In the present study, the in vitro activities of an array of ceftolozane and tazobactam concentrations were tested alone and in combi-
nation in a dynamic fashion over 48 h against four strains of *E. coli* expressing different β-lactamases. Our study design incorporated a wide range of concentrations to ensure that all relevant PD parameters were captured for mathematical modeling purposes in the future.

The ability of inhibitors to inactivate specific β-lactamase enzymes varies markedly within and between classes; for tazobactam, irreversible β-lactamase inhibition ("suicide inhibition") has been demonstrated against class A β-lactamases from *Pseudomonas aeruginosa* (2, 7, 28), the exposure of 2890 to ceftolozane monotherapy resulted in extensive regrowth, even at extremely high concentrations (256 mg/liter) far in excess of the MIC (Fig. 1F). Owing to the relatively stable ceftolozane concentrations used during the time-killing experiments, regrowth was likely characterized by the amplification of ceftolozane-resistant subpopulations. Indeed, the phenomenon of heteroresistance to colistin (29), vancomycin (30), and carbapenems (31) has been reported and defined as the presence of a resistant subpopulation of bacteria within a susceptible strain based on MICs. Although heteroresistance has yet to be understood, consideration may be given to the administration of elevated ceftolozane doses when employed for the treatment of infections that entail a higher bacterial burden.

Interestingly, despite previous suggestions of improved stability of ceftolozane against AmpC β-lactamase from *Pseudomonas aeruginosa* (2, 7, 28), the exposure of 2890 to ceftolozane monotherapy resulted in extensive regrowth, even at extremely high concentrations (256 mg/liter) far in excess of the MIC (Fig. 1F). Owing to the relatively stable ceftolozane concentrations used during the time-killing experiments, regrowth was likely characterized by the amplification of ceftolozane-resistant subpopulations. Indeed, the phenomenon of heteroresistance to colistin (29), vancomycin (30), and carbapenems (31) has been reported and defined as the presence of a resistant subpopulation of bacteria within a susceptible strain based on MICs. Although heteroresistance has yet to be understood, consideration may be given to the administration of elevated ceftolozane doses when employed for the treatment of infections that entail a higher bacterial burden.

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### TABLE 2 Hill plot PD parameter estimates describing the effect of ceftolozane in the presence of increasing tazobactam concentrations (0 to 64 mg/liter) at low and high inocula

<table>
<thead>
<tr>
<th>Strain</th>
<th>Tazobactam concn (mg/liter)</th>
<th>Low inoculum of ceftolozane (SE)</th>
<th>High inoculum of ceftolozane (SE)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$E_0$</td>
<td>$E_{max}$</td>
<td>$H^*$</td>
</tr>
<tr>
<td>2805 0</td>
<td>2.68</td>
<td>9.16</td>
<td>10.0</td>
</tr>
<tr>
<td>1</td>
<td>2.62</td>
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<td>10.0</td>
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<td>4</td>
<td>2.35</td>
<td>8.83</td>
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</tr>
<tr>
<td>16</td>
<td>2.48</td>
<td>8.96</td>
<td>9.53</td>
</tr>
<tr>
<td>64</td>
<td>1.83</td>
<td>8.31</td>
<td>10.0</td>
</tr>
<tr>
<td>2890 0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>1</td>
<td>2.61 (0.734)</td>
<td>2.20 (1.12)</td>
<td>1.00 (1.25)</td>
</tr>
<tr>
<td>4</td>
<td>2.40 (1.56)</td>
<td>9.47 (5.25)</td>
<td>1.00 (1.36)</td>
</tr>
<tr>
<td>16</td>
<td>2.51 (0.751)</td>
<td>6.69 (0.87)</td>
<td>3.23 (2.72)</td>
</tr>
<tr>
<td>64</td>
<td>1.96 (1.60)</td>
<td>6.98 (2.07)</td>
<td>5.10 (5.98)</td>
</tr>
<tr>
<td>2842 0</td>
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<td>10.0 (27.1)</td>
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<tr>
<td>4</td>
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<tr>
<td>16</td>
<td>3.10 (0.119)</td>
<td>9.29 (0.100)</td>
<td>10.0 (0.361)</td>
</tr>
<tr>
<td>64</td>
<td>2.26 (0.003)</td>
<td>8.44 (0.108)</td>
<td>10.0 (0.112)</td>
</tr>
<tr>
<td>2807 0</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
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</table>

