Interaction of *Staphylococcus aureus* and *Acinetobacter baumannii* during *In Vitro* β -Lactam Exposure

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ABSTRACT We sought to determine if *Acinetobacter baumannii* is capable of altering the pharmacodynamics of an antistaphylococcal β -lactam. Two strains of methicillin-susceptible *Staphylococcus aureus* (MSSA) and two *A. baumannii* isolates were studied in 24-h static time-killing experiments under monoculture or coculture conditions. Bacterial killing of meropenem was described using an empirical pharmacokinetics/pharmacodynamics model that was developed using Hill functions. A mechanism-based pharmacodynamic model was also used to describe the effect of meropenem on each species of bacterium, interspecies interactions, and strain-based covariate effects. Monte Carlo simulations of bacterial killing effects were generated based on the population pharmacokinetics of meropenem in 2,500 simulated critically ill subjects over 48 h. Against one of the two MSSA isolates, the magnitude of bacterial killing (E_d) decreased from -4.61 (95% confidence interval [CI], -5.85 to -3.38) to -2.23 (95% CI, -2.85 to -1.61) when cultured in the presence of carbapenem-resistant *A. baumannii* (CRAB). Similarly, the data were best described by a mechanism-based model where the number of *A. baumannii* cells produced a systematic increase in the *S. aureus* concentration for a 50% maximum killing effect (KC_{50}) of 3.53-fold, thereby decreasing MSSA sensitivity to meropenem. A covariate effect by the CRAB isolate resulted in a more pronounced increase in the MSSA KC_{50} for meropenem (31.8-fold increase). However, Monte Carlo simulations demonstrated that a high-intensity meropenem regimen is capable of sustained killing against both MSSA isolates despite protection from *A. baumannii*. Thus, *A. baumannii* and MSSA engage in complex interactions during β -lactam exposure, but optimal antimicrobial dosing is likely capable of killing MSSA despite the potentially beneficial interplay with *A. baumannii*.

KEYWORDS *Acinetobacter*, *Staphylococcus aureus*, beta-lactams, mathematical modeling, pharmacodynamics, polymicrobial

Staphylococcus aureus is a notoriously pathogenic organism that is capable of invasive infections in a diversity of sites within the body (1). The optimal treatment of *S. aureus* infections depends on a multitude of factors, including the site of infection, the resistance mechanisms of an isolate cultured from a patient, and the immunocompetence of the host. However, the presence of other pathogens at the same site of infection may obscure the optimal selection of antibacterials. Not only is *S. aureus* commonly cultured in polymicrobial skin and soft tissue infections, but *Staphylococcus* species have also been implicated in polymicrobial pneumonia, bacteremia, and prosthetic joint infections (2–8).

The majority of studies that have evaluated antimicrobial activity against *S. aureus* in the presence of other pathogens have focused on the duo of *S. aureus* and *Pseudomonas aeruginosa* (9–15). Previous investigations have demonstrated that *P.*

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aeruginosa is capable of modulating the killing of *S. aureus* by multiple antibacterials (11, 16–19). Moreover, previous investigations have also observed that *P. aeruginosa* may alter the pharmacodynamics (PD) of antistaphylococcal agents through the secretion of exoproducts that are not directly related to *P. aeruginosa* resistance mechanisms (16, 19). Understanding the selection of antibacterials during polymicrobial infections is therefore likely not as simple as evaluating the resistance mechanisms possessed by each pathogen.

While coculturing of *S. aureus* and *P. aeruginosa* has been well studied, the interactions between *S. aureus* and *Acinetobacter baumannii* (a nonfermenting Gram-negative pathogen similar to *P. aeruginosa*) remain largely unknown. Similar to *S. aureus*, *A. baumannii* has been implicated in a variety of polymicrobial infections (20–23). Although it is difficult to quantify the incidence of coculturing *S. aureus* and *A. baumannii* clinically, the two pathogens have been coisolated from diabetic foot infections (24–26), pneumonia (6, 27, 28), bacteremia (29), and other sites of infection as well (30, 31). A previous study reported that *A. baumannii* strains are capable of protecting other Gram-negative pathogens from β -lactam exposure, but the extension of this protective effect to *S. aureus* remains unexplored (32).

