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PHARMACOLOGY

Minocycline Alone and in Combination with Polymyxin B, Meropenem, and Sulbactam against Carbapenem-Susceptible and -Resistant *Acinetobacter baumannii* in an *In Vitro* Pharmacodynamic Model

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ABSTRACT *Acinetobacter baumannii* is recognized as an urgent public health threat by the Centers for Disease Control and Prevention (CDC). Current treatment options are scarce, particularly against carbapenem-resistant *Acinetobacter baumannii* (CRAB). We simulated the impact of minocycline standard (200 mg load + 100 mg Q12h) and high (700 mg load + 350 mg Q12h) doses, polymyxin B (2.5 mg/kg Q12h), sulbactam (1 g Q6h and 9 g/24 h as continuous infusion), and meropenem (intermittent 1 or 2 g Q8h and 6 g/24 h as continuous infusion) alone or in combination against CRAB and non-CRAB isolates by simulating human therapeutic dosing regimens in a 72-h, *in vitro* pharmacodynamic (IVPD) model. There were no monotherapy regimens that demonstrated bactericidal activity against the tested non-CRAB and CRAB strains. Resistance development was common in monotherapy regimens. Against the CRAB isolate, the triple combination of high-dose minocycline (*f*AUC/MIC 21.2), polymyxin B (*f*AUC/MIC 15.6), and continuous-infusion sulbactam (67% $T_{>MIC}$) was the most consistently active regimen. Against non-CRAB, the triple therapy regimen of high-dose minocycline (*f*AUC/MIC 84.8) with continuous-infusion meropenem (100% $T_{>MIC}$) and continuous-infusion sulbactam (83% $T_{>MIC}$), as well as the double therapy of high-dose minocycline (*f*AUC/MIC 84.8) with continuous-infusion meropenem (100% $T_{>MIC}$), resulted in persistently bactericidal activity. In conclusion, triple therapy with high-dose minocycline, continuous-infusion sulbactam, and polymyxin B produced the most significant kill against the carbapenem-resistant *Acinetobacter baumannii*, with no regrowth and minimal resistance development.

KEYWORDS minocycline, polymyxin B, beta-lactams, continuous infusion, *Acinetobacter baumannii*

Carbapenem-resistant *Acinetobacter baumannii* (CRAB) was recently escalated to an urgent-level threat by the Centers for Disease Control and Prevention (CDC) due to both its propensity for being intrinsically drug resistant as well as its remarkable ability to acquire resistance against most classes of antimicrobials (1). *A. baumannii* causes a variety of health care-associated infections with notable clinical syndromes, including bloodstream infections (BSIs) and pneumonia, as well as surgical site infections, skin and soft tissue infections, urinary tract infections, and, less commonly, meningitis or peritonitis among patients receiving peritoneal dialysis (2). Infections are most frequently seen among the critically ill and immunocompromised patients, and are

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TABLE 1 Isolate characteristics and baseline isolate minimum inhibitory concentrations (MIC)

			MIC μg/ml					
Isolate[a]	Source	MDR status[a]	Minocycline	Polymyxin B	Meropenem	Sulbactam[b]	Levofloxacin[a]	Amikacin
CRAB	Human blood	MDRO	2	2	128	4	6	128
Non-CRAB	Human blood	Non-MDRO	0.5	0.5	0.5	2	NA	NA

[a]CRAB, carbapenem-resistant *Acinetobacter baumannii*; Non-CRAB, non-carbapenem-resistant *Acinetobacter baumannii*; MDR, multidrug resistant; MDRO, multidrug resistant organism; NA, not applicable.
[b]Tested as ampicillin-sulbactam.

associated with high mortality rates (3). Specifically, nosocomial BSIs caused by *A. baumannii* have reported mortality rates as high as 34% (3), and as high as 43% among intensive care unit (ICU) patients (4). The CDC reported that in addition to an attributable cost of $281 million, there was an estimated 8,500 CRAB cases with 700 deaths among hospitalized patients in 2017 (1). This is particularly concerning, as mortality rates have been shown to double when infection is caused by CRAB compared to non-CRAB (5). Inappropriate empirical antimicrobial selection resulting in delayed therapy has been described as the driver of excess mortality rather than the resistance patterns (6, 7), highlighting the importance of early targeted treatment. To date, there is no "gold standard" treatment for *Acinetobacter* infections, and empirical therapy is driven by local susceptibility patterns. In an era of a sparse antimicrobial pipeline and increasingly limited treatment options against *A. baumannii*, one strategy to elucidate optimal antimicrobial therapy is through reevaluation of existing antimicrobial agents.

Minocycline is an example of such an existing drug that is of particular interest due to its safety profile (2, 8, 9). It is available as both an intravenous and oral formulation. It has been successfully used alone, but mostly in combination with other active agents against multidrug-resistant (MDR) *A. baumannii* (8). However, the optimal dose and role in combination therapy are unknown. Therefore, it is the primary goal of this study to evaluate minocycline alone and in combination with other commonly utilized antimicrobials, including meropenem, sulbactam, and polymyxin B, against CRAB and non-CRAB isolates using standard and pharmacodynamically optimized doses of the antimicrobial agents. Furthermore, we evaluated the combination of meropenem and sulbactam, taking into consideration the favorable safety profile associated with beta-lactams, and we hypothesized that combination therapy would yield greater activity than monotherapy.

(This work was presented in part at the 28th European Congress of Clinical Microbiology and Infectious Diseases, 22 April 2018, in Madrid, Spain.)

RESULTS

Susceptibility testing. Susceptibility results for both the CRAB and non-CRAB isolates are reported in Table 1. In addition to carbapenem resistance, the CRAB isolate was a multidrug-resistant organism (MDRO), as shown by nonsusceptibility to $\geq$1 agent in $\geq$3 antimicrobial classes, including aminoglycosides (e.g., nonsusceptible to tobramycin and amikacin), antipseudomonal carbapenems (e.g., nonsusceptible to meropenem and imipenem), and antipseudomonal fluoroquinolones (e.g., nonsusceptible to levofloxacin) (Table 1) (10).

