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ORIGINAL ARTICLE

Prediction of *In Vivo* and *In Vitro* Infection Model Results Using a Semimechanistic Model of Avibactam and Aztreonam Combination Against Multidrug Resistant Organisms

SKB Sy[1], L Zhuang[1], H Xia[2], M-E Beaudoin[3], VJ Schuck[3] and H Derendorf[1]*

The combination of aztreonam-avibactam is active against multidrug-resistant *Enterobacteriaceae* that express metallo-β-lactamases. A complex synergistic interaction exists between aztreonam and avibactam bactericidal activities that have not been quantitatively explored. A two-state semimechanistic pharmacokinetic/pharmacodynamic (PK/PD) logistic growth model was developed to account for antimicrobial activities in the combination of bacteria-mediated degradation of aztreonam and the inhibition of aztreonam degradation by avibactam. The model predicted that changing regimens of 2 g aztreonam plus 0.375 and 0.6 g avibactam as a 1-hour infusion were qualitatively similar to that observed from *in vivo* murine thigh infection and hollow-fiber infection models previously reported in the literature with 24-hour log kill $\geq$1. The current approach to characterize the effect of avibactam in enhancing aztreonam activity from time-kill study was accomplished by shifting the half-maximal effective concentration (EC_{50}) of aztreonam in increasing avibactam concentration using a nonlinear equation as a function of avibactam concentration, providing a framework for translational predictions.
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Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ *In vitro* and *in vivo* infection models have shown efficacy of aztreonam-avibactam combination against *Enterobacteriaceae* harboring metallo-β-lactamases.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ How preclinical information of a drug combination efficacy can be described using a semimechanistic system model and how well does a mathematical model compare to animal models of infection in predicting bacterial response to human dosing regimens of aztreonam-avibactam.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ This study indicates that it is feasible to determine the 24-hour bacterial response to clinical dosing regimens using *in vitro* susceptibility information from the checkerboard assay that evaluates susceptibility of bacteria to the combination antibiotics.

HOW MIGHT THIS CHANGE DRUG DISCOVERY, DEVELOPMENT, AND/OR THERAPEUTICS?

☑ This model prospectively defines dosing regimens that can be effective against microbial infection in an *in vivo* setting, as it provides testable predictions for expected bacterial response to aztreonam-avibactam regimens.

The current shortage of antibiotic options against multidrug-resistant bacteria, and, in particular, the rise of bacterial isolates that are resistant to all available single-agent therapies and some β-lactam (BL)/β-lactamase inhibitor combination is prompting a global health crisis. The older generation of BL/β-lactamase inhibitor combinations, such as ticarcillin/clavulanate, amoxicillin/clavulanate, ampicillin/sulbactam, and piperacillin/tazobactam have coverage against only class A β-lactamase enzymes and are ineffective against class B, C, and D β-lactamases.[1–3] Bacterial isolates that expressed the extended spectrum β-lactamases, *Klebsiella pneumoniae* carbapenemase, and AmpC β-lactamases are resistant to these combination chemotherapies.[1,3] Bacteria carrying these β-lactamases present a challenge to treat secondary antimicrobial resistance against β-lactamase inhibitors.

Ceftazidime/avibactam combination was approved by the US Food and Drug Administration in 2015 for the treatment against complicated intra-abdominal and complicated urinary tract infections,[4] and in 2016 by the European Medicines Agency for complicated intra-abdominal, complicated urinary tract infection, hospital and ventilator-acquired pneumonia, and aerobic gram-negative infections with limited treatment options.[5] Avibactam has a much larger range of coverage against β-lactamases, encompassing enzymes belonging to class A, C, and even some D, including extended spectrum β-lactamases, serine-based carbapenemases, and *Klebsiella pneumoniae* carbapenemases.[6–8] The inhibition of β-lactamases by avibactam occurs by the formation of a highly stable covalent bond with the enzyme's active serine; the covalent adduct formation inactivates the β-lactamases.[7] Avibactam has no measureable

[1]Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, Florida, USA; [2]Department of Molecular Genetics and Microbiology, College of Medicine, University of Florida, Gainesville, Florida, USA; [3]AstraZeneca, Waltham, Massachusetts, USA. *Correspondence: H. Derendorf (hartmut@cop.ufl.edu)
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activity against metallo-β-lactamases.[9,10] Given that aztreonam is impervious to hydrolysis by metallo-β-lactamases, combination with avibactam presents a strategic approach to the treatment of infections caused by isolates with multiple β-lactamase profiles, including those containing class B β-lactamases.[5] The time-dependent pharmacodynamic (PD) indices ($fT>$MIC; $fT>C_T$) for aztreonam/avibactam were 50% $fT>$MIC for aztreonam and 50% $fT>C_T$ at 2–2.5 mg/L threshold avibactam concentration (C_T) against highly resistant *K. pneumoniae* and *Escherichia coli*.[11,12] These target PD indices for both aztreonam and avibactam are consistent with the humanized dosing of 2 g/0.375 g and 2 g/0.6 g aztreonam/avibactam against metallo-β-lactamase-producing gram-negative organisms in the mouse thigh infection model.

As there are several published *in vitro* and *in vivo* studies that characterize the antimicrobial activities of aztreonam/avibactam,[11–13] no mathematical model has yet been developed to describe the actions of combined chemotherapies. Additionally, the PD indices are based merely on summary endpoints of pharmacokinetic (PK) parameters and point estimates of the effect, wherein many interactions between drug concentration and bacteria responses are lost.[14] A better approach should consider connecting the full time-course of the combination to the dose-dependent bacterial response using a semimechanistic PK/PD model to guide dose selection in the clinic.[15–17]

This study aimed to build a semimechanistic PK/PD model of aztreonam/avibactam with the following steps: (1) quantify the time course of growth and killing of four highly drug-resistant clinical isolates in response to aztreonam/avibactam combination using a semimechanistic model[13,20]; (2) qualify this model by simulating the PK profiles of humanized dosing in *in vitro* and *in vivo* infection models, as well as the corresponding bacterial response using the developed model. An overview of antimicrobial PK/PD is furnished in the **Supplementary Material** to explain the PK/PD approach in antimicrobial therapy, strategy, and significance of the current modeling approach.