The objective of the current investigation was to culture methicillin-susceptible *S. aureus* (MSSA) with *A. baumannii* during β -lactam exposure to determine if *A. baumannii* is capable of altering the pharmacodynamics of antibacterials with antistaphylococcal activity. A secondary objective was to determine if the presence of *S. aureus* would alter the activity of β -lactam antimicrobials against *A. baumannii*. Finally, the investigation also sought to determine if a simulated regimen of a carbapenem would be effective against MSSA during coculture with *A. baumannii*. A carbapenem was selected as the β -lactam of interest because carbapenems are one of the drugs of choice against β -lactam-susceptible *A. baumannii*, and carbapenems also possess activity against MSSA (33).

RESULTS

Time-killing experiments. The killing of MSSA by meropenem in the time-killing experiments is depicted in Fig. 1a. Based on the observed time-killing data, meropenem concentrations of ≥ 0.25 mg/liter killed >2.6 log₁₀ CFU/ml of CDC MSSA by 8 h regardless of the presence of *A. baumannii*. By 24 h, meropenem concentrations of ≥ 4.0 mg/liter achieved CDC *S. aureus* reductions of ≥ 3.1 log₁₀ CFU/ml in monoculture and coculture experiments.

Unlike the consistent killing of CDC *S. aureus*, the activity of meropenem against the ATCC *S. aureus* isolate was more variable. Although meropenem concentrations of ≥ 0.25 mg/liter resulted in maximal reductions of >3.5 log₁₀ CFU/ml during monoculture and coculture with the ATCC *A. baumannii* strain, only 64 mg/liter of meropenem was capable of killing >3.5 log₁₀ CFU/ml of ATCC *S. aureus* during coculture with carbapenem-resistant *A. baumannii* (CRAB). Similarly, a meropenem concentration of 0.0625 mg/liter achieved a maximal reduction of >1.5 log₁₀ CFU/ml during monoculture and coculture with the ATCC *A. baumannii* isolate, whereas the same concentration was capable of only a 0.3-log reduction against the ATCC *S. aureus* isolate when cultured with CRAB.

The killing of meropenem against both *A. baumannii* isolates is shown in Fig. 1b. Against the ATCC *A. baumannii* isolate, meropenem concentrations of ≥ 4.0 mg/liter killed >1.6 log₁₀ CFU/ml of *A. baumannii* by 8 h in monoculture and coculture experiments. In contrast, none of the meropenem concentrations were able to kill CRAB during monoculture; however, 64 mg/liter of meropenem was capable of achieving 0.7- and 1.4-log₁₀ CFU/ml reductions against CRAB during coculture with the CDC *S. aureus* and ATCC *S. aureus* isolates, respectively.

Integrated PK/PD analysis. The results of the integrated pharmacokinetics/pharmacodynamics (PK/PD) analysis are presented graphically in Fig. 2, and the parameter estimates from the Hill functions are listed in Table 1. Against the ATCC *S. aureus* isolate, the magnitude of the effect for meropenem (E_{Δ}) was statistically significantly

FIG 1 (a) *S. aureus post hoc* population fits and observed data. Model fits (lines) based on population parameters are overlaid with the geometric means of the observed time-kill data (points) and observed geometric standard deviations (error bars) for each time-kill. Each column represents a culture condition (monoculture, coculture with the ATCC isolate, or coculture with the CRAB isolate), whereas each row represents the specific strain being observed. (b) *A. baumannii post hoc* population fits and observed data.

higher during monoculture (E_{Δ} , -4.61 [95% confidence interval {CI}, -3.38 to -5.85]) and coculture with the ATCC *A. baumannii* isolate (E_{Δ} , -4.16 [95% CI, -3.35 to -4.97]) than during coculture with CRAB (E_{Δ} , -2.85 [95% CI, -1.61 to -2.85]). In contrast, the maximal activity of meropenem against the CDC *S. aureus* isolate was not significantly different during monoculture (E_{Δ} , -3.89 [95% CI, -2.77 to -5.01]) or coculture with either the ATCC *A. baumannii* (E_{Δ} , -3.92 [95% CI, -3.28 to -4.55]) or the CRAB (E_{Δ} , -5.53 [95% CI, -4.14 to -6.91]) isolate. The maximal activities of meropenem against the ATCC *A. baumannii* isolate were also similar during monoculture (E_{Δ} , -2.88 [95% CI, -2.43 to -3.33]) and coculture with ATCC *S. aureus* (E_{Δ} , -3.33 [95% CI, -2.94 to -3.73]) or CDC *S. aureus* (E_{Δ} , -2.95 [95% CI, -2.30 to -3.59]). The killing of meropenem against the CRAB isolate was not sufficient enough to describe the killing profile using a Hill function.

