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1 **Pharmacokinetics-Pharmacodynamics of Tazobactam in Combination with**
2 **Cefepime in an *In Vitro* Infection Model**
3
4 Running title: PK-PD of Tazobactam
5
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19 **ABSTRACT (word count = 250)**

20 We previously demonstrated that for tazobactam administered in combination
21 with ceftolozane, the pharmacokinetic-pharmacodynamic (PK-PD) index that best
22 described tazobactam efficacy was the percentage of the dosing interval that
23 tazobactam concentrations were above a threshold (%T>threshold). Using data
24 from studies of Enterobacteriaceae-producing ESBL, a relationship between
25 tazobactam %T>threshold and reduction in log_{10} CFU from baseline, for which
26 tazobactam threshold concentration was the product of the isolate's ceftolozane-
27 tazobactam MIC value and 0.5, was identified. However, since the kinetics of
28 cephalosporin hydrolysis vary among ESBLs and compounds, it is likely that the
29 translational relationship to derive the tazobactam threshold concentration varies
30 among enzymes and compounds. Using a one-compartment *in vitro* infection
31 model, the PK-PD of tazobactam administered in combination with cefepime was
32 characterized and a translational relationship across ESBL-producing
33 Enterobacteriaceae was developed. Four clinical isolates, two *Escherichia coli*
34 and two *Klebsiella pneumoniae*, known to produce CTX-M-15 β-lactamase
35 enzymes and displaying cefepime MIC values of 2 to 4 mg/L in the presence of 4
36 mg/L tazobactam, were evaluated. Tazobactam threshold concentrations from
37 0.0625-1 times the tazobactam-potentiated cefepime MIC value were
38 considered. The threshold that best described the relationship between
39 tazobactam %T>threshold and change in log_{10} CFU from baseline was the
40 product of 0.125 and the cefepime-tazobactam MIC (R^2=0.813). The magnitude
41 of %T>threshold associated with net bacterial stasis and a 1-log_{10} CFU/mL

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42 reduction from baseline at 24 hours was 21.9 and 52.8%, respectively. These
43 data will be useful to support the identification of tazobactam dosing regimens in
44 combination with cefepime for evaluation in future clinical studies.

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45 **INTRODUCTION**

46 Due to the increasing prevalence of β-lactamase-producing Enterobacteriaceae,
47 there is renewed interest in β-lactam-β-lactamase inhibitor combinations. This
48 interest encompasses combinations involving new cephalosporins or inhibitors
49 and new combinations of old agents. Tazobactam is a penicillanic acid sulfone β-
50 lactamase inhibitor that has been used in combination with piperacillin for over
51 two decades [1]. Tazobactam was approved by the United States Food and Drug
52 Administration for clinical use with the anti-pseudomonal cephalosporin
53 ceftolozane in 2014 [2]. Cefepime-tazobactam combinations, with an 8:1 ratio,
54 are available from multiple manufacturers in India, but are not licensed elsewhere
55 [3].
56
57 Tazobactam extends the spectrum of ceftolozane activity to include many
58 extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae. Like
59 ceftolozane, cefepime is unstable in the presence of many ESBL enzymes but
60 has greater inherent stability to Enterobacterial AmpC enzymes [4].
61 Administering tazobactam in combination with cefepime increases the agent's
62 spectrum of activity to include many ESBL-producing Enterobacteriaceae [5, 6].
63
64 Recently, we demonstrated that the percentage of the dosing interval that
65 tazobactam concentrations remained above a threshold (%T>threshold) was the
66 pharmacokinetic-pharmacodynamic (PK-PD) measure best associated with
67 efficacy for tazobactam when administered in combination with ceftolozane [7].