Pharmacodynamic models. Results for monotherapy and combination therapy for non-CRAB and CRAB strains in 72-h *in vitro* pharmacodynamic models are shown in Fig. 1 and 2, and Fig. 3 and 4, respectively. Quantitative changes in the bacterial population, expressed as change in log CFU/ml, for each antimicrobial regimen are described in Table 2 for non-CRAB isolates and Table 3 for CRAB isolates.

Non-carbapenem-resistant *Acinetobacter baumannii* (non-CRAB). The average bacterial density of the starting inoculum was 5.96 $\pm$ 0.06 standard deviation (SD) $\log_{10}$ CFU/ml across both *A. baumannii* isolates. Despite susceptible MICs, only polymyxin B at 24 h demonstrated a significant reduction in count ($\log_{10}$ CFU/ml), followed by regrowth (Table 2). Among combination therapies, standard-dose minocycline (i.e.,

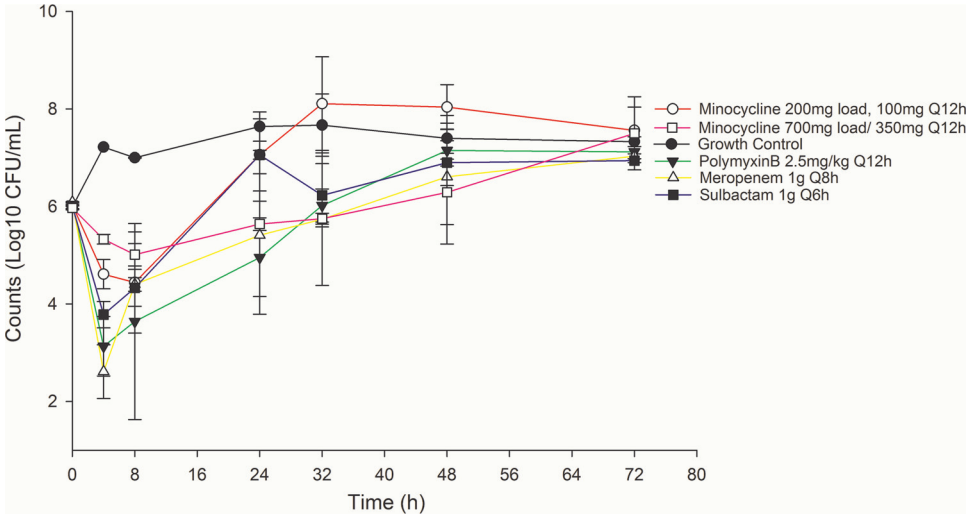

FIG 1 Activities of tested monotherapies against non-carbapenem-resistant *Acinetobacter baumannii* (non-CRAB).

200 mg load with 100 mg every 12 hours [Q12h]) in combination with polymyxin B, as well as pharmacodynamically optimized combination therapies that included high-dose minocycline (i.e., 700 mg load followed by 350 mg Q12h) plus continuous-infusion meropenem and continuous-infusion sulbactam, and high-dose minocycline plus continuous-infusion (CI) meropenem, demonstrated significant reduction in bacterial counts at 24 h, 48 h, and 72 h. Although high-dose minocycline with sulbactam was associated with an initial drop in bacterial counts, regrowth occurred by hours 48 and 72. Regrowth was associated with an increase in MIC for all regimens, except high-dose minocycline with meropenem 6 g/24 h CI and sulbactam 9 g/24 h CI, and high-dose minocycline with meropenem 6 g/24 h CI.

Carbapenem-resistant *Acinetobacter baumannii* (CRAB). Among monotherapy options, polymyxin B was associated with a significant reduction in bacterial counts at 24 h and 72 h (Table 3). Standard-dose minocycline with polymyxin B initially resulted in reduced bacterial counts, but regrowth was noted at 48 h and 72 h. While high-dose minocycline with meropenem 6 g/24 h CI and sulbactam 9 g/24 h CI yielded a significant drop in bacterial count at 24 h and 72 h, high-dose minocycline with polymyxin

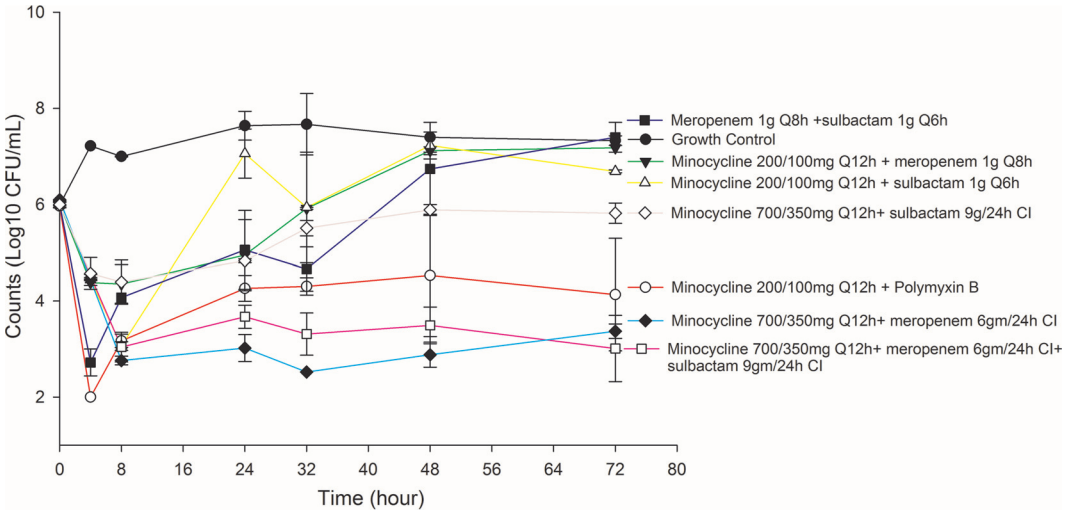

FIG 2 Activities of tested combination therapies against non-carbapenem-resistant *Acinetobacter baumannii* (non-CRAB).