Mechanism-based modeling. The final model utilized a two-subpopulation structure for each of the bacterial species studied: susceptible and resistant (Fig. 1a and b and Fig. 3). For the *A. baumannii* submodel, each subpopulation had a unique mean

FIG 2 Empirical PK/PD model fits of log ratio areas. Log ratio areas from each time-kill study were modeled as a function of the meropenem concentration. Black lines indicate monoculture conditions, whereas red or black represents an *S. aureus* isolate cocultured with CRAB or ATCC *A. baumannii*, respectively. Yellow and green lines represent *A. baumannii* cocultured with CDC *S. aureus* and ATCC *S. aureus*, respectively. Each panel represents the bacterial species being observed.

generation time (MGT), maximum killing effect ($K_{\max}$), and concentration for 50% $K_{\max}$ (KC_{50}). Between the different strains, a covariate relationship on the KC_{50} for the susceptible subpopulation ($KC_{50,S}$) by CRAB was found to decrease sensitivity roughly 76-fold for the susceptible subpopulation (Table 2). Because of the limited spectrum of the killing effect at the meropenem concentrations studied, the $KC_{50,R}$, which defines the drug sensitivity of the resistant subpopulation, was fixed to a value of 32 mg/liter. The maximum observed concentration utilized in experiments was 64 mg/liter and resulted in only modest bacterial killing, with regrowth by 24 h. In addition, a covariate effect of strain type on the mean generation times of both susceptible and resistant populations was found to produce a statistically significantly improved model fit, with CRAB dividing more rapidly.

The *S. aureus* submodel was structured similarly to the *A. baumannii* submodel but also included an additional drug effect that inhibited growth (see the equations in the supplemental material). Because of the difficulty in properly estimating the growth inhibition parameters in the context of the current experimental design, the concentration for 50% inhibition of maximum growth (GC_{50}) was fixed to 0.125 mg/liter. The maximum growth inhibition was estimated to be 98.6% (13% relative standard error [RSE]), indicating a substantial decrease in the growth rate for arms treated with meropenem concentrations of ≥ 0.125 mg/liter compared to the experiment growth controls.

In order to properly describe observed differences in killing effects between mono- and coculture conditions, two general types of bacterial interaction models were explored. These interaction effects were broadly categorized as *A. baumannii*-on-*S. aureus* (AB-on-SA) or *S. aureus*-on-*A. baumannii* (SA-on-AB) effects. First, the bacterial interaction model of an AB-on-SA effect was explored. A stimulation effect on the KC_{50} of meropenem killing of *S. aureus* was introduced into the model based on observed differences in empirical Bayesian estimates of killing parameters between culture conditions and based on the hypothesized protection by *A. baumannii* due

TABLE 1 Summary PK/PD statistics^a

Parameter	Coculturing organism	Lower CI	Estimate	Upper CI	Statistical significance
ATCC <i>S. aureus</i> vs meropenem					
E_{Δ}	None	-5.85	-4.61	-3.38	
$E_{\max}$		-3.36	-4.03	-4.71	
EC_{50}		2.14e-2	6.16e-2	1.02e-1	
E_{Δ}	ATCC <i>A. baumannii</i>	-4.97	-4.16	-3.35	
$E_{\max}$		-3.29	-3.73	-4.17	
EC_{50}		-3.72e-4	9.40e-3	1.92e-2	
E_{Δ}	CRAB	-2.85	-2.23	-1.61	*
$E_{\max}$		2.68	3.02	3.36	*
EC_{50}		5.73e-2	9.99e-2	1.43e-1	
CDC <i>S. aureus</i> vs meropenem					
E_{Δ}	None	-5.01	-3.89	-2.77	
$E_{\max}$		-3.48	-4.09	-4.70	
EC_{50}		3.23e-2	7.42e-2	1.16e-1	
E_{Δ}	ATCC <i>A. baumannii</i>	-4.55	-3.92	-3.28	
$E_{\max}$		3.81	4.16	4.50	
EC_{50}		1.93e-2	3.06e-2	4.19e-2	
E_{Δ}	CRAB	-6.91	-5.53	-4.14	
$E_{\max}$		-4.29	-5.04	-5.79	
EC_{50}		3.35e-2	6.89e-2	1.04e-1	
ATCC <i>A. baumannii</i> vs meropenem					
E_{Δ}	None	-3.33	-2.88	-2.43	
$E_{\max}$		-2.61	-2.91	-3.21	
EC_{50}		9.23e-1	1.74	2.56	
E_{Δ}	ATCC <i>S. aureus</i>	-3.73	-3.33	-2.94	
$E_{\max}$		-2.80	-3.03	-3.26	
EC_{50}		3.79e-1	5.74e-1	7.70e-1	*
E_{Δ}	CDC <i>S. aureus</i>	-3.59	-2.95	-2.30	
$E_{\max}$		-2.79	-3.18	-3.57	
EC_{50}		3.04e-1	6.69e-1	1.03	