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68 The tazobactam threshold concentration that allowed co-modeling of
69 Enterobacteriaceae producing various ESBL enzymes was the product of the
70 individual isolate's ceftolozane-tazobactam MIC value and 0.5 [8]. However,
71 since the kinetics of β-lactam hydrolysis, maximum reaction velocity (V_{max}) and
72 Michaelis-Menten Constant (K_m), varies among compounds and enzymes [9], it is
73 likely that the tazobactam threshold concentration and translational relationships
74 will vary among cephalosporins. Another source of potential variation is the
75 permeation rates of the cephalosporin relative to the inhibitor, as cefepime is said
76 to rapidly permeate cellular membranes [10].
77
78 Using a one-compartment *in vitro* infection model, the objectives of this study
79 were two-fold. The first objective was to confirm that %T>threshold described
80 the PK-PD of tazobactam when administered with cefepime. The second
81 objective was to identify a tazobactam threshold concentration that would allow
82 co-modeling of isolates and thereby, identify candidate tazobactam dosing
83 regimens for future combination with cefepime.

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84 **METHODS**

85 **Bacteria, antimicrobial, and β-lactamase inhibitor.** Cefepime and tazobactam
86 were obtained from Toronto Research Chemicals (Toronto, Canada) and ACS
87 Dobfar (Tribiano MI, Italy), respectively.
88
89 The challenge panel of four clinical Enterobacteriaceae isolates with CTX-M-15
90 ESBLs was obtained from JMI laboratories (North Liberty, Iowa, USA). The panel
91 was comprised of two *Klebsiella pneumoniae* and two *Escherichia coli* isolates
92 chosen based upon cefepime MIC value when assayed with tazobactam (4
93 mg/L).
94
95 **Media and *in vitro* susceptibility studies.** Susceptibility studies were
96 conducted in accordance with Clinical and Laboratory Standards Institute
97 guidelines [11] using cation-adjusted Mueller-Hinton broth (BD laboratories,
98 Franklin Lakes, New Jersey, USA) in a broth micro-dilution method. Isolate
99 susceptibility to cefepime was determined alone and in combination with a fixed
100 tazobactam concentration (4 mg/L). All susceptibility studies were conducted in
101 triplicate over a 2-day period.
102
103 **Whole genome sequencing, epidemiology typing and resistance genes.**
104 Total genomic DNA, extracted using QIAmp genomic DNA kit (Qiagen, Hilden,
105 Germany), was used to prepare paired end True-Seq library and cluster
106 generation. Samples were sequenced using Illumina® MiSeq instrument

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107 (SeqWright, Houston, TX, USA). Sequences were aligned into multiple contigs
108 using Lasergene NGen Denovo assembly protocol (DNAStar, Madison, WI). The
109 ResFinder web server (www.genomicepidemiology.org) was used to identify
110 acquired antimicrobial resistance genes in the respective assembled genomes,
111 using a threshold of 98.0% identity. Assembled genomes were also utilized for
112 determining the multilocus sequence typing (MLST) and analyzing the
113 sequences of protein membrane genes (OmpC, OmpF and OmpK37), *ampC* (in
114 *E. coli*) and *acrA*. DNA and protein analysis was performed using the
115 Lasergene® software package (DNAStar; Madison, Wisconsin). Amino acid
116 sequences obtained were compared to those of *E. coli* ATCC 25922 and *K.*
117 *pneumoniae* ATCC 13833. One additional clinical isolate of each species was
118 also used as control strains and both isolates exhibited a cefepime-tazobactam
119 MIC results of 1 mg/L.
120
121 **Determination of transcription levels of the intrinsic AmpC (*E. coli* only),**
122 **AcrA efflux pump and outer membrane protein genes.** The transcription
123 levels of *amp*C, *acrA*, and *ompC*, *ompF* and *ompK*37 were determined by
124 comparing the transcription levels of these selected targets to those from control
125 isolates (cefepime-tazobactam MIC, 1 mg/L). Total genomic RNA samples were
126 extracted from each isolate using the RNeasy Mini Kit in a fully-automated
127 robotic workstation (Quiacube; Qiagen, Valencia, CA) and residual DNA was
128 eliminated with RNase-free DNase (Promega, Madison, WI). Sample quality and
129 quantification of the genomic RNA were assessed using the Agilent 21000