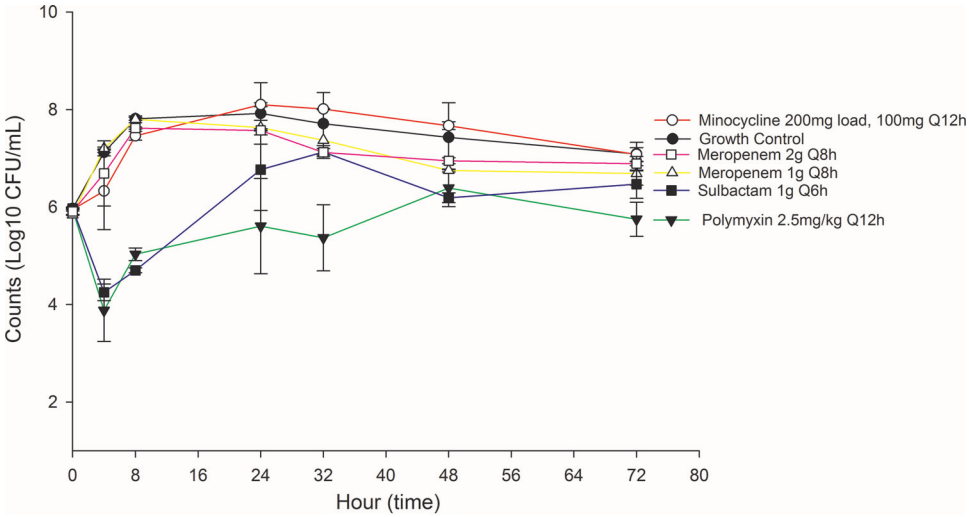

FIG 3 Activities of tested monotherapies against carbapenem-resistant *Acinetobacter baumannii* (CRAB).

and continuous-infusion sulbactam demonstrated the highest and most consistent reduction in bacterial counts.

Detection of resistance. Resistance was detected for all monotherapy regimens, with polymyxin B displaying a 128-fold increase in MIC by hour 72 for CRAB isolates (i.e., MIC increased from 2 to 256 μg/ml). All regimens that exhibited regrowth were associated with a rise in MIC with few exceptions. Regimens that did not result in resistance included: (i) high-dose minocycline plus continuous-infusion meropenem and continuous-infusion sulbactam, as well as (ii) high-dose minocycline plus meropenem against non-CRABs. Regimens that resulted in minimal resistance against CRABs included high-dose minocycline plus polymyxin B and continuous-infusion sulbactam. Detailed MICs at 24, 48, and 72 h are in Table 4 and 5.

Pharmacodynamic/pharmacokinetic parameters. The pharmacodynamic/pharmacokinetic (PK/PD) parameters for tested agents are displayed in Table 6. For the CRAB isolate, the achieved area under the concentration-time curve for the free frac-

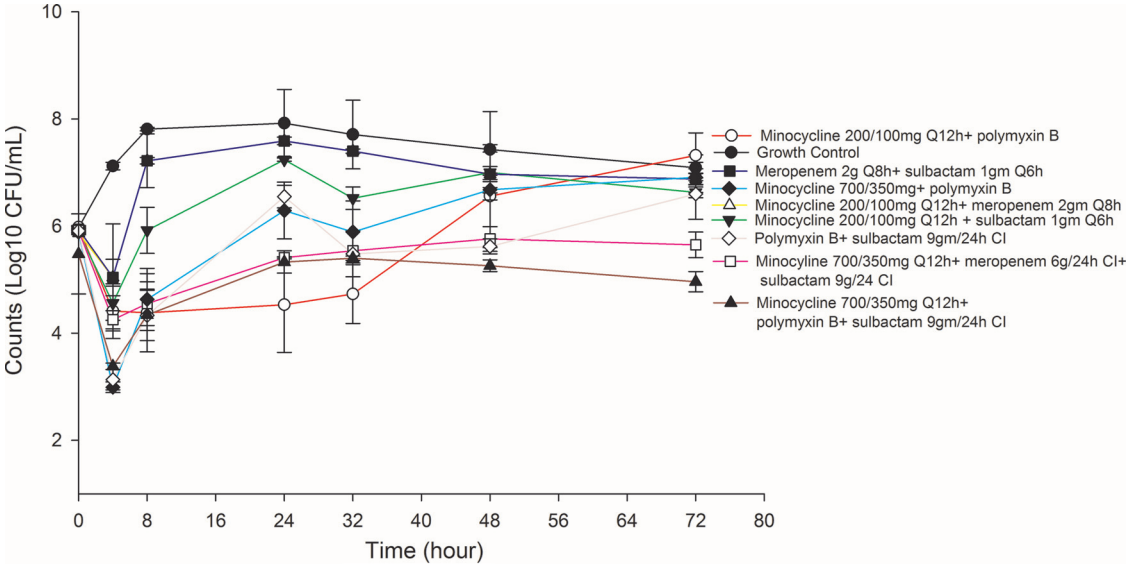

FIG 4 Activities of tested combination therapies against carbapenem-resistant *Acinetobacter baumannii* (CRAB).