^a* indicates statistical significance compared to monoculture as indicated by nonoverlapping 95% confidence intervals. $LRA = E_0 + [(E_{\max} \times \text{meropenem}) / (EC_{50} + \text{meropenem})]$, and $E_{\Delta} = E_{\max} - E_0$. EC_{50} , 50% effective concentration.

to its complement of oxacillinases. Subsequently, an SA-on-AB effect was explored, using similar methods to identify key aspects of the model (e.g., growth or killing) that were distinct between mono- and coculture conditions. Ultimately, the data were best described with a modeled SA-on-AB effect that increased the meropenem $K_{\max}$ on *A. baumannii* with increasing numbers of *S. aureus* bacteria.

In all cases, the ATCC isolates for each species were considered the “typical” isolates for the purposes of the covariate search. Within each subpopulation model, the automated covariate search found statistically significant differences in growth times for both *S. aureus* and *A. baumannii* depending on the strain. Automated covariate searching found that there was a 31.8-fold increase of the AB-on-SA effect on the KC_{50} of *S. aureus* when cocultured with CRAB, compared to a baseline increase of 3.52-fold when cocultured with ATCC *A. baumannii*.

Monte Carlo simulations. Meropenem at 2 g in a 3-h prolonged infusion (3hPI) every 8 h (q8h) was simulated in 2,500 simulated critically ill subjects over 48 h of therapy (34). The resulting simulations were then plotted with the median and 10th/90th prediction intervals (Fig. 4). Simulation of a clinical regimen resulted in a less pronounced difference in *A. baumannii* killing between mono- and cocultures. In comparison, both

FIG 3 Mechanism-based pharmacodynamic model diagram. The *S. aureus* submodel is encircled in red, whereas the *A. baumannii* submodel is encircled in green. For each parameter affected by a covariate relationship, a subscripted prefix has been appended, with “s” signifying a covariate effect caused by the CDC *S. aureus* strain and “a” signifying a covariate effect caused by the CRAB strain. IC, inhibitory concentration.

S. aureus isolates had an observable decrease in the rate of killing in coculture compared to monoculture.

DISCUSSION

Although both *S. aureus* and *A. baumannii* are implicated in polymicrobial infections, the interaction of the two organisms in the presence of antibacterials has been poorly explored. Previous investigations have suggested that the ability of bacteria to cooperate during antimicrobial exposure partially depends on the resistance mechanisms possessed by each organism. Specifically, multiple studies have observed that β -lactamase-producing bacteria are capable of protecting neighboring organisms from β -lactam antibiotics (32, 35, 36). In contrast, several investigations have also found that β -lactamase-producing strains of *Haemophilus influenzae* are poorly able to protect *Streptococcus pneumoniae* from aminopenicillins (35, 37). It is therefore likely that cooperation during β -lactam exposure is partially dependent on the specific β -lactamases harbored by organisms in mixed cultures. A previous investigation demonstrated that OXA-58-producing *A. baumannii* strains are capable of protecting *Escherichia coli* from carbapenems (32).

In the present study, the mechanism-based model indicated that the ATCC *A. baumannii* isolate harboring a chromosomally encoded OXA-51 enzyme was able to provide only a minimal amount of protection to MSSA during meropenem exposure, whereas the CRAB isolate that possessed an OXA-51 enzyme and a plasmid-mediated OXA-23 enzyme offered a more substantial shielding effect. Every *A. baumannii* strain produces an OXA-51-like chromosomal oxacillinase that may confer carbapenem resistance if the enzyme is overexpressed, but the acquisition of a plasmid bearing genes for OXA-23, OXA-40, or OXA-58 is typically associated with carbapenem resistance in *A. baumannii* (38, 39). A previous study observed that plasmid-mediated OXA-58 was excreted outside the *A. baumannii* cell and was capable of protecting neighboring Gram-negative pathogens from β -lactam exposure (32). In the present study, the OXA-23-harboring isolate may have conferred more substantial protection to *S. aureus* due to a higher rate of meropenem hydrolysis, and the production of the plasmid-mediated oxacillinase may have resulted in more carbapenemase secretion into the extracellular space. Clinical use of rapid diagnostics that detect specific β -lactamase enzymes