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130 Bioanalyzer utilizing the RNA 6000 Nano Kit according to manufacturer
131 instructions (Agilent, Santa Clara, CA). Reverse-transcription PCR was
132 performed in triplicate using QuantiTect SYBR Green RT-PCR Kit (Qiagen,
133 Germantown, MD) in the StepOne Plus instrument (Lie Technologies, Foster
134 City, CA).
135
136 **One-comparment *in vitro* infection model and sample processing.** The one-
137 compartment *in vitro* infection model utilized in these studies has been described
138 previously [12]. Briefly, the model consists of a central infection compartment
139 containing growth medium, the challenge isolate and magnetic stir bars to ensure
140 the homogeneity of drug concentrations and ensure even dispersion of bacteria
141 within the compartment. This central infection compartment was attached to a stir
142 plate and the entire unit was placed within a temperature and humidity controlled
143 incubator set at 35°C. Drug-free growth medium was pumped into the central
144 infection compartment via a computer-controlled peristaltic pump while growth
145 medium was simultaneously removed through an exit port and captured in a
146 waste container. The challenge isolates were aseptically inoculated into the
147 central infection compartment, and the peristaltic rate of diffusion was set at a
148 flow rate that allowed for the simulation of human concentration-time profiles for
149 the drug(s) under study. The test compounds were infused via computer
150 controlled syringe pumps, allowing simulation of the desired half-lives, dosing
151 frequencies, and concentrations. Samples for CFU determination and drug
152 concentration assay were collected from the central infection compartment using

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153 a sterile syringe and needle through a rubber septum at pre-determined time-
154 points.
155
156 The initial inoculum was 1.0 x 10^6 CFU/mL of the challenge isolate, prepared
157 from a culture grown overnight on trypticase soy agar supplemented with 5%
158 lysed sheep blood (BD Laboratories, Franklin Lakes, New Jersey). Isolates were
159 taken from these overnight cultures and grown to mid-logarithmic phase in a flask
160 of Mueller-Hinton broth set in a shaking water bath at 35°C and 125 rotations per
161 minute. The bacterial concentration was determined by optical density referenced
162 against previously-confirmed growth curves for each challenge isolate.
163
164 After inoculation into the one-compartment *in vitro* infection model, bacteria were
165 exposed to changing concentrations of cefepime and tazobactam simulating
166 human half-lives of 2 hours for cefepime [13] and 1 hour for tazobactam [14].
167
168 One milliliter samples were collected for CFU determination at 0, 2, 4, 6, 8, 12,
169 and 24 hours. Each sample was centrifuged, washed, and re-suspended with
170 sterile normal saline twice to prevent drug carryover and was then cultured on
171 trypticase soy agar enriched with 5% sheep blood, as well as Mueller-Hinton
172 agar infused with cefepime at four times the potentiated MIC value and
173 tazobactam at a fixed concentration of 4 mg/L. Plated samples were incubated at
174 35°C for 24 hours and colonies were counted for enumeration of bacterial
175 density. A few colonies were collected from the drug-containing agar plates to

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176 survey for any decrease in sensitivity to cefepime-tazobactam. One milliliter
177 samples were collected from the growth compartment for drug assay at 1, 3, 5, 7,
178 9, 11, 13, and 24 hours, sterile-filtered, then immediately frozen at -80°C until
179 assayed for drug concentration.
180
181 **Mutation frequency studies.** The mutation frequency to drug resistance was
182 estimated by plating 4 mL of log-phase growth suspension containing an average
183 concentration of 6.17 x 10^8 CFU/ml onto agar containing four times the baseline
184 cefepime MIC value with a fixed 4 mg/L of tazobactam. The bacterial density in
185 the suspension was determined by quantitative culture, and the ratio of colonies
186 on the drug-containing plates to that of the starting inoculum provided an
187 estimate of the drug resistance frequency within a total population. The assay
188 was performed in duplicate and a subset of isolates from each trial were taken
189 from the drug-containing plates and re-tested by standard MIC methodology to
190 confirm decreased susceptibility.
191
192 **Dose-ranging studies.** Duplicate dose-ranging studies were conducted in order
193 to determine the dose-response relationship for each challenge isolate. In these
194 studies, a fixed cefepime dose of either a 1 or 2 g was administered either alone
195 or in combination with tazobactam, using an every 8 hour (q8h) schedule. The
196 modelled tazobactam doses ranged from 8 to 4,000 mg following the same q8h
197 schedule. Both cefepime and tazobactam were administered over a 1 hour
198 infusion.