TABLE 2 Inoculum change in non-CRAB compared to growth control

	Change in bacterial density (log10 CFU/ml) over 24, 48, and 72 h		
Antimicrobial regimen	**24 h**	**48 h**	**72 h**
Monotherapy			
Minocycline 200 mg load, 100 mg Q12h (SD)	–0.59	+0.64	+0.23
Minocycline 700 mg load, 350 mg Q12h (HD)	−2.01	−1.11	+0.17
Meropenem 1 g Q8h	−2.24	−0.79	−0.30
Polymyxin 2.5 mg/kg Q12h	−2.7[d]	−0.26	−0.21
Sulbactam 1 g Q6h	-0.59	−0.5	−0.39
Dual Therapy			
Minocycline SD + meropenem 1 g Q8h	−2.69[d]	−0.28	−0.15
Minocycline SD + sulbactam 1 g Q6h	−0.58	−0.17	−0.64
Minocycline SD + polymyxin B 2.5 mg/kg Q12h[a]	−3.39[d]	−2.87[d]	−3.2[d]
Meropenem 1g Q8h + sulbactam 1 g Q6h	−2.59[d]	−0.66	+0.07
Minocycline HD + meropenem 6 g/24 h CI[c]	−4.63[d]	−4.52[d]	−3.96[d]
Minocycline HD + sulbactam 9 g/24 h CI	−2.82[d]	−1.51	−1.51
Triple Therapy			
Minocycline HD + meropenem 6 g/24 h CI + sulbactam 9g/24h CI[b]	−3.97[d]	−3.92[d]	−4.32[d]

[a]Enhanced activity compared to minocycline SD alone at 24 h, 48 h.
[b]Enhanced activity compared to minocycline HD alone at 48 h, 72 h.
[c]Enhanced activity compared to minocycline HD alone at 24 h, 48 h, and 72 h; enhanced activity compared to polymyxin B alone at 72 h. CI, continuous infusion; HD, high dose; SD, standard dose.
[d]$P \leq 0.05$.

tion of the drug divided by the MIC (*f*AUC/MIC) was 7.04 and within 2% of the targeted *f*AUC/MIC of 7.18 for minocycline 200 mg load, 100 mg Q12h. High-dose (HD) minocycline (700 mg load, 350 mg Q12h) achieved an $fAUC_{24h}$ of 21.21, while targeted *f*AUC/MIC was 21.68. Published studies recommend an *f*AUC/MIC of 20 to 25. The achieved $fAUC_{24h}$ for polymyxin B was 31.26 μg · hour/ml, approximately 35% above the targeted $fAUC_{24h}$ of 21.84 μg · hour/ml, which is above the $fAUC_{24h}$ published parameters of 4 to 22 μg · hour/ml. The achieved *f*AUC/MIC associated with polymyxin B $fAUC_{24h}$ was 15.63, which was above the targeted *f*AUC/MIC of 10.92. Lastly, with a recommended target of the cumulative percentage of a 24-h period for which the

TABLE 3 Inoculum change in CRAB compared to growth control

	Change in bacterial density (log10 CFU/ml) over 24, 48, and 72 h		
Antimicrobial regimen[c]	**24 h**	**48 h**	**72 h**
Monotherapy			
Minocycline 200 mg load, 100 mg Q12h (SD)	+0.17	+0.24	−0.01
Meropenem 1 g Q8h	−0.30	−0.69	−0.40
Meropenem 2 g Q8h	−0.36	−0.49	−0.21
Polymyxin B 2.5 mg/kg Q12h	−2.32[d]	−1.05	−1.34[d]
Sulbactam 1 g Q6h	−1.15	−1.25	−0.62
Dual Therapy			
Minocycline SD + meropenem 2 g Q8h	−0.34	−0.47	−0.22
Minocycline SD + sulbactam 1 g Q6h	−0.69	−0.44	−0.46
Minocycline SD + polymyxin B 2.5 mg/kg Q12h[a]	−3.40[d]	−0.88	+0.24
Meropenem 2 g Q8h[b] + sulbactam 1 g Q6h	−0.93	−0.62	−0.29
Minocycline HD + polymyxin B	−1.63	−0.76	−0.19
Polymyxin B + sulbactam 9 g/24 h CI	−1.38	−1.81[d]	−0.50
Triple Therapy			
Minocycline 700 mg load, 350 mg Q12h (HD) + meropenem 6 g/24 h CI + sulbactam 9 g/24 h CI	−2.51[d]	−1.68	−1.45[d]
Minocycline HD + polymyxin B + sulbactam 9 g/24 h CI	−2.60[d]	−2.18[d]	−2.13[d]

[a]Enhanced activity compared to minocycline SD alone at 24 h
[b]Meropenem exposure maximized for CRAB isolates due to resistance
[c]CI, continuous infusion; HD, high dose; SD, standard dose.
[d]$P \leq 0.05$.

TABLE 4 MIC ranges at 24, 48, and 72 h for CRAB isolates

Regimen[c]	MIC in μg/ml[a,b]								
	Minocycline (susceptible MIC = 2)			Polymyxin B (susceptible MIC = 2)			Sulbactam (susceptible MIC = 4)		
	24h	48h	72h	24h	48 h	72 h	24h	48 h	72 h
Monotherapy									
Minocycline 200 mg load, 100 mg Q12h (SD)	4–6	12–16	12–24	–	–	–	–	–	–
Meropenem 1 g Q8h	–	–	–	–	–	–	–	–	–
Meropenem 2 g Q8h	–	–	–	–	–	–	–	–	–
Polymyxin B 2.5 mg/kg Q12h	–	–	–	48–96	256–512	128–256	–	–	–
Sulbactam 1 g Q6h	–	–	–	–	–	–	64	64	24–96
Dual therapy									
Minocycline SD + meropenem 2 g Q8h	12	8–12	8	–	–	–	–	–	–
Minocycline SD + sulbactam 1 g Q6h	6	8–32	8–16	–	–	–	8–16	12–>256	12–>256
Minocycline SD + polymyxin B 2.5 mg/kg Q12h	4	16	24–64	3-4	16	16	–	–	–
Meropenem 2 g Q8h + sulbactam 1 g Q6h	–	–	–	–	–	–	3–16	32–96	16–32
Minocycline HD + polymyxin B	2–4	8	16	2	2	4	–	–	–
Polymyxin B + sulbactam 9 g/24 h CI	–	–	–	4–6	8–16	8–16	6–12	>256	>256
Triple therapy									
Minocycline 700 mg load, 350 mg Q12h (HD) + meropenem 6 g/24 h CI + sulbactam 9 g/24 h CI	6	8	8	–	–	–	4–6	12	12
Minocycline HD + polymyxin B + sulbactam 9 g/24 h CI	6–8	6–8	4–6	8–12	4	4	4	3–4	3–4

[a]All MICs determined using E-tests and represent the range determined by duplicate runs. The meropenem MIC is 128 μg/ml.
[b]CLSI breakpoints used for susceptibility: minocycline ≤4; polymyxin B ≤2, meropenem ≤2; and ampicillin/sulbactam ≤8/4; –, not applicable.
[c]HD, high dose; SD, standard dose; CI, continuous infusion.