TABLE 2 Parameter estimates^a

Parameter	Description	Unit	Value	RSE (%) ^b
<i>S. aureus</i> ^c				
LogINOC _{SA}	Starting inoculum for <i>S. aureus</i>	Log ₁₀ CFU/ml	6.06	1.26
LogMF _{SA,R}	Mutation frequency for resistant subpopulation		2.22	4.91
MGT _{SA,S}	Mean generation time for susceptible subpopulation	min	60.7	13.3
βMGT _{SA,S_CDCMSSA}	CDC MSSA covariate effect on MGT _{SA,S}		−0.616	22.3
MGT _{SA,R}	Mean generation time for resistant subpopulation	min	178	15
K _{maxSA,S}	Maximum killing effect for susceptible subpopulation	h ^{−1}	2.03	13.1
K _{maxSA,R}	Maximum killing effect for resistant subpopulation	h ^{−1}	0.359	17.7
KC _{50SA,S}	Meropenem concn for 50% K _{maxSA,S}	mg/liter	0.0212	43.4
KC _{50SA,R}	Meropenem concn for 50% K _{maxSA,R}	mg/liter	4.43	43.1
Hill _{SA}	Shape parameter for meropenem killing effect		0.933	22.1
GR _{maxSA,R}	Maximum inhibition of growth by meropenem		0.986	13.6
GC ₅₀	Meropenem concn for 50% GR _{maxSA,R}	mg/liter	0.125	Fixed
<i>A. baumannii</i> ^c				
LogINOC _{AB}	Starting inoculum for <i>A. baumannii</i>	Log ₁₀ CFU/ml	6.34	0.558
LogMF _{AB,R}	Mutation frequency for resistant subpopulation		3.35	10.5
MGT _{AB,S}	Mean generation time for susceptible subpopulation	min	120	8.13
βMGT _{AB,S_CRAB}	CRAB covariate effect on MGT _{AB,S}		−1.4	6.11
MGT _{AB,R}	Mean generation time for resistant subpopulation	min	266	16
βMGT _{AB,R_CRAB}	CRAB covariate effect on MGT _{AB,R}		−2.11	4.41
K _{maxAB,S}	Maximum killing effect for susceptible subpopulation	h ^{−1}	1.13	4.6
K _{maxAB,R}	Maximum killing effect for resistant subpopulation	h ^{−1}	0.304	9.79
KC _{50AB,S}	Meropenem concn for 50% K _{maxAB,S}	mg/liter	0.331	7.29
βKC _{50AB,S_CRAB}	Clinical <i>A. baumannii</i> covariate effect on KC _{50AB,S}		4.34	3.3
KC _{50AB,R}	Meropenem concn for 50% of K _{maxAB,R}	mg/liter	32	Fixed
Hill _{AB}	Shape parameter for meropenem killing effect		2.85	19.5
Bacterial interaction ^d				
LogCFU _{max}	Maximum bacterial population	Log ₁₀ CFU/ml	8.51	0.373
E _{maxAB-on-SA}	Maximum increases in KC _{50SA,S} and KC _{50SA,R} based on <i>A. baumannii</i> inoculum		1.26	76
βE _{maxAB-on-SA_CRAB}	CDC MSSA covariate effect on E _{maxAB-on-SA}		2.2	27.6
LogAB ₅₀	<i>A. baumannii</i> inoculum for 50% E _{maxAB-on-SA}	Log ₁₀ CFU/ml	5.52	24.6
E _{maxSA-on-AB}	Maximum increase in K _{maxAB,S} based on <i>S. aureus</i> inoculum		0.0625	79.2
βE _{maxSA-on-AB_CDCMSSA}	CDC MSSA covariate effect on E _{maxSA-on-AB}		−0.276	87.1
βE _{maxSA-on-AB_CRAB}	Clinical <i>A. baumannii</i> covariate effect on E _{maxSA-on-AB}		3.43	22.6
LogSA ₅₀	<i>S. aureus</i> inoculum for 50% E _{maxSA-on-AB}	Log ₁₀ CFU/ml	2	Fixed
Residual error				
σ _{AB}	Additive error to <i>A. baumannii</i> observations	Log ₁₀ CFU/ml	0.388	3.17
σ _{SA}	Additive error to <i>S. aureus</i> observations	Log ₁₀ CFU/ml	0.829	2.96

^aCovariates modeled as $\log(\theta) = \log(\theta_{\text{pop}}) + (\text{CRAB} = 1 \text{ or } \text{CDC } S. \text{ aureus} = 1) \times \beta + \eta$.^bLinearization method used to compute standard errors/log likelihoods.^cIV was fixed to 0.05 for all bacterium-specific parameters.^dIV was fixed to zero for bacterial interaction parameters.

and other resistance mechanisms may be a potential avenue for tailoring pathogen-specific antibacterials during polymicrobial infections.