MATERIALS AND METHODS

Bacteria strains and time-kill kinetic studies

The data source for the mathematical model development came from previously reported constant-concentration (static) time-kill kinetic studies of avibactam and aztreonam combinations against four multidrug-resistant clinical isolates: *Escherichia coli* ARC3600 (NDM-1, CMY-6, and OXA-1), ARC3807 (NDM-1, SHV-12, TEM-1, OXA-9, and CMY-42), *Klebsiella pneumoniae* ARC3802 (NDM-1, SHV-2a, SHV-11, CTX-M-15, and TEM-1), and *Pseudomonas aeruginosa* ARC3928 (OXA-2 and GES-1).[13] The minimum inhibitory concentrations (MICs) of aztreonam alone against these isolates were 32, 2,048, 128, and 512 mg/L, respectively, and the MICs of aztreonam-avibactam (avibactam at 4 mg/L) were 0.5, 4, 0.25, and 32 mg/L.[13] The static time-kill study concentration of aztreonam and avibactam investigated as both single-agent and combination ranged from 0.015–8,192 mg/L and from 1–64 mg/L, respectively, in twofold increments. The degradation of both aztreonam and avibactam in the time-kill studies were monitored during the static time-kill study. The PD hollow-fiber infection model studies of aztreonam-avibactam against *K. pneumoniae* ARC3802 came from Singh *et al*.[11]; the neutropenic and immunocompetent mouse thigh infection models of human-simulated drug concentrations were previously reported by Crandon and Nicolau.[12]

In vitro pharmacodynamic model

The schematic representation of the effect of aztreonam and avibactam on bacterial density dynamics, as well as the degradation of aztreonam due to β-lactamase activities of the bacteria, is shown in **Supplementary Material Figure S1**. The core PK/PD model to describe the *in vitro* bacterial density changes came from the logistic growth model,[18,19] and the structural model for the bacterial population included two compartments representing two subpopulations of bacteria: P_1 (actively growing and responding to aztreonam concentration as affected by the concentration of avibactam) and P_2 (resting or dormant and not responding to changes in aztreonam or avibactam concentrations). Aztreonam degradation that is dependent on the bacteria density was also modeled mathematically as a function of the density of P_1, assuming that $P_1 \gg P_2$. The majority of bacteria was assumed to be in the P_1 state at the start of the experiment. The initial condition for the secondary state P_2 was $1/10^7$ of P_1.[20] The logistic growth model was modified to incorporate bactericidal effect of both aztreonam and avibactam, which were described separately by two sigmoidal maximum effect (E_{max}) models.

The modified logistic growth model was formulated as follows:

$$\frac{dP_1}{dt} = -\left[\frac{E_{max,ATM}\cdot ATM^{\gamma}}{(A\exp(-\alpha AVI)+B\ \exp(-\beta AVI))^{\gamma}+ATM^{\gamma}}+\frac{E_{max,AVI}\cdot AVI^{\phi}}{EC_{50AVI}^{\phi}+AVI^{\phi}}-k_{12}\right]P_1+I\left[k_{growth,1}\left(1-\frac{P_1+P_2}{N_{max}}\right)\right]P_1 \quad (1)$$

$$\frac{dP_2}{dt} = \left[k_{growth,2}\left(1-\frac{P_1+P_2}{N_{max}}\right)\right]P_2+k_{12}P_1 \quad (2)$$

The description of model parameters and symbols can be found in **Table 1** and in the **Supplementary Materials**. The fitness of P_1 is assumed to be greater than P_2 by setting $k_{growth,2}$ to 1/20 of $k_{growth,1}$ estimated value.[21] The initial condition of the resting state was 0.1 $\log_{10}$ cfu/mL. The bactericidal effect of aztreonam was described by a sigmoidal E_{max} model for all bacteria isolates. The synergistic effect of avibactam was characterized such that increasing avibactam concentrations decrease the half-maximal effective concentration (EC_{50}) of aztreonam. The decreasing aztreonam EC_{50} was described by a bi-exponential function of avibactam concentration on the aztreonam EC_{50}. Another sigmoidal E_{max} model was used to describe the antimicrobial effect of avibactam, which was only observed in *E. coli* ARC3600 and ARC3807. I is the delay function to allow

Table 1 Parameter estimates for the bacterial population dynamic model developed from four clinical isolates of multidrug resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa* time-kill kinetics and aztreonam degradation

		Parameter ± SE			
Parameter	**Description**	***E. coli* ARC3600**	***E. coli* ARC3807**	***K. pneumoniae* ARC3802**	***P. aeruginosa* ARC3928**
N_{max}, log_{10} cfu/mL	Maximum achievable carrying capacity in the system	10.2 ± 0.175	10.0 ± 0.263	10.0 ± 0.198	11.6 ± 1.55
$k_{growth,1}$, h^{-1}	Bacterial growth rate constant associated with log_{10} of active population	1.28 ± 0.223	0.984 ± 0.0887	0.461 ± 0.0249	0.113 ± 0.0461
$E_{max,ATM}$, h^{-1}	Maximum kill rate constant due to aztreonam	0.266 ± 0.0318	0.287 ± 0.00926	0.313 ± 0.0059	0.142 ± 0.0293
A, mg/L	First parameter of bi-exponential function to characterize aztreonam EC_{50} in monotherapy	52.8 ± 7.85	2560 ± 474	213 ± 9.0	320 ± 28.3
B, mg/L	Second parameter of bi-exponential function to characterize aztreonam EC_{50} in monotherapy	2.36 ± 0.549	31.4 ± 14.0	0.321 ± 0.0318	27.4 ± 9.21
α, L/mg	Exponential constant associated with A parameter that describes the relationship between avibactam concentration and potency of aztreonam	14.9 ± 2.64	5.06 ± 0.5	7.63 ± 0.298	1.32 ± 0.140
β, L/mg	Exponential constant associated with B parameter that describes the relationship between avibactam concentration and potency of aztreonam	0.480 ± 0.0409	0.401 ± 0.0633	0.174 ± 0.0149	0.0642 ± 0.0425
γ	Hill coefficient that determined the steepness of the slope of the sigmoidal E_{max} curve associated with avibactam enhancement of aztreonam potency	1.95 ± 0.509	1.30 ± 0.0555	2.98 ± 0.0912	2.79 ± 0.999
δ, h^{-1}	Exponential constant of the delay function to retard growth of active population	0.0569[a] fix 0.0119[b] fix 0.0160[c] fix	0.0296[a] fix 0.0331[b] fix 0.0232[c] fix	0.132 fix	0.270 fix
k_{12}, h^{-1}	Rate constant for the conversion of bacterial cells from active to resting states	0.005 fix	0.005 fix	0.005 fix	0.005 fix
$E_{max,AVI}$, h^{-1}	Maximum kill rate constant due to avibactam	0.262 ± 0.0355	0.180 ± 0.0156	–	
$EC_{50,AVI}$, mg/L	Concentration of avibactam that produces 50% of $E_{max,AVI}$	14.2 ± 0.540	26.6 ± 3.02	–	
Φ	Hill coefficient that determined the steepness of the slope of the sigmoidal E_{max} curve associated with avibactam bactericidal effect	5.71 ± 0.822	1.98 ± 0.0477	–	
Deg_{max}, h^{-1}	Maximum degradation rate constant of aztreonam	–	–	0.190 ± 0.00677	0.406 ± 0.00774
K_m, log_{10} cfu/mL	Log_{10}-transformed cfu number density that yielded 50% of the maximum degradation rate	–	–	7.5 fix	6.87 fix
φ	Hill coefficient that determined the slope of sigmoidal E_{max} model for aztreonam degradation	–	–	3.59 ± 0.11	4.15 ± 0.662