TABLE 5 MIC Ranges at 24, 48, and 72 h for non-CRAB isolates

	MIC in μg/ml[a,b]											
	Minocycline (susceptible MIC = 0.5)			Polymyxin B (susceptible MIC = 0.5)			Meropenem (susceptible MIC = 0.5)			Sulbactam (susceptible MIC = 2)		
Regimen[c]	24 h	48 h	72 h	24 h	48 h	72 h	24 h	48 h	72 h	24h	48h	72h
Monotherapy												
Minocycline 200 mg load, 100 mg Q12h (SD)	6–8	4–6	4–6	–	–	–	–	–	–	–	–	–
Minocycline 700 mg load, 350 mg Q12h (HD)	1	24	24–32	–	–	–	–	–	–	–	–	–
Meropenem 1 g Q8h	–	–	–	–	–	–	0.5–0.75	1.5	1–1.5	–	–	–
Polymyxin B 2.5 mg/kg Q12h	–	–	–	0.75–2	1.5–16	4–12	–	–	–	–	–	–
Sulbactam 1 g Q6h	–	–	–	–	–	–	–	–	–	3–4	6	6–12
Dual therapy												
Minocycline SD + meropenem 1 g Q8h	0.75–1.5	1.5–2	2	–	–	–	1	0.75–2	2	–	–	–
Minocycline SD + sulbactam 1 g Q6h	1.5–2	1.5	1.5–2	–	–	–	–	–	–	3	3	8
Minocycline SD + polymyxin B 2.5 mg/kg Q12h	0.38–0.75	1	0.75	16–24	12	12	–	–	–	–	–	–
Meropenem 1g Q8h + sulbactam 1 g Q6h	–	–	–	–	–	–	1.5	1.5	1	3–4	4	3
Minocycline HD + meropenem 6 g/24 h CI	NG	NG	NG	–	–	–	NG	NG	NG	–	–	–
Minocycline HD + sulbactam 9 g/24 h CI	1.5	1.5	1.5	–	–	–	–	–	–	2–3	3	3
Triple therapy												
Minocycline HD + meropenem 6 g/24 h CI + sulbactam 9 g/24 h CI	0.5	0.5	0.5	–	–	–	0.5	0.5	0.5	2	2	2

[a]All MICs determined using E-tests and represent the range determined by duplicate runs.
[b]CLSI breakpoints used for susceptibility: minocycline ≤4; polymyxin B ≤2, meropenem ≤2; and ampicillin/sulbactam ≤8/4; –, not applicable; NG, not enough growth to determine.
[c]HD, high dose; SD, standard dose; CI, continuous infusion.

TABLE 6 Values of achieved and targeted pharmacokinetic parameters

Regimen[a]	fC_{max} (μg/ml)		Half-life (h)		K_e		% Protein binding	*f*AUC (μg · h/ml)		*f*AUC/MIC CRAB (non-CRAB)		% $T_{>MIC}$ (CRAB [non-CRAB])	
	Targeted	Achieved	Targeted	Achieved	Targeted	Achieved	Targeted	Targeted	Achieved	Targeted	Achieved	Targeted	Achieved
Minocycline 200 mg load plus 100 mg Q12h	0.84 (0.6–1.59)	0.78[b]	11	10.56	0.06	0.07	76	14.4	14.1	7.2 (28.7)	7.0 (28.1)	—	—
Minocycline 700 mg load, 350 mg Q12h (HD)	2.94	2.73[b]	11	10.56	0.06	0.07	76	43.4	42.4	21.7 (86.7)	21.2 (84.8)	—	—
Polymyxin B 2.5 mg/kg/day	1.75	1.77[b]	4.3	7.44	0.16	0.1	79	21.8	31.3	10.9 (43.7)	15.6 (62.5)	—	—
Meropenem 1g Q8h	48.02	38.36[c]	1	1	0.69	0.69	2	335.8	287.5	—	—	0 (≥50)	0[d] (100)
Sulbactam[e] 1g Q6h	26.66	35.14[c]	1	1	0.69	0.69	38	253.7	338.8	—	—	≥60 (≥60)	67 (83)

[a]Pharmacokinetic parameters extrapolated to other regimen based on achieved K_e.
[b]Determined with in house bioassay.
[c]Determined by HPLC analysis.
[d]Unable to achieve $T_{>MIC}$ as this isolate was meropenem resistant.
[e]Tested as ampicillin-sulbactam.

concentration exceeds the MIC ($fT_{>MIC}$) of 60%, meropenem achieved 0% $fT_{>MIC}$, as expected with a MIC of 128 μg/ml, while the sulbactam $fT_{>MIC}$ was 67%.

For non-CRAB isolates, the achieved *f*AUC/MIC of 28.14 was within 2% of the targeted *f*AUC/MIC of 28.74 for minocycline 200 mg load, 100 mg Q12h, and HD minocycline (700 mg load, 350 mg Q12h) achieved $f\text{AUC}_{24h}$ of 84.84 while targeted *f*AUC/MIC was 86.70. The achieved *f*AUC/MIC for polymyxin B was 62.52, and the targeted *f*AUC/MIC was 43.68. Free $fT_{>MIC}$ was 100% for meropenem and 83% for sulbactam for the non-CRAB isolate, and met recommendations of >60%. Pharmacokinetic parameters were extrapolated based on the achieved elimination rate constant (K_e) for all other dosing regimens used for pharmacodynamic models.