Aside from the resistance mechanisms possessed by pathogens in polymicrobial infections, there are likely other variables that influence how organisms will alter the pharmacodynamics of drugs that act on neighboring pathogens. Pseudomonal release of endopeptidases, rhamnolipids, and 2-heptyl-4-hydroxyquinolone *N*-oxide has previously been shown to modulate the activity of antistaphylococcal agents (16, 19). The current study also utilized static *in vitro* time-killing experiments that may be influenced by the quantity of bacteria, and the presence of additional organisms during co-culture experiments may have attenuated antimicrobial activity in a manner analogous to the inoculum effect observed in other investigations (40). An integrated PK/PD analysis found that the CRAB isolate significantly reduced the maximal activity of meropenem against one of the two investigated MSSA isolates. Although other investigations into mechanisms of bacterial cooperation during antimicrobial exposure have focused on the organism that is resistant to the antibacterial of interest, the current study

FIG 4 A 2,500-subject Monte Carlo simulation. Meropenem in a 2-g 3hPI q8h was simulated over 48 h in 2,500 subjects for each mono- and coculture scenario explored in the time-kill studies. The resulting medians (solid lines) and 90th/10th prediction intervals (shaded regions) were plotted for each *A. baumannii* and *S. aureus* isolate.

suggests that the drug-susceptible pathogen may also influence the extent of cooperation through as-yet-unknown mechanisms. Further investigations into nuanced differences between MSSA strains are needed to determine why the magnitude of attenuated drug susceptibility varies between *S. aureus* isolates.

Unlike the protection from carbapenems that *A. baumannii* offered to MSSA, the ability of meropenem to kill *A. baumannii* increased in the presence of *S. aureus*. To better understand the possible clinical implications of the augmented killing of *A. baumannii*, Monte Carlo simulations evaluated the performance of the highest-dose meropenem regimen approved by the U.S. Food and Drug Administration (34). Despite the increased susceptibility to meropenem during MSSA coculture, both the ATCC and CRAB isolates were capable of surviving the meropenem regimen. In contrast to the survival of the *A. baumannii* isolates, the simulated meropenem regimen eradicated both MSSA isolates despite the protective effect offered by *A. baumannii*. Pending additional studies, optimal β -lactam dosing may be a viable option for overcoming the *A. baumannii* shielding effect and killing MSSA. It is also noteworthy that the meropenem MIC of 4 mg/liter for the ATCC *A. baumannii* isolate was the lowest concentration of meropenem that was able to prevent the growth of the ATCC *A. baumannii* monoculture by 24 h in time-killing experiments, which suggests

concordance between the susceptibility testing and the performance of meropenem in the time-killing experiments.

The majority of *in vitro* experiments and mathematical models on anti-infective pharmacodynamics in the current literature typically utilize a representative bacterial isolate and single culture conditions. However, in many disease states, patients can experience infections with multiple bacterial species simultaneously. Mathematical modeling of coinfection is challenging as multiple, interdependent components must be established in a systematic manner while maintaining the most parsimonious model structure. This study utilized two isolates of *S. aureus* and *A. baumannii* each, which were studied under four monoculture conditions and four coculture conditions. Models using strain-specific features as covariates have been reported previously but not in the context of coculture (41). In parallel, previous studies have focused on the development of mathematical models to describe antimicrobial pharmacodynamics in coculture but without the implementation of covariate effects for multiple isolates (18). In the present study, bacterial interaction was handled as a structural component of the model, where bacterial counts of one isolate are the driving force affecting the other isolate. In contrast, an automated covariate searching method allows rapid testing of strain effects on the species-specific submodel and the AB-on-SA or SA-on-AB interactions. This method could be expanded more mechanistically to use key molecular determinants of resistance of either species in an expanded model utilizing a much larger number of well-characterized clinical isolates.