E_{max}, maximum effect.
[a]Avibactam only; [b]aztreonam only; [c]both aztreonam and avibactam present.

the model to bend to the shape of the bacteria during the initial decline and also the regrowth of bacteria at the later timepoints was characterized such that:

$$I=\begin{cases}1, & \textit{if aztreonam and avibactam}=0 \text{ (all conditions)}\\ 1-\exp(-\delta t), & \textit{otherwise}\end{cases} \quad (3)$$

The degradation of aztreonam during the time-kill experiment in *K. pneumoniae* ARC3802 and *P. aeruginosa* 3928 was best described by a second-order nonlinear function dependent on both aztreonam concentration and bacterial density of P_1 (Eq. 4):

$$\frac{dATM}{dt}=-\frac{Deg_{max}\cdot P_1^{\varphi}}{K_m^{\varphi}+P_1^{\varphi}}ATM \quad (4)$$

Qualitatively, K_m was the value of P_1 at which rapid aztreonam degradation commenced. The φ parameter

determined the shape of the function. When φ is a large value (>3), the Hill function behaved as a switch over the range of values of P_1 from just below K_m to just above K_m.

As shown in Eq. 4, the degradation of aztreonam during the time-kill experiment was dependent on both the aztreonam concentration and the bacteria density in P_1 state. When avibactam was present in the system, no degradation was observed. Consequently, Deg_{max} was set to 0 in the presence of avibactam.

Given that the progeny was derived from a single inoculum and was assumed to be genetically identical, as long as they are from the same strain, as well as the bacteria in all drug combinations were from the same colony, the bacteria response to various combinations of drug concentrations was assumed to be reproducible with minimal variability. No interconcentration variability was incorporated to any of the model parameters.

The residual variability that described the difference between the observed and model-predicted value was an additive model. The data used for model development were based on the $\log_{10}$-transformed data and the residual errors conform to a normal distribution after transformation.

Model qualification

The external data for model qualification were extracted from literature sources. The study of Crandon and Nicolau[12] evaluated human-simulated aztreonam-avibactam doses in murine thigh infection models against 14 NDM-expressing *Enterobacteriaceae* and 13 multidrug-resistant *P. aeruginosa*. The range of MIC of aztreonam alone against these microorganisms ranged from 8 to >256 mg/L and the MICs of aztreonam-avibactam combination (with 4 mg/L avibactam) were between 0.13 and 32 mg/L. The human-simulated dose equivalent of 2 g aztreonam and 0.375 or 0.6 g avibactam every 6 hours as a 1-hour infusion was administered to both neutropenic and immunocompetent mice. Aztreonam exposures in the mouse infection models were derived from a previously published population PK model in patients with cystic fibrosis,[22] whereas avibactam exposures were simulated from a population PK model of ceftazidime-avibactam combination.[23] The typical human free-drug concentration-time profiles of aztreonam and avibactam in administration of 2 g aztreonam alone and in combination with 0.375 or 0.6 g avibactam were used as PK input for the PD model. The simulations also included control groups, wherein no drug was administered. The initial inoculum was 6.5 $\log_{10}$ cfu/mL.

The second data source investigated constant 4 mg/L avibactam concentration with dynamically changing aztreonam concentrations against *K. pneumoniae* ARC3802 (reported MIC aztreonam alone/combination at 4 mg/L avibactam: 256 mg/L/0.5 mg/L) in the hollow-fiber infection model (HFIM).[11] The PK model for aztreonam concentration in HFIM was a one-compartment intravenous bolus starting from specific maximum free aztreonam concentrations (fC_{max}) with a drug elimination half-life of 2 hours. The simulation of the PK model of aztreonam assumed a one-compartment model with a volume of distribution (V) of 1 L and clearance of 0.347 L/h, which is equivalent to an elimination rate constant (k_e) of the natural logarithm of 2 divided by the half-life of 2 hours, because $k_e = CL/V$. The regimens of aztreonam with constant 4 mg/L avibactam and as monotherapy are described in the **Supplementary Material**. The evaluation was based on comparison of 24-hour bacteria density in $\log_{10}$ cfu/mL between model prediction and HFIM, assuming an initial inoculum size of 6.5 $\log_{10}$ cfu/mL.

Predictability of minimum inhibitory concentration-fitted aztreonam half-maximal effective concentration function on the 24-hour bacteria density

A bi-exponential model (Eq. 5) was fitted to the MIC of aztreonam with 0–8 mg/L avibactam against *E. coli* ARC3807 and *K. pneumoniae* ARC3802:

$$A\exp(-\alpha AVI)+B\ \exp(-\beta AVI) \quad (5)$$

The parameter estimates A, B, α, and β obtained from the model fit were substituted into Eq. 1 to determine the time-course of bacterial population dynamics in response to the same static concentrations of the combination previously evaluated in the time-kill kinetic studies,[13] assuming a starting inoculum size of 6.3 $\log_{10}$ cfu/mL, which is similar to the condition in the time-kill study.

The 24-hour bacteria density in $\log_{10}$ cfu/mL from the prediction using the MIC-fitted bi-exponential model substituted into Eq. 1 was then compared to the same target metrics determined by the PD model developed from the time-kill kinetic information.

RESULTS

Pharmacodynamic model development from static time-kill data

The concentration ranges of aztreonam and avibactam in static time-kill kinetic studies[13] were designed to provide optimized information for model development. The avibactam concentrations in twofold increments in the combination were 1–8 mg/L. Depending on the MIC of aztreonam at the respective avibactam concentrations, the drug concentration of aztreonam used in the static time-kill kinetic studies ranged from 0.25-fold to 4-fold MIC. The bacteria responses at concentrations lower than 0.25-fold MIC were similar to growth control, whereas bacteria responses at greater than 4-fold MIC resulted in significant bacteria kill. The experimental design of the static time-kill kinetic studies covered the necessary concentration range to observe bacteria kill and regrowth patterns. Significant aztreonam degradation was observed at ≤MIC at the respective avibactam concentrations in *K. pneumoniae* ARC3802 and *P. aeruginosa* ARC3928, but no degradation was observed in the two *E. coli* isolates.[13]

The time-course of the bacterial population dynamics and aztreonam degradation during the course of time-kill kinetic studies were modeled by a two-state PK/PD model for the bacterial system that affects the drug degradation process. The model parameter estimates and SE of the estimates are summarized in **Table 1**. We utilized a nonlinear aztreonam EC_{50} function dependent on avibactam concentration for the sigmoidal E_{max} function in the PK/PD model to