DISCUSSION

Acinetobacter baumannii is associated with a wide variety of intrinsic and acquired resistance mechanisms (2). Carbapenem resistance is associated with higher mortality rates than carbapenem-susceptible *A. baumannii* isolates, with an adjusted odds ratio of 2.49 (95% confidence interval [CI], 1.61 to 3.84) (5). *A. baumannii* can develop resistance to carbapenems through several mechanisms, including plasmid and chromosomally encoded carbapenemases (e.g., OXA-like class D beta-lactamases), metallo beta-lactamases, porin changes, penicillin-binding protein alterations, as well as efflux pumps. Due to this propensity for multidrug resistance, empirical therapy may miss coverage of CRAB isolates, creating a notable delay in therapy that may be responsible for the increased mortality, as previously demonstrated in *Enterobacteriaceae* (7). Furthermore, even upon organism identification and susceptibility reporting, optimal anti-CRAB therapy remains unclear. Therefore, our study aimed to evaluate the optimal monotherapy or combination therapy against CRAB and non-CRAB isolates by looking at standard doses as well as pharmacodynamically optimized doses (11–14).

This work identifies several critical dosing strategies to consider. First, despite having low MICs, non-CRAB monotherapy generally led to regrowth that was associated with development of resistance within 24 to 72 h, depending on the regimen, but favoring pharmacodynamically optimized combination therapy. The pharmacodynamic index most associated with activity for polymyxin B is $f\text{AUC}_{24h}$ and *f*AUC/MIC for minocycline, although standard-dose minocycline (e.g., 200 mg) has been associated with development of resistance, similar to the observations within this study suggesting that an *f*AUC/MIC goal of 20 to 25 may be needed (11, 15). A previous dose-finding study found high-dose minocycline capped at 700 mg daily, after a loading dose up to 700 mg, was safe when used for its anti-inflammatory properties in ischemic stroke management for 6 total doses (14). Our study showed that, although regrowth occurs with monotherapy, combining high-dose minocycline with continuous-infusion meropenem with or without sulbactam led to the highest reduction in bacterial counts against non-CRAB strains. For CRAB isolates, triple therapy may be required as a 2-log kill was sustained at 24, 48, and 72 h only when treated with high-dose minocycline in combination with polymyxin B and continuous-infusion sulbactam. However, the safety profile for long-term therapy in human studies is needed.

Meropenem and sulbactam are time-dependent antimicrobial agents that exhibit activity when the free concentrations are maintained above the MIC ($fT_{>MIC}$) at least 40 to 60% of the time (16, 17). Continuous or extended infusion meropenem was found to have superior cumulative fraction of response as determined by population pharmacokinetic modeling and Monte Carlo simulation (13). Therefore, this is a recommended dosing strategy, particularly for less-susceptible organisms, including *A. baumannii*, and *Pseudomonas aeruginosa*. Similarly, prolonging sulbactam infusion has been associated with favorable target attainment probability (12). While the current study did not use extended infusion (e.g., over 4 h) dosing strategies, it does highlight that including continuous-infusion beta-lactam therapy as double and/or triple therapy may yield greater reductions in bacterial count than intermittent dosing (e.g., Q8h 30 min infusion).

CRAB isolates remain difficult to treat, and optimal therapy that minimizes toxicity needs to be elucidated. While polymyxin B monotherapy demonstrated activity against the CRAB isolate, development of resistance was noted over the course of 72 h. Current literature comparing polymyxin monotherapy versus combination therapy against clinical outcomes is limited. However, as observed within this study, rapid emergence of resistance, particularly when used as monotherapy, is concerning and has resulted in clinical failure (18, 19). Furthermore, heteroresistance among clinical isolates (20) and a paradoxical amplification of resistance associated with increased polymyxin B exposure was previously observed, highlighting the pitfalls of monotherapy against *A. baumannii* (21). Our study demonstrated that triple therapy, particularly with high-dose minocycline, polymyxin B, and continuous-infusion sulbactam, resulted in the greatest reduction of bacterial counts without emergence of resistance among CRABs. This finding is supported by previous data of a 14-day hollow fiber model, where administration of high-dose sulbactam (tested as ampicillin/sulbactam) as a component of a combination regimen combated polymyxin B resistance, indicating that dose manipulation may aid in overcoming some resistance mechanisms (22). Of note, while tested as ampicillin/sulbactam, anti-*Acinetobacter* activity is attributed to the sulbactam component through the binding of penicillin binding protein 1 (PBP1) and PBP3 (23).

Our study has several limitations. This is an *in vitro* pharmacodynamic model with a limited duration of 72 h that does not consider patient's own immune function, limiting extrapolation to clinical outcomes. Additionally, this study simulated only two strains of *Acinetobacter baumannii* at a fixed initial inoculum of 10^6 CFU/ml (i.e., final observations may not predict the results of a higher burden of infection). It evaluated only four antimicrobial agents, and assumed normal renal function. Therefore, it is unclear whether other antimicrobial agents provide more optimal reductions in bacterial counts, and how impaired renal function might alter pharmacodynamic effects against these isolates. Nonetheless, this study provides important insight on the difficulties associated with eradicating *A. baumannii*. Both CRAB and non-CRAB isolates demonstrate propensity for rapidly inducible resistance mechanisms, posing a serious threat to public health. Data indicate there is an unmet clinical need to identify optimal therapy for both CRAB and non-CRAB isolates in order to improve patient outcomes.