Although the current study was able to observe alterations in meropenem pharmacodynamics during the coculture of *A. baumannii* with one of two investigated MSSA isolates, there are several important limitations that must be accounted for before extrapolating the results to the clinical setting. The current modeling work is limited by the use of experiments based on static drug concentrations. Expanding the investigation to utilize dynamic dosing of a β -lactam in a hollow-fiber infection model could improve the translatability of these findings and permit the development of a pharmacodynamic model built on humanized dosing regimens. A robust pharmacokinetic analysis in the dynamic model would also allow a better understanding of how β -lactam degradation from oxacillinase enzymes impacts the survival of MSSA during β -lactam exposure. These dynamic concentrations will also enhance the ability for a mechanism-based mathematical model to better describe the growth and direct killing effects of meropenem at the extremes (i.e., maximum concentration of the free, unbound fraction of the drug in serum [$fC_{\max}$] and minimum concentration of the free, unbound fraction of the drug in serum [$fC_{\min}$]) over the full course of therapy. Future studies will also benefit from increasing the number of isolates in each submodel. By increasing the number of strains, the covariate selection method can be improved by using molecular determinants of resistance as covariates rather than using strain type empirically. These future studies would significantly improve the model's reliability for a broader range of isolates. Further studies identifying the mechanisms of strain-specific interactions would also be warranted based on the hypotheses generated from the modeling results. The initial ratio of *A. baumannii* to *S. aureus* was fixed at 1:1 in the current study, whereas *S. aureus* may outnumber *A. baumannii* in a polymicrobial infection. Varying the starting concentrations of both organisms in future investigations will better determine the number of *A. baumannii* cells needed to confer a clinically meaningful protective effect. Finally, the population pharmacokinetics used for the Monte Carlo simulations were obtained from critically ill adults, whereas patients receiving treatment for polymicrobial infections outside critical care units may possess meropenem pharmacokinetics different from those of the study population (42).

In closing, the current study investigated the interaction of MSSA and *A. baumannii* during carbapenem exposure to help elucidate the optimal treatment of polymicrobial infections. An integrated PK/PD analysis and a novel mechanism-based model were used to determine that *A. baumannii* was capable of protecting MSSA from β -lactam exposure; however, the magnitude of the shielding effect was dependent not only on

the level of meropenem resistance possessed by the *A. baumannii* isolate but also on which MSSA isolate was investigated. Monte Carlo simulations subsequently demonstrated that a high dose of meropenem is able to overcome the protective effect exerted by *A. baumannii* and achieve sustained killing against MSSA. Clinically, the results of this study suggest that dose-optimized β -lactam antibacterials may be a therapeutic option for MSSA and β -lactam-susceptible *A. baumannii* despite the simultaneous presence of *S. aureus* and *A. baumannii* at the same site of infection. Subsequent investigations in dynamic *in vitro* models and animal models are needed to confirm the results of the current study and better define the optimal treatment of mixed infections involving *A. baumannii* and *S. aureus*.

MATERIALS AND METHODS

Bacterial isolates. Two MSSA isolates, ATCC 25923 (ATCC *S. aureus*) and *Staphylococcus aureus* CDC AR Isolate Bank number 0484 (CDC *S. aureus*), and two *A. baumannii* isolates, ATCC 19606 (ATCC *A. baumannii*) and 03-149.1 (CRAB), were used in the study. ATCC *A. baumannii* was previously shown to have a meropenem MIC of 4 mg/liter and to possess the chromosomal oxacillinase OXA-51, whereas CRAB has a meropenem MIC of 16 mg/liter and produces the chromosomal oxacillinase OXA-51 and the plasmid-mediated oxacillinase OXA-23 (43). The ATCC *A. baumannii* isolate is also a model organism that has been used in investigations of antimicrobial pharmacodynamics (44, 45), drug resistance (46), genomics (47, 48), and microbial interactions (49).

Time-killing experiments. Using an inoculum of 10^6 CFU/ml, time-killing experiments were conducted using a meropenem concentration array against MSSA and *A. baumannii* isolates alone. Time-killing experiments were then conducted against every iteration of a pathogen duo that consisted of one MSSA isolate and one of the *A. baumannii* isolates as described previously (18). Mueller-Hinton broth adjusted with calcium (25 mg/liter) and magnesium (12.5 mg/liter) was used for all experiments, and fresh solutions of meropenem (AK Scientific) were prepared on the day of each experiment. Meropenem concentration arrays consisted of 0.063, 0.25, 1.0, 4.0, 16, and 64 mg/liter of meropenem. Samples were collected at 0, 2, 4, 6, 8, and 24 h; serially diluted in saline; and plated onto selective agar. Mueller-Hinton agar containing 4.0 mg/liter of vancomycin was used to enumerate *A. baumannii* colonies, whereas Mueller-Hinton agar imbued with 8.0 mg/liter of polymyxin B was used to quantify MSSA. After 24 h of incubation at 37°C, colonies were enumerated with a lower limit of detection of 40 CFU/ml. All experiments were conducted at least in duplicate.

