Pharmacodynamics of the Antibacterial Effect of and Emergence of Resistance to Doripenem in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in an *In Vitro* Pharmacokinetic Model

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An *in vitro* dilutional pharmacokinetic model of infection was used to study the pharmacodynamics of doripenem in terms of the ability to kill *Pseudomonas aeruginosa* or *Acinetobacter baumannii* and also changes in their population profiles. In dose-ranging studies, the cumulative percentages of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions (T_{MIC} s) required for doripenem to produce a 24-h bacteriostatic effect and a -2 -log-unit reduction in viable count were $25\% \pm 11\%$ and $35\% \pm 13\%$, respectively, for *P. aeruginosa* (MIC range, 0.24 to 3 mg/liter) and $20\% \pm 11\%$ and $33\% \pm 12\%$, respectively, for *Acinetobacter* spp. (MIC range, 0.45 to 3.0 mg/liter). A T_{MIC} of >40 to 50% produced a maximum response with both species at 24 h or 48 h of exposure. After 24 h of exposure to doripenem at a T_{MIC} in the range of 12.5 to 37.5%, *P. aeruginosa* and *A. baumannii* population profiles revealed mutants able to grow on $4\times$ MIC-containing medium; such changes were further amplified by 48 h of exposure. Dose-fractionation experiments targeting T_{MIC} s of 12.5%, 25%, or 37.5% as six exposures, two exposures, or a single exposure over 48 h with a single strain of *P. aeruginosa* indicated that changes in population profiles were greatest with multiple exposures at T_{MIC} targets of 12.5 or 25%. In contrast, multiple exposures at 37.5% T_{MIC} most effectively suppressed total bacterial counts and changes in population profiles. Simulations of human doses of doripenem of 500 mg, 1,000 mg, 2,000 mg, and 3,000 mg every 8 h over 96 h showed marked initial killing up to 6 h but growback thereafter. Changes in population profiles occurred only in the regimen of 500 mg every 8 h against *P. aeruginosa* but occurred with all dose regimens for *A. baumannii* strains. A doripenem T_{MIC} of ≥ 40 to 50% is maximally effective in killing *P. aeruginosa* or *A. baumannii* and suppressing changes in population profiles in short-term experiments for up to 48 h; however, a T_{MIC} of 12.5 to 25% amplifies population changes, especially with exposures every 8 h. In longer-term experiments, up to 96 h, even doripenem doses of 4 to 6 times those used in human studies proved incapable of pathogen eradication and prevention of changes in population profiles. The association of a T_{MIC} of 25 to 37.5% with changes in population profiles has implications in terms of future clinical breakpoint setting.

Preclinical studies on antibacterial pharmacokinetics and pharmacodynamics underpin the doses of drug and drug dose frequencies used in clinical trials for developmental drugs and afterwards in clinical practice. Such preclinical studies are most often performed over short durations, that is, up to 24 to 48 h, and most commonly focus on bacterial killing. While such approaches have proven utility, they do not address the longer-term effects of antibiotic dosing used in clinical practice or the risks of emergence of resistance in target pathogens at sites of infection during treatment. Such preclinical data are likely to have important impacts on drug use clinically.

Doripenem is the newest carbapenem antibiotic approved for clinical use in Europe and North America. Studies of *in vitro* potency show that doripenem has potency similar to that of imipenem against Gram-positive pathogens and potency similar to that of meropenem against Gram-negative pathogens (7, 8). These studies suggest that doripenem will have particular clinical utility against hospital-acquired pathogens, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*.

Doripenem MICs for *P. aeruginosa* are in the range of 0.06 to 8 mg/liter, with MIC₅₀ values being 0.25 mg/liter and MIC₉₀s being 0.5 to 4 mg/liter (7, 8, 15, 17, 18). Activity against carbapenem-resistant isolates and those producing metallo- β -lactamases is reduced (7, 8). MICs against *A. baumannii* are in the range of 0.03 to ≥ 32 mg/liter, with MIC₅₀s being 0.25 to 0.5 mg/liter and MIC₉₀s

being 1 to 16 mg/liter (5, 7, 8). *Acinetobacter* sp. strains with a range of carbapenemases, such as IMP, OXA-23 to OXA-27, and OXA-40, have doripenem MICs of ≥ 16 mg/liter (13). Recent studies on clinical isolates from the United States showed that doripenem was 4-fold more active than meropenem, 4- to 16-fold more potent than imipenem against *P. aeruginosa*, and 2-fold more potent than both against *Acinetobacter* spp. (15).