In conclusion, carbapenem-resistant *Acinetobacter* was recently escalated to an urgent public health threat by the CDC, representing a large burden among immunocompromised patients (1). This escalation in threat severity is attributed to the ease of resistance development, lack of current effective antibiotics, and lack of antibiotics in the development pipeline. Consequently, our study aimed to study minocycline alone and in various combinations with polymyxin B, meropenem, and sulbactam. Our data confirm that *in vitro* resistance can develop rapidly across both CRAB and non-CRAB isolates. This was associated with most regimens failing to sustain kill at 24, 48, and 72 h. Against CRAB isolates, the triple combination of high-dose minocycline, polymyxin B, and continuous-infusion sulbactam was the most consistently active regimen, while high-dose minocycline with continuous-infusion meropenem with and without continuous-infusion sulbactam was most persistently bactericidal against non-CRAB isolates. Our results should be applied to clinical practice cautiously, as confirmation from clinical outcome trials is necessary.

MATERIALS AND METHODS

Bacterial strains. Two clinical isolates of *Acinetobacter baumannii* (NR-13382 and NR-17786) were tested. Both were isolated from human blood and obtained from the Biodefense and Emerging Infection Research Resources Repository, Manassas, VA (BEI). Strain NR-13382 was both multidrug resistant (MDR) in accordance with previous definitions (10) and a CRAB, while strain NR-17786 was a non-MDR, non-CRAB.

Media and antimicrobials. Minocycline HCl powder (The Medicines Company; lot number M3401602), meropenem (Fresenius Kabi, LLC; lot number 0004D51), ampicillin/sulbactam (Pfizer Inc.; lot number 16/17000), and polymyxin B (X-Gen Pharmaceuticals; lot number AB7600) drug products were used in the experiments. Drug products were supplied by the Providence Veterans Affairs Medical

Center Pharmacy Department. Antimicrobial stability was ensured in accordance with drug monographs and Trissel's Stability of Compounded Formulations, accessed via Micromedex. Continuous infusion regimens were replaced with new drug approximately every 8 to 10 h. Cation-adjusted (calcium, 25 μg/ml and magnesium, 12.5 μg/ml) Mueller-Hinton broth (CAMHB; Difco Laboratories, Sparks, MD, USA) was used to determine susceptibility testing and for *in vitro* models.

For non-CRAB isolates, monotherapy regimens included: (i) minocycline 200 mg load, followed by 100 mg Q12h (i.e., standard dose, SD); (ii) minocycline 700 mg load, followed by 350 mg Q12h (i.e., high-dose, HD); (iii) meropenem 1 g Q8h; (iv) polymyxin 2.5 mg/kg Q12h; or (v) sulbactam 1g Q6h. Dual therapy regimens included: (i) minocycline SD + meropenem 1g Q8h; (ii) minocycline SD + sulbactam 1g Q6h; (iii) minocycline SD + polymyxin 2.5 mg/kg Q12h; (iv) meropenem 1g Q8h + sulbactam 1g Q6h; (v) minocycline HD + meropenem 6 g/24 h continuous infusion (CI); or (vi) minocycline HD + sulbactam 9 g/24 h CI. Finally, triple therapy maximizing drug exposure included minocycline HD + meropenem 6 g/24 h CI + sulbactam 9 g/24 h CI. Initial doses were selected based on MIC. For example, lower drug exposures were utilized for non-CRAB isolates because eradication was anticipated. These doses were evaluated against pharmacodynamically optimized doses (i.e., continuous infusion and higher doses) to assess differences in isolate eradication.

For CRAB isolates, monotherapy regimens included: (i) minocycline 200 mg load, followed by 100 mg Q12h (i.e., standard dose, SD); (ii) meropenem 1 g Q8h; (iii) meropenem 2 g Q8h; (iv) polymyxin 2.5 mg/kg Q12h; or (v) sulbactam 1g Q6h. Dual therapy regimens included: (i) minocycline SD + meropenem 2 g Q8h; (ii) minocycline SD + sulbactam 1g Q6h; (iii) minocycline SD + polymyxin B 2.5 mg/kg Q12h; (iv) meropenem 2 g Q8h + sulbactam 1g Q6h; (v) minocycline HD + polymyxin B 2.5 mg/kg Q12h; or (vi) polymyxin B 2.5 mg/kg Q12h + sulbactam 9 g/24 h CI. Finally, triple therapy maximizing drug exposure included (i) minocycline HD + meropenem 6 g/24 h CI + sulbactam 9 g/24 h CI and (ii) minocycline HD + polymyxin B 2.5 mg/kg Q12h + sulbactam 9 g/24 h CI. A higher dose of meropenem was selected for dual therapy due to carbapenem resistance and the necessity for optimized exposures. Additionally, more polymyxin B-containing regimens were included because of inherent challenges of eradicating CRAB isolates. Pharmacodynamically optimized doses were chosen to maximize exposures while maintaining clinically achievable concentrations previously utilized in practice.

Susceptibility testing. MIC was determined via broth microdilution in accordance with Clinical and Laboratory Standards Institute (CLSI) standards using cation-adjusted Mueller-Hinton broth (24, 25). Combination MICs were not conducted. To assess the activity of ampicillin, isolates were tested against sulbactam alone prior to use of ampicillin/sulbactam and no difference in MIC was noted. Baseline isolate characteristics are further depicted in Table 1.