Pharmacokinetics/pharmacodynamics analyses. Three-parameter Hill functions were fit using the nlme package in R (version 4.0.0) (50). The 24-h time-kill data were summarized using the log ratio area (LRA) method, as previously shown (18, 51). Briefly, the observed bacterial concentrations were numerically integrated using the log-linear trapezoidal method from 0 to 24 h. The resulting area was normalized to the average area under the growth control and log transformed. The LRA was then modeled as a function of the meropenem concentration. The model was parameterized to utilize the term E_{delta} in place of E_0 , as the normalization process could potentially create an E_0 that is either slightly positive or slightly negative due to averaging of the growth control. Ultimately, this parameterization makes comparisons of the magnitudes of reduction easier.

Mechanism-based modeling of antimicrobial exposure during coculture. Modeling was performed using the stochastic approximation expectation maximization algorithm within Monolix 2019R2 (Lixoft SAS, Antony, France), with standard errors and likelihood calculated using the linearization method (52). The mathematical model was constructed sequentially. First, individual models for each ATCC isolate were built using a simple two-subpopulation structure: susceptible and resistant. Each subpopulation had a unique mean generation time (MGT). In order to generate the most parsimonious model, the killing function (modeled using a Hill function) was first made identical for both subpopulations of each bacterial species. The three parameters of a Hill function, K_{max} , KC_{50} , and γ , were changed in a stepwise fashion for each subpopulation based on a statistically significant improvement in the model objective function ($-2 \times \log$ likelihood). Once individual models for each of the ATCC isolates were constructed to describe monoculture, the models were combined, and the data were expanded to include coculturing of the organisms. By comparing empirical Bayesian estimates of parameters from mono- and cocultures, we inferred any changes in growth rates and meropenem killing effects due to the presence of both bacteria together. These were handled by the addition of a coculture effect of the effector bacteria (i.e., the bacteria whose presence is causing a change in parameter estimates for the cocultured bacteria) to the receptor bacteria (i.e., the bacteria that are manifesting a change in growth/killing parameters due to coculture conditions).

Once a finalized structural model was completed to describe the mono- and coculturing of ATCC isolates for both *S. aureus* and *A. baumannii*, the modeling data set was again expanded to include the additional strain for each species. In this phase of the modeling, observed differences in parameter estimates were handled using a covariate effect. The covariate search was handled using the COSSAC (Conditional Sampling use for Stepwise Approach based on Correlation tests) method supplied as an automatic covariate model-building procedure. Covariates were selected based on changes in the log likelihood, with significance levels of 0.05 and 0.01 used for forward and backward selection, respectively. Covariate relationships were locked such that the *S. aureus* or *A. baumannii* strain type could affect only its own growth or killing parameters because any effect due to coculturing conditions was

handled previously through structural changes to the model. However, the relationships of the covariates on the parameters governing coculture interaction were tested for both CDC *S. aureus* and CRAB. For example, the mean generation time of *S. aureus* could be affected by either a covariate effect of the *S. aureus* strain type or a coculture effect of *A. baumannii*. The coculture effect could then be affected by a covariate effect of either the *S. aureus* or *A. baumannii* strain type.

Monte Carlo simulations. To provide a clinical context for the observed pharmacodynamic effects of coculturing, meropenem pharmacokinetics were simulated based on a previously reported population study (42). Simulations were conducted using the RxODE package in R (53). The model included patient covariates for creatinine clearance, weight, and albumin. Creatinine clearance was simulated assuming an interindividual variability (IIV) of a 30% coefficient of variation (CV), whereas weight and albumin were simulated with an IIV of a 10% CV. A high-dose regimen of 2 g in a 3-h prolonged infusion q8h was then simulated in 2,500 subjects. The resulting PK profiles were then used to drive the newly generated mechanism-based pharmacodynamic model for all four possible coinfection scenarios with the two *A. baumannii* plus two *S. aureus* strains. Briefly, the pharmacodynamic model (see the equations in the supplemental material) was driven by static concentrations that were introduced into the model as a time-independent covariate. For simulations, the free concentration was instead driven by the product of the concentration in the central compartment based on simulation from the population model and the fraction of unbound drug (assumed to be 98%). Each of the 2,500 simulated PK profiles was then used to drive the pharmacodynamics. Prediction intervals for bacterial killing were calculated at the 10th, 50th, and 90th levels.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

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We have no transparency declarations.

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