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199

200 **Analytical method.** All samples were assayed by liquid chromatography-tandem
201 mass spectrometry LC/MS/MS (Waters Xevo TQ-S, Milford MA) and drug levels
202 were quantitated using external standardization. Standard curves ranged from
203 0.500 to 200 mg/L for cefepime and from 0.0100 to 20.0 mg/L for tazobactam.
204 The standard curves were linear over their respective ranges (r^2=0.974 and
205 0.988 or greater) for cefepime and tazobactam, respectively. The lower limit of
206 quantification was 0.500 mg/L for cefepime and 0.0100 mg/L for tazobactam. The
207 intra-assay percent coefficient of variation (%CV) for cefepime quality control
208 samples at concentrations of 5.0, 25.0, and 100 mg/L was 15.2% or less. The
209 intra-assay %CV for tazobactam quality control samples at concentrations of
210 0.05, 0.5, and 5.0 mg/L was 7.04% or less. Inter-assay %CVs for the cefepime
211 quality control samples at concentrations of 5.00, 25.0, and 100 mg/L were
212 8.70% or less and 5.83 % or less for tazobactam quality control samples at
213 0.0500, 0.500, and 5.00 mg/L. Diluted quality control samples for tazobactam
214 (100 mg/L) exhibited a inter-assay %CV of 5.24% or less and an intra-assay
215 %CV of 6.19% or less in runs which required dilution of samples into the
216 calibration curve range.

217

218 **Pharmacokinetic-pharmacodynamic analysis.** Data from the dose-ranging
219 studies were evaluated using Hill-type models and non-linear least squares
220 regression. The data were weighted using the inverse of the estimated
221 measurement variance. Relationships between change in $\log_{10}$ CFU at 24 hours

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222 and tazobactam %T>threshold were evaluated. Tazobactam %T>threshold was
223 identified through an iterative process in which candidate tazobactam threshold
224 concentrations, representing the product of the tazobactam-potentiated cefepime
225 MIC value and 1, 0.5, 0.25, 0.125, and 0.0625 for each individual isolate, were
226 evaluated. Discrimination among tazobactam threshold concentrations was
227 based on the evaluation of the dispersion of data along the %T>threshold axis
228 and optimization of r^2 values for the relationship between change in $\log_{10}$ CFU at
229 24 hours and tazobactam %T>threshold.

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230 **RESULTS**

231 *In vitro* susceptibility testing, whole genome sequencing, epidemiology typing and
232 determination of transcription levels of intrinsic AmpC, AcrA, and outer
233 membrane protein levels. Table 1 shows the β-lactamase enzyme(s) identified
234 within the challenge panel and the MIC values for cefepime and tazobactam
235 alone and combined with 4 mg/L of tazobactam. All four isolates carried CTX-M-
236 15, and it was the sole enzyme in *E. coli* 30854. The remaining isolates produced
237 additional enzymes including OXA-1/30, TEM-1 and SHV-1 and -28. MIC values
238 ≥ 256 mg/L for tazobactam and ≥ 32 mg/L for cefepime alone were recorded for
239 all four isolates. When cefepime was studied in combination with 4 mg/L
240 tazobactam, the MIC values of the isolate panel ranged from 2 to 4 mg/L,
241 representing the high end of the susceptible range for cefepime against
242 Enterobacteriaceae [11].

243

244 An array of resistance determinants were detected, especially in isolates 39930
245 and 25021 as shown in **Table 1**. Higher expression levels of OmpC (47- to 62-
246 fold more than the control strain) were observed in both *E. coli*. Moreover, *E. coli*
247 30854 also expressed the intrinsic AmpC gene 17-fold more than the control
248 strain. In addition, *K. pneumoniae* 604 and 25021 showed lower expression
249 levels (approximately a third) of OmpK37 when compared with that of a control
250 strain.