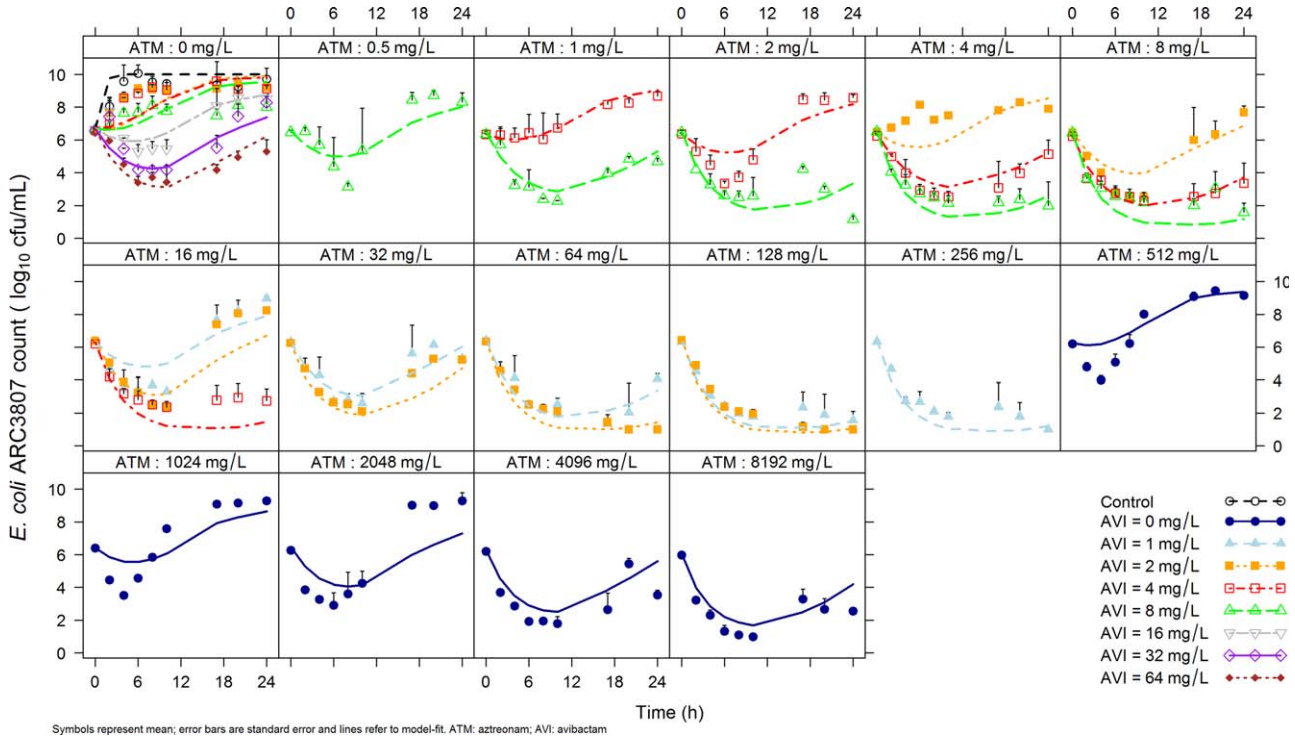

Figure 1 Model-prediction and observed static time-kill curves of aztreonam and avibactam against *E. coli* ARC3807 over 24 hours. The points show the experimental data; color-matched lines show the results of the pharmacokinetic/pharmacodynamic model described in the text.

describe how avibactam conferred aztreonam efficacy. This empirical approach described well the increase in bacterial susceptibility toward aztreonam, as shown in representative **Figures 1–3** for *E. coli* ARC3807, *K. pneumoniae* ARC3802, and *P. aeruginosa* ARC3928. The model fit of bacteria response for *E. coli* ARC3600 can be found in **Supplementary Figure S2**. This EC_{50} function was characterized by a bi-exponential model to shift the EC_{50} of aztreonam kill to a lower value at each incremental avibactam concentration. The resulting EC_{50} values of aztreonam follow closely the MIC values at respective avibactam concentrations. For example, aztreonam MIC against *E. coli* ARC3807 decreased from 2,048 to 4 mg/L at 0 and 4 mg/L avibactam[13] and aztreonam EC_{50} was predicted to decrease from 2,591 to 4.6 mg/L for the same range of avibactam concentration. The predicted aztreonam EC_{50} values against the other three isolates were also very close to the MIC values at respective avibactam concentrations.

A separate bactericidal effect of avibactam was incorporated to the model using another sigmoidal E_{max} model in the two *E. coli* isolates, wherein avibactam was shown to have activity. The EC_{50} values of avibactam were 14 and 25 mg/L (**Table 1**), which were close approximation of the MIC values of avibactam (16 mg/L) for both *E. coli* ARC3600 and ARC3807, respectively.[13]

The drug degradation was characterized by a nonlinear function that was dependent on both the active bacterial population and the remaining drug in the system for *K. pneumoniae* ARC3802 and *P. aeruginosa* ARC3928, as shown in the lower panel of **Figures 2 and 3**. The addition of avibactam resulted in no drug degradation of aztreonam in both isolates, even at the lowest concentrations of 1 and 2 mg/L avibactam in both isolates, respectively. The maximum degradation rate in the drug degradation model was set to 0 when avibactam was present in the system. Drug degradation model was not incorporated in the two *E. coli* isolates because no aztreonam degradation was observed in the time-kill study.[13]

Models with successful minimization or convergence without boundary problems were accepted as the final model. Models were fitted separately to drug degradation and bacterial response information for the data from *K. pneumoniae* and *P. aeruginosa* isolates. A model that did not include the drug degradation process was initially developed for bacterial response data, assuming no drug degradation. In the second phase of model development, the parameters determined from the initial model that did not include drug degradation were fixed to obtain parameters for aztreonam degradation. The densities of the active population that resulted in half the maximum degradation were 7.5 and 6.87 log_{10} cfu/mL, respectively, indicating that aztreonam was efficiently removed even when the drug was just added. The 32 and 64 mg/L aztreonam panels in **Figure 2** for *K. pneumoniae* ARC3802 show a very rapid decline in remaining aztreonam in the closed system. For *P. aeruginosa* ARC3928, the 128 to 512 mg/L aztreonam panels in **Figure 3** show an effective drug removal even when bacteria density was decreasing.