***In vitro* pharmacodynamic model.** An *in vitro* 72-h pharmacodynamic (IVPD) model using a 250-ml one-compartment chamber with ports for removal of medium, administration of antimicrobials, and collection of bacterial samples was employed using previously established methodology for monotherapy and combination therapy (26–28). The chamber was prefilled with medium, and isolate inoculation as well as intermittent antimicrobial boluses were slowly (over ~1 min) administered via injection port. Continuous-infusion antimicrobials were combined with broth. All models were simulated in duplicate to ensure reproducibility. Prior to each experiment, *A. baumannii* colonies from an overnight growth on tryptic soy agar (TSA) (Difco, Becton, Dickinson Co., Sparks, MD, USA) were suspended in 0.9% sodium chloride to obtain a 0.5-McFarland standard suspension for an initial starting inoculum of 10^6 CFU/ml. Models were placed in a 37°C water bath with a magnetic stir bar for continuous mixing of medium for the duration of the experiment. Antibiotic-containing broth was continuously replaced using a peristaltic pump (Masterflex; Cole-Parmer Instrument, Chicago, IL) at a rate simulating the half-life elimination for the respective antibiotic. Combination regimen experiments were set for the rate of the drug with the shortest half-life, and the drug with the longer half-life was supplemented in accordance with previously established methods (27, 28). Of note, because three different half-lives could not be simulated, for the combination of high-dose minocycline with polymyxin B and continuous-infusion sulbactam, polymyxin B was utilized as the fastest half-life drug and sulbactam concentration was maintained as a constant (i.e., the broth contained sulbactam throughout the experiment). Consequently, the aforementioned regimen did not receive a loading dose of sulbactam, while all other continuous infusion regimens did. All antimicrobials were administered to simulate humanized doses corresponding to exposures reflecting approximate free drug concentrations (i.e., fC_{max}), based on protein binding affinity at standard doses and pharmacodynamically optimized doses (Table 6).

Pharmacodynamic analysis. Approximately 1-ml samples were collected from each model at 0, 4, 8, 24, 32, 48, and 72 h and serially diluted with 0.9% sodium chloride. Bacterial counts were determined by inoculating three 20-μl drops of each dilution onto TSA plates. Plated samples were incubated at 37°C for 24 h and colonies were subsequently counted (CFU per milliliter) with a limit of detection of 2.0 $\log_{10}$ CFU/ml (29–31). Growth curves were conducted for each pathogen at the fastest and slowest half-life of each model. Antimicrobial carryover was accounted for by serial dilution of all plated samples. When the anticipated dilution was near the MIC, vacuum filtration was utilized to remove antimicrobial agents through the use of a 0.45-μm filter and sterile water. Filters were plated on TSA plates and incubated at 37°C for 24 h. Time-kill curves of colony counts (log 10 CFU/ml) versus time were plotted for each model (SigmaPlot V13.0, Systat Software, Inc.). Bactericidal activity (i.e., 99.9% kill) was defined as a $\geq$3 $\log_{10}$ CFU/ml reduction in colony counts compared to the initial inoculum, while bacteriostatic activity was defined as a $<$3 $\log_{10}$ CFU/ml reduction in colony count (26). Models without change in colony count were deemed inactive (26). When comparing combination regimens, enhanced activity was defined as a $\geq$2 $\log_{10}$ CFU/ml increase in kill compared to the most active single agent within that

combination, while improvement was defined as a one to two $\log_{10}$ CFU/ml increase in kill compared to the most active single agent. Colony count reductions were measured over a 72-h period at 24, 48, and 72 h.

Pharmacokinetic analysis. Samples for pharmacokinetic analyses were obtained through the injection port at 0, 0.5, and 4 h for verification of target antibiotic concentrations. All samples were stored at −80°C until analysis. Meropenem and ampicillin-sulbactam concentrations were determined by a previously described and validated high-pressure liquid chromatography (HPLC) method (Center for Anti-Infective Research and Development, Hartford, CT) (32). Polymyxin B concentrations were determined by a modified microbioassay utilizing *Micrococcus luteus* ATCC 49732 as the reference organism (33, 34). Bioassay models were run at a higher concentration of 30 μg/ml in order to achieve zones of inhibition in the agar. As previously described, the bacteria were incorporated into molten cation-adjusted MHA to achieve a final concentration of approximately 5 log 10 CFU/ml (34). Three standard solutions (60, 30, and 15 μg/ml) and duplicates of three test samples from the model were pipetted into a 6-mm diameter hole in the agar. Plates were incubated at 35°C for 24 h and the zones of inhibition were measured using calipers. Minocycline concentrations were determined by standard agar diffusion assay using a lawn of 0.5 McFarland of *Micrococcus luteus* ATCC 49732 on cation-adjusted MHA (33). Bioassay models were run at a higher concentration of 30 μg/ml in order to achieve zones of inhibition on the agar. Three standard solutions (30, 15, and 7.5 μg/ml) and duplicates of three test samples from the model were pipetted onto a 6-mm blank disk on the agar. Plates were incubated at 35°C for 24 h, and the zones of inhibition were measured using calipers. Standard curves for the assays were made by plotting the inhibition zone diameter versus the standard drug concentrations. Both assays were linear over their concentration ranges; minocycline (R^2 = 0.9643) and polymyxin B (R^2 = 0.9944).

Based on previously described pharmacodynamic parameters, we targeted a higher *f*AUC/MIC of 20 to 25 for minocycline monotherapy (11). However, in order to test for enhanced activity of minocycline in combination with beta-lactams, we targeted a lower *f*AUC/MIC. Optimal polymyxin B *f*AUC/MIC for *Acinetobacter* is recommended to be around 12 to 48 (15, 35, 36). For sulbactam (12, 16) and meropenem (13, 17), we targeted a $fT_{>MIC}$ of at least 40 to 60%. The AUC was calculated based on the trapezoidal rule in Excel.

Resistance. Samples from 0, 24, 48, and 72 h were taken and approximately 100 μl was plated on Mueller-Hinton agar (MHA) for MIC determination via Etest for each antimicrobial agent.

Statistical analysis. Bacterial colony counts (i.e., $\log_{10}$ CFU/ml) were compared at 24, 48, and 72 h by two-way analysis of variance using Tukey's *post hoc* test. A P value of $\leq$0.05 was considered significant. Data were plotted and graphed using SigmaPlot V13.0 software (Systat Software, Inc.). All data were analyzed using SPSS statistical software (SPSS version 24 Inc. Chicago, IL).

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