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252 Sequence analysis of genes of interest demonstrated that all isolates included in
253 the study had several alterations in the OmpK36 (OmpC analogue of *E. coli*)-
254 encoding gene. Several alteration, deletions and insertions were noted, including
255 an insertion in the L5 region of OmpC in both *E. coli*, and mutations and six
256 amino acid deletions in L5 and L6, respectively in both *K. pneumoniae* isolates.
257 Other genes investigated (*ampC*, *acrA*, OmpF and OmpK37) showed sequences
258 similar to the control isolates.
259
260 **Pharmacokinetics.** The targeted cefepime and tazobactam pharmacokinetic
261 profiles were well-simulated in the *in vitro* infection model for all studied dosing
262 regimens. **Figure 1** shows the relationship between observed and targeted drug
263 concentrations. As evidenced by the high coefficient of determination values
264 (cefepime, R^2=0.967; tazobactam, R^2=0.991), there was excellent precision but
265 with a modest tendency to underpredict concentrations, as evidenced by the
266 slope values (cefepime, 14.18%; tazobactam, 13.53%).
267
268 Drug concentrations for cefepime and tazobactam were each fit to a one-
269 compartment model with zero-order input and first-order elimination. The
270 pharmacokinetic data for each agent were well described by this model.
271
272 **Mutation frequency studies.** The mean densities the of drug-resistant
273 subpopulation observed at four times the baseline cefepime-tazobactam MIC for

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274 each challenge isolate are presented in **Table 2**; these frequencies ranged from
275 1.3 x 10^{-7} to 3.5 x 10^{-8} CFU.
276
277 **Dose-ranging studies.** Bacteria in the no-treatment control arms grew well in
278 each case, reaching a density exceeding 1.0 x 10^{8} CFU/mL by 12 hours (**Figure**
279 **2 A-D**).The cefepime and tazobactam monotherapy control regimens behaved as
280 expected: i.e., the tazobactam arms performed similarly to the no-treatment
281 control arms and the cefepime arms provided some initial cell kill but with full
282 regrowth by 24 hours.
283
284 The range of tazobactam doses used in combination with the fixed cefepime
285 dosing regimens provided a full spectrum of drug effects for each challenge
286 isolate. For example, the low-intensity cefepime (1 g)-tazobactam (8 mg) regimen
287 behaved similarly to cefepime (1 g) alone while intermediate-intensity cefepime
288 (1 g)-tazobactam (15.6 to 125 mg) dosing regimens resulted in net bacterial
289 stasis at the 24 hour time point (**Figure 2A**) and the, high-intensity cefepime (1
290 g)-tazobactam (250-500 mg) regimens achieved slightly more than a 1 $\log_{10}$
291 CFU/mL reduction from baseline (**Figure 2A**).
292
293 Drug-resistant isolates were observed for all controls, including the cefepime and
294 tazobactam monotherapy dosing regimens. Drug-resistant isolates were also
295 observed for three of the cefepime-tazobactam dosing regimens and typically
296 occurred in low-intensity tazobactam regimens (8 to 250 mg) (**Figure 2 A-D**). The

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297 MIC values of the isolates collected from the drug-containing plates from the
298 mutation frequency studies and dose-ranging studies were determined and are
299 presented in **Table 2**.
300
301 **Pharmacokinetic-pharmacodynamic analysis.** The relationships between
302 change in log_{10} CFU over 24h and the tazobactam %T>threshold for tazobactam
303 threshold concentrations from 0.0625- to 1-times the cefepime-tazobactam MIC
304 are presented in **Figure 3**. The coefficient of determination (r^2) and scatter of
305 data about the fitted function across the X-axis were most optimal for the Hill
306 functions describing the relationships between the change in log_{10} CFU/mL from
307 baseline and the product of the cefepime-tazobactam MIC and either 0.0625 or
308 0.125. Emphasis was placed on a tazobactam threshold concentration of 0.125
309 times the cefepime-tazobactam MIC. The basis for the focus on this threshold
310 was the modestly better scatter of data across the range of tazobactam
311 %T>thresholds than that based on the data using the tazobactam threshold
312 concentration of 0.0625 times the MIC, without less apparent clustering of data
313 points at the lower (0) and upper (100) margins of the range. The %T>thresholds
314 based on the tazobactam threshold concentration of 0.125 times the cefepime-
315 tazobactam MIC associated with net bacterial stasis and a 1-log_{10} CFU reduction
316 in bacterial burden at 24 hours were 21.9 and 52.8%, respectively. The
317 parameter estimates (standard errors) for the relationship between change in
318 log_{10} CFU and tazobactam %T>threshold were E_0 2.69 (0.22), E_{max} 12 (38.3),
319 Hill's constant 0.48 (0.65), and EC_{50} 277.51 (3241.81).