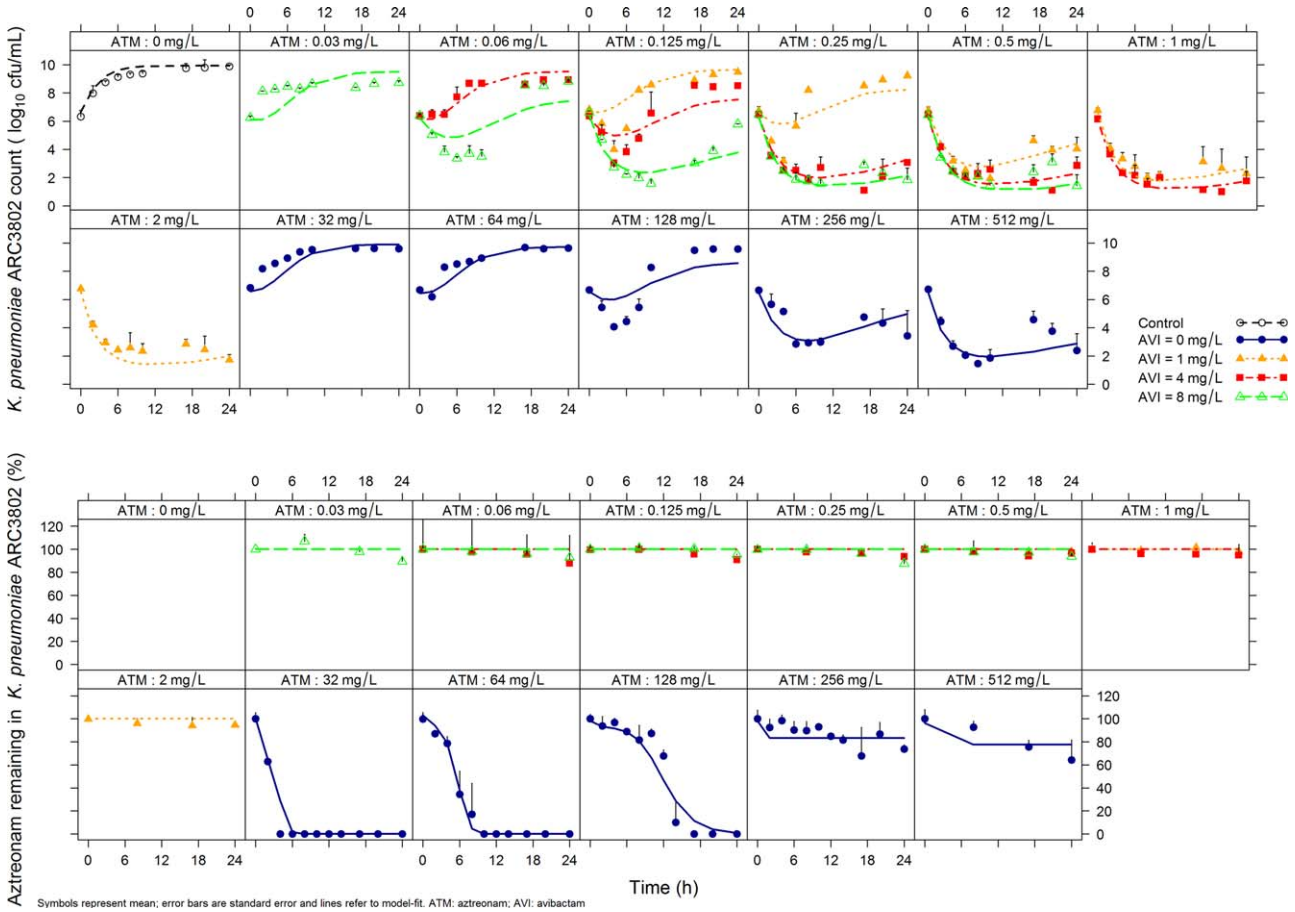

Figure 2 Model-prediction and observed static time-kill curves of aztreonam and avibactam against *K. pneumoniae* ARC3802 over 24 hours (top) and aztreonam concentration remaining in the system during the time-kill experiment (bottom). The points show the experimental data; color-matched lines show the results of the pharmacokinetic/pharmacodynamic model described in the text.

Once the degradation model parameters were obtained, the degradation model was incorporated to the bacteria response model with the parameters from the drug degradation model fixed. The reported parameters in **Table 1** are final estimates of the integrated model.

Model prediction of *in vivo* murine and *in vitro* hollow-fiber infection models

The first part of external validation of the PD model of aztreonam-avibactam activities in the four isolates was carried using *in vivo* efficacies data from murine thigh infection models, reported by Crandon and Nicolau.[12] Their study used both immunocompetent and neutropenic mice whose thigh were inoculated with MDR *P. aeruginosa*; NDM-containing *Enterobactericeae* studies were conducted only with neutropenic mice. In the current simulation exercise, the free drug concentration-time profiles from the same dosing regimens were used to simulate bacteria response for the four isolates that were used in the PD model development. The human-simulated aztreonam and avibactam free drug concentration profiles of 2 g aztreonam, 375 mg, or 600 mg avibactam every 6 hours as 1-hour infusions are shown in **Supplementary Figure S3**.

Figure 4 shows the change in 24-hour bacterial density from the initial inoculum size of ~6.5 cfu/mL for the four bacterial isolates used for model development in the current study, under the "*in silico*" section. For comparison purposes, the bacterial responses from the *in vivo* thigh infection models are also shown in **Figure 4**. The maximum free aztreonam concentration after a 2 g dose in an hour infusion was ~95.5 mg/L, which is still below the MIC in aztreonam alone against the isolates evaluated in our model, with the exception of *E. coli* ARC3600. As expected, the regimen containing aztreonam alone resulted in a net positive change at 24 hours (data not shown). The two aztreonam regimens containing avibactam resulted in at least 1 $\log_{10}$ kill at 24 hours, including *P. aeruginosa* ARC3928, wherein the decrease in aztreonam MIC in the presence of avibactam against this isolate was not as much as the other three isolates. No significant difference in efficacy between the two avibactam-containing regimens (0.375 vs. 0.6 g) was observed, consistent with the *in vivo* efficacies in the murine infection models. Aztreonam MIC values with 4 mg/L avibactam against *Enterobacteriaceae* in this study are well below the breakpoints for susceptibility (MIC breakpoint $\leq$4 mg/L).[24] Consequently, the $\log_{10}$ kill in the *E. coli* and *K. pneumoniae* isolates are well over 3 $\log_{10}$ units in the