*H* Hill’s constants were constrained between the limits of 1 and 10.

a —, no parameter estimates calculated because there was negligible activity that was not modeled well by a Hill-type function.
both inocula. Ceftolozane and tazobactam concentrations of 64 mg/liter and 16 mg/liter reflect achievable maximum concentration of drug (C_{max}) values obtained in healthy volunteers receiving the equivalent of 1.0-g and 0.5-g doses of ceftolozane and tazobactam, respectively, which were the doses investigated in phase III clinical trials (16, 32, 33). Taken together, these results highlight the utility of tazobactam to increase the susceptibility of β-lactama-producing E. coli strains to ceftolozane therapy at both inocula.

Although the %T_{\text{threshold}} has been identified as the PK/PD index most predictive of the activity of ceftolozane-tazobactam, our results show a clear concentration dependence, with enhanced killing at elevated ceftolozane concentrations. The %T_{\text{threshold}} was first proposed by VanScoy et al. (12) as the PK/PD index that best described ceftolozane-tazobactam killing in E. coli strains expressing the CTX-M-15 β-lactamase (12). Further work with E. coli and Klebsiella pneumoniae strains expressing various β-lactamases led to the unifying conclusion that the percentage of time above the ceftolozane-tazobactam MIC × 0.5 is predictive of the combination’s efficacy, regardless of the β-lactamase expression profile of the pathogen (11). An in vivo study utilizing a neutropenic mouse model corroborated %T_{\text{threshold}} as the ideal PK/PD index, and the authors were able to identify the % time above the MIC (%T_{\text{MIC}}) targets required to achieve specific magnitudes of bacterial killing (34). In the present study, the concentration dependence observed in the time-kill experiments may partially be ascribed to the elevated bacterial burden (10^6 CFU/ml) that exceeded the bacterial load utilized in previous in vitro (10^5 CFU/ml) and in vivo (10^{6.2} to 10^{7.1} CFU/ml) studies (11, 12, 34). The static concentrations used in the current investigation may also account for some of the discordance with the results from prior studies. However, previous investigations evaluating the PK/PD of β-lactam–β-lactamase inhibitor combinations have asserted that stoichiometric inhibition of β-lactama- mase enzymes is determined by exposure of the inhibitor over time, which is best predicted by the area under the concentration-time curve (AUC) (35). A prior study utilizing piperacillin and tazobactam also found that dose fractionating the administration of both agents while maintaining the same drug exposure did not alter the killing of a TEM-producing strain of E. coli (36). The activity of ceftolozane-tazobactam may therefore not be completely described by time-dependent killing, and a hybrid index that accounts for the AUC or C_{max} may improve the predictive capability of the %T_{\text{threshold}} index.

In summary, we systematically described the concentration-effect relationship of ceftolozane and tazobactam alone and in combination, revealing the ability of ceftolozane-tazobactam to achieve potent bactericidal activity against E. coli strains expressing different types of β-lactamase enzymes. While these results are promising, our time-kill studies utilized static concentrations of ceftolozane-tazobactam, making the extrapolation of our results into the clinical setting difficult. To the best of our knowledge, only two studies, by VanScoy et al. (10, 37), have looked at ceftolozane-tazobactam in a hollow-fiber infection model. Further studies evaluating the performance of the combination regimen against other pathogens containing β-lactamase enzymes are needed to completely understand the niche of ceftolozane-tazo- bactam among other β-lactams.

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**REFERENCES**