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320 **Discussion**

321 The objectives of these studies were two-fold. The first was to use a one-
322 compartment *in vitro* infection model to confirm that %T>threshold described the
323 PK-PD of tazobactam when administered in combination with cefepime. The
324 second was to identify a tazobactam threshold concentration that would allow co-
325 modeling across isolates, and using this, %T>threshold targets that could be
326 used to identify candidate tazobactam dosing regimens in combination with
327 cefepime for future study.
328
329 We confirmed that the PK-PD index associated with efficacy for tazobactam
330 against Enterobacteriaceae was %T>threshold when tazobactam administered in
331 combination with cefepime. These findings are consistent with those for
332 tazobactam paired with ceftolozane [3]. The two tazobactam threshold
333 concentrations that allowed the entire challenge panel to be co-modeled with the
334 most optimal fit of the model to the data were those based on the product of the
335 cefepime-tazobactam MIC and either 0.0625 or 0.125. For reasons described
336 above, 0.125 x cefepime-tazobactam MIC was considered the more optimal
337 threshold. This multiple is significantly lower than the 0.5 x MIC previously
338 identified for ceftolozane-tazobactam [8]. Moreover, the %T>threshold
339 tazobactam concentration associated with net bacterial stasis and a 1-$\log_{10}$ CFU
340 reduction from baseline were lower for cefepime-tazobactam (net bacterial stasis,
341 21.9; 1 $\log_{10}$ CFU reduction from baseline, 52.8) than for ceftolozane-tazobactam
342 (net bacterial stasis, 65.9; 1-$\log_{10}$ CFU reduction from baseline, 77.3) [8]. The

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343 tazobactam doses administered q8h that correspond to the % T > MIC*0.125
344 required to achieve net bacterial stasis and a 1-$\log_{10}$ CFU reduction from
345 baseline were 31.25 to 62.5 mg and 125 to 250 mg, respectively, for isolates with
346 potentiated MICs of 2 and 4 mg/L. It is worthwhile to note that one isolate,
347 *K. pneumoniae* 604, was common to the studies described herein and those
348 previously-conducted for ceftolozane-tazobactam [8].
349
350 When the results of both sets of evaluations are considered, these data imply
351 that a lower tazobactam exposure was required for a given level of drug effect
352 when tazobactam was combined with cefepime rather than ceftolozane. Possible
353 explanations, which are not mutually exclusive, are that cefepime and
354 ceftolozane may differ in the following ways: (i) in their lability to the ESBLs
355 represented; (ii) in their affinity for these enzymes, which determines the extent
356 to which they may outcompete the inhibitor for enzyme binding; and (iii) in their
357 relative acylation and deacylation rates, which may determine the extent that the
358 enzyme is held in a form invulnerable to attack by tazobactam or relative
359 permeation rate into the bacterial periplasm.
360
361 There are two limitations of the studies described herein that deserve comment.
362 The first limitation is that the one-compartment *in vitro* infection model utilized for
363 these studies does not account for the effect of an immune system and is
364 conducted using Mueller-Hinton broth media that optimizes bacterial growth. The
365 impact of the former is that the magnitude of the %T>threshold for tazobactam