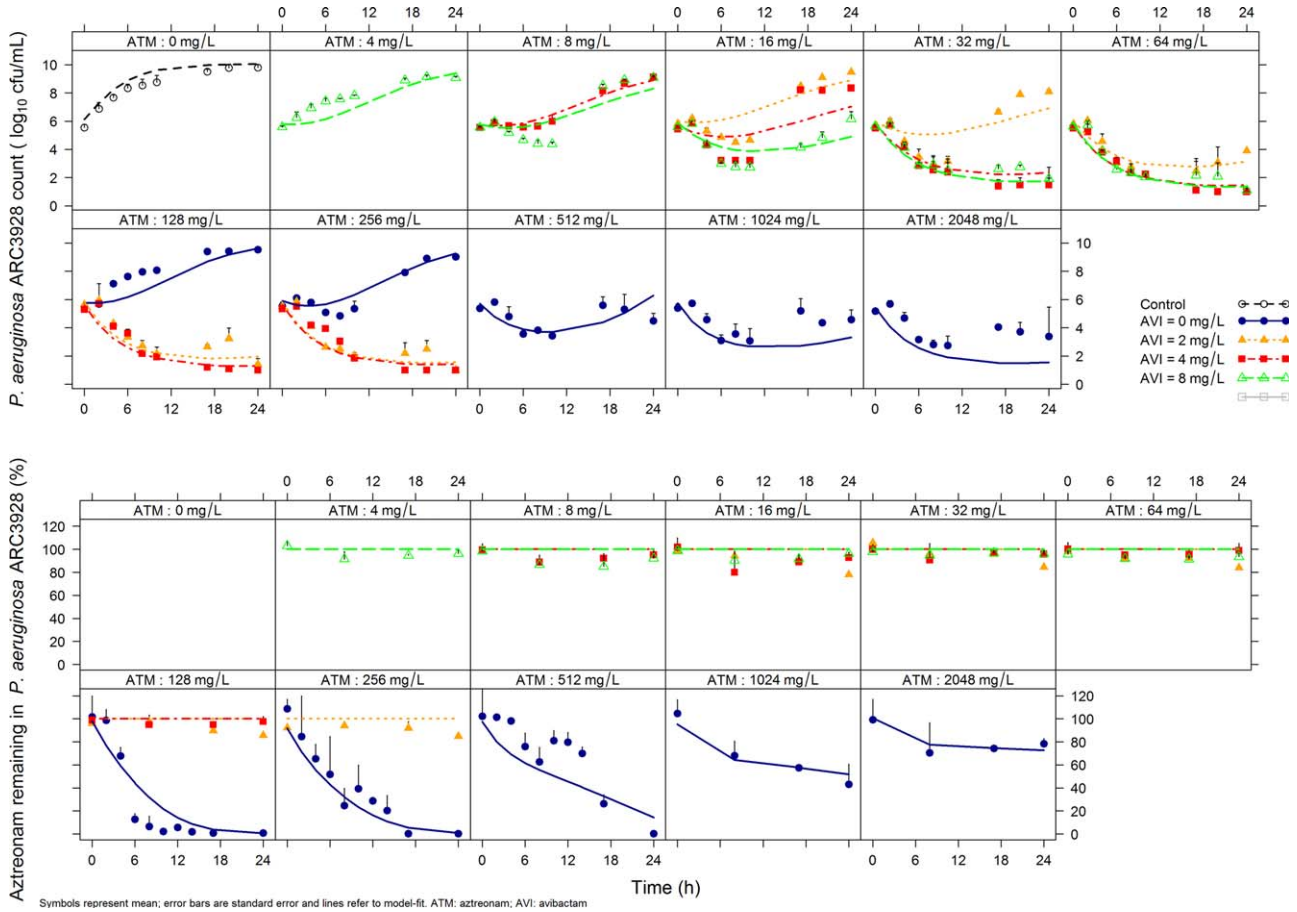

Figure 3 Model-prediction and observed static time-kill curves of aztreonam and avibactam against *P. aeruginosa* ARC3928 over 24 hours (top) and aztreonam concentration remaining in the system during the time-kill experiment (bottom). The points show the experimental data; color-matched lines show the results of the pharmacokinetic/pharmacodynamic model described in the text.

aztreonam-avibactam combination. In contrast, aztreonam MIC in the presence of 4 mg/L avibactam against *P. aeruginosa* ARC3928 was 32 mg/L. The 24-hour $\log_{10}$ kill in this isolate was 1 and 1.75 $\log_{10}$ unit in the 0.375 and 0.6 g avibactam with 2 g aztreonam combination, respectively, for this isolate.

The second external validation was accomplished by comparison of 24 hours $\log_{10}$ bacteria density of *K. pneumoniae* ARC3802 from model prediction and from HFIM in response to various aztreonam regimens with constant 4 mg/L avibactam concentration. The aztreonam profiles were assumed to be identical to a one-compartment model with intravenous bolus administration and starting with aztreonam concentration at the free maximum drug concentration (fC_{max}) listed in Singh *et al.*[11] The model-predicted 24-hour bacterial densities, shown in **Figure 5**, were very close to the observed values from the HFIM for the q24-hour and q6-hour dosing regimens, as well as the monotherapy treatments (i.e., aztreonam alone or avibactam alone). A discrepancy between the observed and model predicted densities was found for the q12-hour aztreonam regimen with constant 4 mg/L avibactam, wherein the model predicted a net 1.3 $\log_{10}$ kill units, whereas the HFIM resulted in a larger kill of 4.8 $\log_{10}$ units. The overall model prediction of the expected bacterial responses were consistent with those observed in both *in vitro* and *in vivo* aztreonam-avibactam efficacy evaluation, even in the case of dynamically changing drug concentrations.

Prediction of 24-hour bacteria density using minimum inhibitory concentration-fitted aztreonam EC_{50} function

The utility of substituting the aztreonam EC_{50} function with a MIC-fitted function was evaluated for the purpose of determining whether MIC-avibactam relationship defined by a nonlinear equation can be swapped with the EC_{50} function determined from the time-kill kinetic studies. This exercise was performed on both *E. coli* ARC3807 and *K. pneumoniae* ARC3802. The bi-exponential models for the MIC values as a function of avibactam concentration are shown in Eqs. 6 and 7:

$$E.\ coli\ \text{ARC3807}: \quad MIC = 1991\exp(-4.057AVI) + 56.87\exp(-0.6541AVI) \tag{6}$$

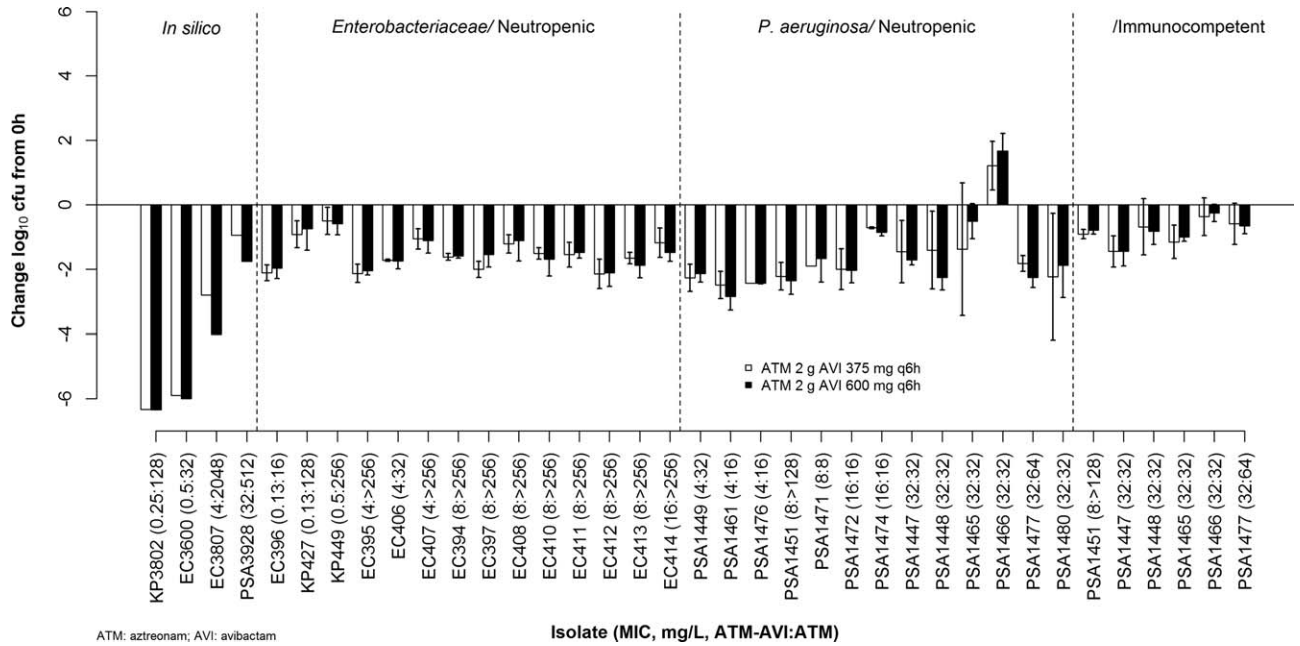

Figure 4 Change in $\log_{10}$ bacteria density at 24 hours from model prediction (*in silico*) and *Enterobacteriaceae* in neutropenic mouse thigh infection model and *P. aeruginosa* in both neutropenic and immunocompetent mouse thigh infection model from Crandon & Nicolau.[12] Model-based responses to the Monte Carlo simulations of free drug concentration-time profiles are represented in the "*in silico*" section. Observed bacterial responses in neutropenic and immunocompetent mouse thigh infection models against *Enterobacteriaceae* and *P. aeruginosa*. The minimum inhibitory concentration of aztreonam in 4 mg/L avibactam and aztreonam alone against the isolates is shown in brackets after the isolate number.

$$K.\ pneumoniae\ \text{ARC3802}: MIC = 128\exp(-6.447AVI) + 0.331\exp(-0.106AVI) \quad (7)$$

These two equations replaced the bi-exponential model for aztreonam EC_{50} function in Eq. 1. The resulting bacteria density at 24 hours predicted using the substituted MIC-derived bi-exponential functions in Eqs. 6 and 7 were compared with the original model fitted values in **Figure 6** for *E. coli* ARC3807 and *K. pneumoniae* ARC3802, respectively, assuming an initial inoculum size of 6.3 $\log_{10}$ cfu/mL. The MIC-derived 24-hour densities were very close to the original estimated densities from the PD model fit to the time-kill kinetic data, indicating that one can use the MIC-derived bi-exponential equation to derive an expected bacterial response that is close to the time-kill kinetic study results.

DISCUSSION

Model development

The modeling approach developed in the present study was designed to efficiently characterize the PD action of two antibiotics in combination. The combined action was characterized by a shift in EC_{50} function, given the substantial increase in microorganism's susceptibility to aztreonam in increasing avibactam concentration. An empirical model was applied to describe the enhanced potency of aztreonam, as represented by the EC_{50} function within the sigmoidal E_{max} model, as a function of avibactam concentration. This approach contrasts our previous approach to characterize the synergistic effect of an aminoglycoside and a BL, wherein we utilized Loewe additivity model.[15] The difference is that both aminoglycoside and BL have intrinsic antimicrobial activity, whereas avibactam has very limited activity against *E. coli* at high concentrations and has no intrinsic activity against *K. pneumoniae* and *P. aerugionosa* isolates.[11,13] The Loewe additivity model is better suited for evaluating synergy in combination antibiotics, wherein each drug has its own effect in a monotherapy setting. The current approach of shifting EC_{50} was also applied to the previous aminoglycoside and BL combination and described well the trend in bacteria killing over a 24-hour time course (unpublished data).

The advantage of the current approach of using a mathematical function to shift the EC_{50} of the active BL agent by a partnering β-lactamase inhibitor is that this function can be approximated by the MIC values at respective avibactam concentrations. Mouton and Vinks[25] had previously shown that MIC and EC_{50} are correlated for the logistic growth model. Because aztreonam EC_{50} values were very close to its MIC values at respective avibactam concentration, the bi-exponential EC_{50} function derived from the time-kill kinetic study can be swapped with a bi-exponential MIC function determined from the checkerboard assay. We have shown that the 24-hour bacterial density can be predicted from a bi-exponential MIC function at various combinations of aztreonam and avibactam concentrations in a virtual time-kill kinetic study setting. The simple approach wherein aztreonam EC_{50} value can be approximated by its MIC against specific microorganisms at respective avibactam

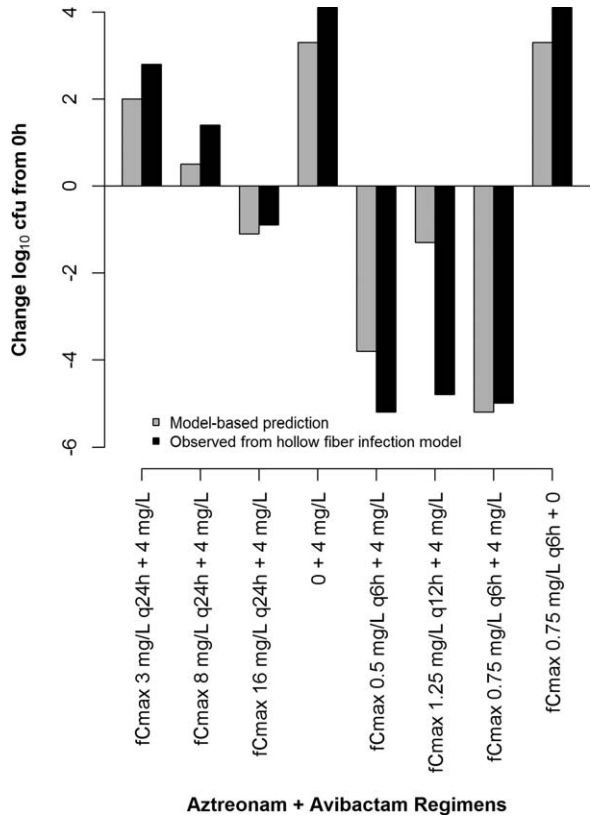

Figure 5 Model-predicted and observed *K. pneumoniae* ARC3802 24-hour $\log_{10}$ cfu/mL (change in $\log_{10}$ cfu/mL from time 0) at 24 hours with starting inoculum of ~6.5 $\log_{10}$ cfu/mL in response to various regimens of aztreonam and avibactam. Observed data were digitally extracted from Singh *et al.*[11] Model-based (gray) and observed (black) 24-hour bacterial responses to the simulation of free changing drug concentration following mono-exponential decline from fC_{max} starting values.

concentration allows for broader simulation of time-course of bacteria response using MIC values at respective avibactam concentration, given that the checkerboard assay for MIC determination is less labor-intensive than running a time-kill kinetic study.