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366 may be overestimated. The second limitation is that the duration of the studies
367 carried out was 24 hours. This duration of the study was insufficient to evaluate
368 the effect of intensity and duration of therapy of each tazobactam dosing regimen
369 on the amplification of pre-existing drug-resistant bacterial subpopulations. The
370 impact of the limited duration of the experiment is that the magnitude of the
371 %T>threshold for tazobactam may in fact be underestimated. Additional studies
372 utilizing immunocompetent and immunosuppressed animal infection and hollow-
373 fiber *in vitro* infection models will be needed to address these limitations.
374
375 In conclusion, we confirmed that the PK-PD index associated with tazobactam
376 efficacy when administered in combination with cefepime was %T>threshold.
377 This finding was consistent with the PK-PD index associated with tazobactam
378 efficacy when administered in combination with ceftolozane. Through this
379 evaluation, we also identified a tazobactam threshold concentration, which was
380 the product of the cefepime-tazobactam potentiated MIC and 0.125. The use of
381 this threshold allowed for data from the entire challenge panel to be co-modeled
382 with the most optimal fit of the model to the data. These data will be useful to
383 identify candidate tazobactam dosing regimens to be administered in
384 combination with cefepime for future study.

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408 D.M.L. is employed by the University of East Anglia, and is on the advisory
409 board/an ad-hoc consultant for Accelerate, Achaogen Inc., Adenium Biotech,
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Table 1. Susceptibility testing results, identified β-lactamase enzymes, and transcription levels of outer membrane proteins and AmpC expression for the Enterobacteriaceae panel

Isolate	Identified β-lactamase enzyme(s)	Omp transcription level[a]	AmpC transcription level[a]	Microbroth MIC values[b] (mg/L)		
				TAZ alone	FEP alone	FEP-TAZ (4 mg/L)
E. coli 30854	CTX-M-15	OmpC (47)	AmpC (17)	512	256	2
E. coli 39930	CTX-M-15, TEM-1, OXA-1/30, SHV-28	OmpC (62)	AmpC (1)	256	128	4
K. pneumoniae 25021	CTX-M-15, TEM-1, OXA-2	OmpK (-33)	ND	512	32	4
K. pneumoniae 604	CTX-M-15, OXA-1/30, SHV-1	OmpK (-33)	ND	512	>512	4

a. 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.

b. All MIC values shown represent modal values.

Omp=Outer membrane protein; FEP=Cefepime; TAZ=Tazobactam; ND = Not Determined

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471 **Table 2**. Mutation frequencies and MIC values for mutants collected from the drug-containing plates utilized in the
472 dose-ranging efficacy and mutation frequency studies

Isolate	Baseline cefepime-tazobactam MIC[a]	Geometric mean of cefepime-tazobactam mutation frequency	Cefepime/ tazobactam MIC for isolates taken from drug-containing plate[a]
E. coli 30854	2	1.3×10^{-7}	8 to 32
E. coli 39930	4	9.4×10^{-7}	32 to 64
K. pneumoniae 25021	4	1.8×10^{-7}	16 to 128
K. pneumoniae 604	4	3.5×10^{-8}	8

a. All MIC values shown, as mg/L, represent modal values determined using broth microdilution and tazobactam at a fixed 4 mg/L concentration.

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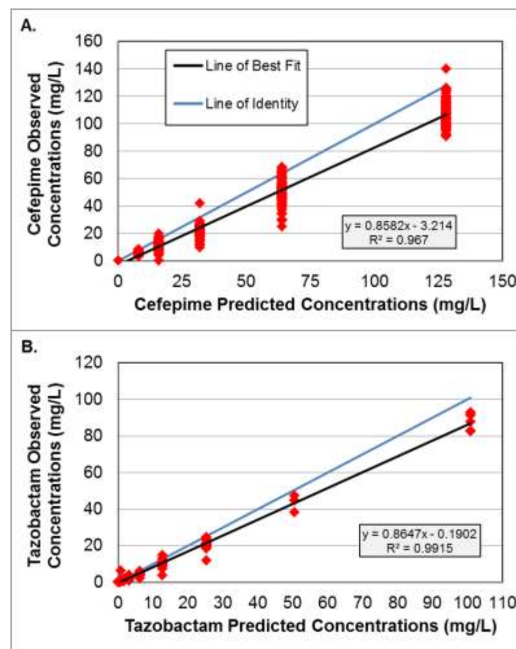
473 **Figure 1.** The relationships between the observed and targeted PK profiles simulated over the 24 hour *in vitro*

474 experiment for cefepime (A) and tazobactam (B)



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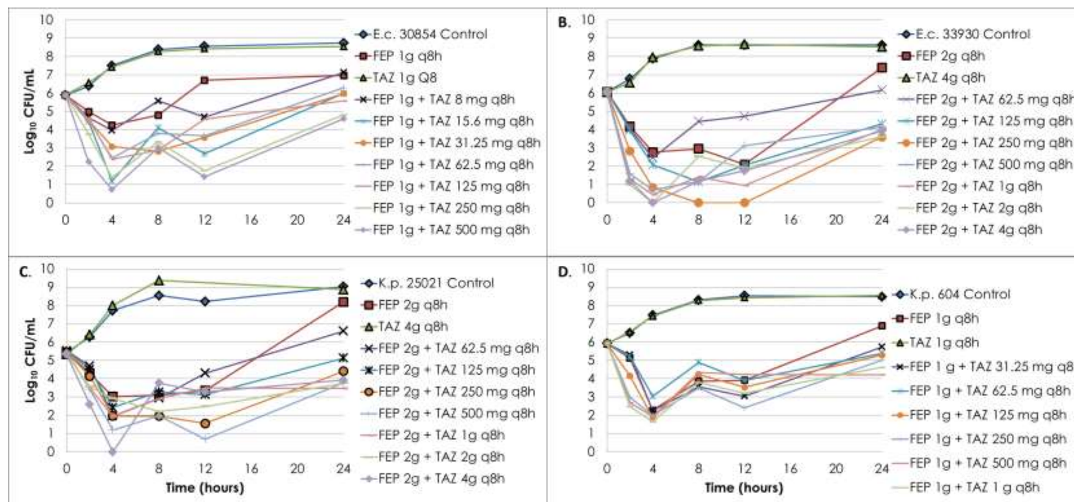
476 **Figure 2.** Averaged dose-ranging study results for the four Enterobacteriaceae isolates examined (**A.** *E. coli* 30854, **B.**
477 *E. coli* 39930, **C.** *K. pneumoniae* 25021, **D.** *K. pneumoniae* 604). The data series with black outlines represent regimens
478 found to contain a drug-resistant sub-population on or before the 24-hour time point. FEP=Cefepime, TAZ=tazobactam.



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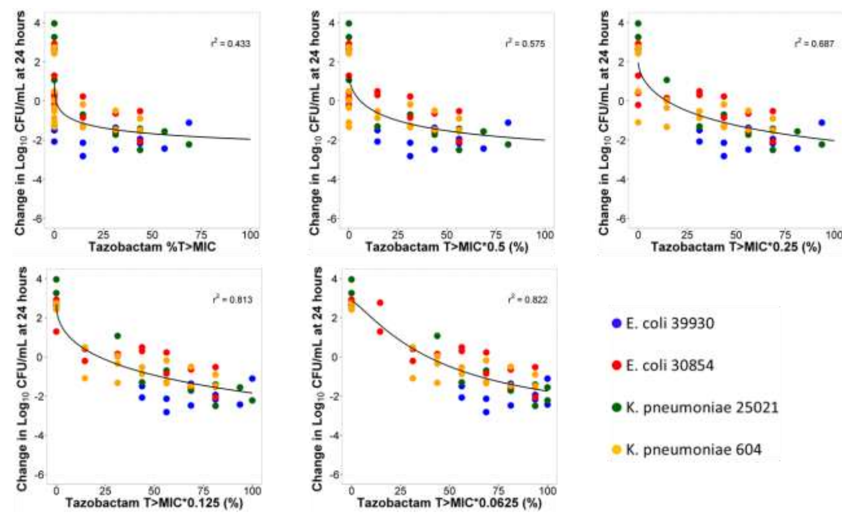
480 **Figure 3.** Relationship between change in $\log_{10}$ CFU after 24 hours of exposure and tazobactam %T>threshold based
481 on data from a one-compartment *in vitro* infection model. The tazobactam threshold concentrations evaluated represent
482 the product of each individual isolates cefepime-tazobactam MIC and either 1, 0.5, 0.25, or 0.125. Different colors
483 represent each of the four different isolates examined



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