Evaluating model against external *in vitro* data

The current approach to model qualification was carried out by examining the predictions of the model applied to external data sources. These sources were: *in vitro* constant concentration time-kill data[13]; and *in vitro* varying concentration and *in vivo* varying concentration 24-hour bacterial response data.[11,12]

The model described well the bacteria responses in both *in vitro* nonvarying and varying concentrations of aztreonam and avibactam. The HFIM with *K. pneumoniae* ARC3802[11] utilized identical bacteria isolate as the one used in the nonvarying concentration time-kill kinetic study.[13] The similarity between the model-predicted 24-hour bacteria response and the observed response in the varying concentration infection model indicates that the model is sufficiently robust to predict the bacteria response in varying-concentration scenarios. The model also captured the time-dependent nature of aztreonam bactericidal activity, as it predicts that more frequent dosing in the q6-hour scenario provides a better kill than the once or twice-daily frequency (**Figure 5**).

Testing model predictability against external *in vivo* data

The simulated bacteria response of the four clinical isolates over 24 hours to varying concentrations of aztreonam and avibactam were compared to bacterial responses to experimentally simulated patient exposures in the mouse thigh infection model (**Figure 4**). The killing predicted by the model for 2 g aztreonam with 375 or 600 mg avibactam every 6 hours were qualitatively similar to the mouse data

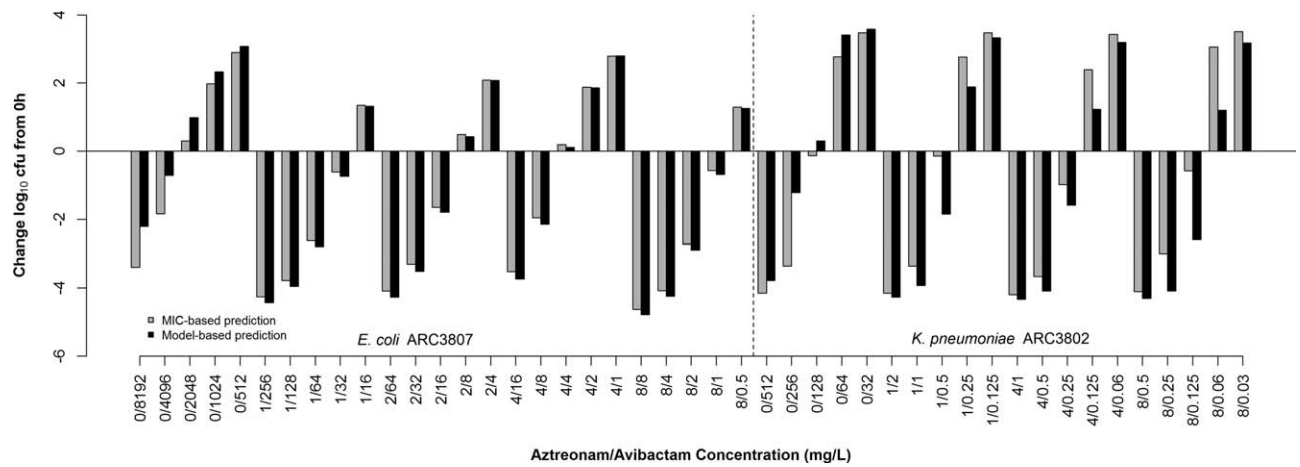

Figure 6 Comparison of the 24-hour bacteria density (change in $\log_{10}$ cfu/mL from 0 hour) of *E. coli* ARC3807 and *K. pneumoniae* ARC3802 for substituted minimum inhibitory concentration (MIC)-fitted aztreonam EC_{50} versus model-fitted aztreonam EC_{50} from time-kill kinetic study. Model-based responses to the simulations of static-free drug concentration using MIC-fitted EC_{50} equation (gray) and the time-kill model-fitted EC_{50} equation (black) for the original concentrations of aztreonam and avibactam used in the time-kill kinetic experiments.

for other isolates tested with similar humanized dosing regimens. There was no marked difference in log kill between 375 and 600 mg avibactam with 2 g aztreonam q6-hour, as shown both by the model and the animal data. The model-predicted killings were much larger than that in the animal model with the exception of *E. coli* ARC3807 and *P. aeruginosa* ARC3928. In the two isolates wherein aztreonam MIC values in combination with 4 mg/L avibactam were less than 1 mg/L, the net kill was predicted to be much greater as expected because these MIC values are markedly lower than the clinical breakpoints.[24] The model-predicted killing in *P. aeruginosa* ARC3928 was comparable to the animal data when aztreonam was administered along with avibactam. The fold decrease in MIC of aztreonam with 4 mg/L avibactam against *P. aeruginosa* ARC3928 isolate was much larger than the decrease observed in the isolates injected in the animals, compared to aztreonam alone. A stark difference in killing was predicted by the model between aztreonam with avibactam and aztreonam alone. The killing of the majority of *P. aeruginosa* isolates in the murine infection model[12] were not different between aztreonam with avibactam and aztreonam alone because there was no change in MIC against these isolates whether avibactam was added or not. The MIC values in these isolates were $\leq$32 mg/L, which is identical to the aztreonam MIC with avibactam against *P. aeruginosa* ARC3928. This aztreonam MIC is higher than the breakpoint for *P. aeruginosa* intermediate susceptibility criteria for standard 0.5 hour infusion[24] and a net kill was still observed in the animal data and was also predicted by the model using a 1-hour infusion regimen. It has been documented that longer infusion durations for time-dependent antimicrobial agents do shift the probability of target attainment and susceptibility profiles,[26] as longer infusion duration can increase the time that drug concentrations remained above the MIC.[27] The overall model-predicted responses in *P. aeruginosa* to aztreonam and avibactam regimens were quantitatively similar to the animal data.

The current model described well the effect of aztreonam-avibactam combination on bacterial population dynamics and aztreonam degradation in the absence of avibactam. Its predictive performance was evaluated by simulations of both *in vitro* and *in vivo* infection models of wide ranging resistance levels of *Enterobacteriaceae* and *P. aeruginosa*. The current approach of using external *in vitro* and *in vivo* infection data for model qualification is suitably relevant for evaluating model predictability. This mathematical model is robust and can be easily extended to predict behaviors of other isolates to different human-simulated regimens of aztreonam-avibactam combination. The current model is a useful translational tool for evaluation of other dosing regimens, which were not evaluated in this study, and can potentially serve as a guide for the design of human trials of aztreonam-avibactam against multidrug-resistant *Enterobacteriaceae* and *P. aeruginosa*.

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Conflict of Interest. Both M.E.B. and V.J.S. own stocks of AstraZeneca. H.D. received a grant from AstraZeneca. Currently, S.K.B.S. is an employee of Nektar Therapeutics; L.Z. and H.X. are employees of the US Food and Drug Administration; M.E.B. is an employee of Merck; and V.J.S. is an employee of Novartis.

Author Contributions. S.K.B.S., L.Z., H.X., and H.D. wrote the manuscript. S.K.B.S., V.J.S., and H.D. designed the research. S.K.B.S., L.Z., H.X., V.J.S., and M.E.B. performed the research. S.K.B.S., L.Z., and H.X. analyzed the data.

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