Preclinical animal pharmacodynamic studies have indicated that a cumulative percentage of a 24-h period that the drug concentration exceeds the MIC under steady-state pharmacokinetic conditions (T_{MIC}) over the range of 20 to 40% (mean, 29%) was associated with a 24-h bacteriostatic effect and that a T_{MIC} of 35 to 54% (mean, 44%) was associated with a -2 -log-unit kill of aerobic Gram-negative rods, including *P. aeruginosa* (1). Against *P. aeruginosa* strains, a T_{MIC} of 20% was

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TABLE 1 Doripenem pharmacokinetic parameters and concentrations

Dose (mg/liter)	Parameter ^a	Value	
		Measured	Target
500	C at 1 h (mg/liter)	22.6 ± 3.6	22.9
	C at 4 h (mg/liter)	2.6 ± 0.6	
	C at 6 h (mg/liter)	0.8 ± 0.2	
	t _{1/2} (h)	1.02 ± 0.10	1.07
	AUC _{0–8} (mg · h/liter)	31.4 ± 3.8	31.5
1,000	C at 1 h (mg/liter)	42.2 ± 3.6	
	C at 4 h (mg/liter)	5.0 ± 0.6	
	C at 6 h (mg/liter)	1.4 ± 0.1	
	t _{1/2} (h)	1.02 ± 0.04	
	AUC _{0–8} (mg · h/liter)	59.8 ± 2.4	
2,000	C at 1 h (mg/liter)	79.5 ± 4.2	
	C at 4 h (mg/liter)	10.1 ± 6.9	
	C at 6 h (mg/liter)	2.6 ± 0.2	
	t _{1/2} (h)	1.02 ± 0.05	
	AUC _{0–8} (mg · h/liter)	119.4 ± 3.0	
3,000	C at 1 h (mg/liter)	111.5 ± 5.7	
	C at 4 h (mg/liter)	14.7 ± 1.0	
	C at 6 h (mg/liter)	3.7 ± 0.2	
	t _{1/2} (h)	0.95 ± 0.07	
	AUC _{0–8} (mg · h/liter)	167.6 ± 8.2	

^a C, concentration; AUC_{0–8}, area under the concentration-time curve from time zero to 8 h.

associated with a 24-h bacteriostatic effect and a T_{MIC} of 40% was associated with a –3-log-unit drop when animal pharmacokinetics were humanized (9). Given the standard dosing of 500 mg infused over 1 h every 8 h in humans and a T_{MIC} target of 35%, then bacterial strains with MICs of ≤2 mg/liter could be regarded as suitable targets for doripenem (2). Strains of *P. aeruginosa* with doripenem MICs of 4 to 16 mg/liter have been studied in animal models, and doses larger than 500 mg have been simulated in a bid to understand if strains with raised MICs could be treated in humans (4). As yet, no studies on the pharmacodynamics of emergence of resistance to doripenem or studies of dosing longer than 24 to 48 h have been published.

We used an *in vitro* pharmacokinetic model of infection to study the relationship between doripenem exposure and emergence of resistance in two key difficult-to-treat pathogens in serious hospital infections, namely, *P. aeruginosa* and *A. baumannii*. We then simulated the impact of high-dose doripenem exposure

over 96 h on the antibacterial effect and risk of emergence of resistance for these bacteria.

MATERIALS AND METHODS

In vitro pharmacokinetic model. A New Brunswick (Hatfield, Herefordshire, England) Bioflo 1000 *in vitro* pharmacokinetic model was used to simulate free serum concentrations of doripenem associated with administration of 500 mg, 1,000 mg, 2,000 mg, and 3,000 mg every 8 h and also to perform dose-ranging simulations and dose-fractionation experiments. The apparatus, which has been described before, consists of a single central chamber connected to a collecting vessel for overflow (10). The contents of the central chamber were diluted with Mueller-Hinton broth (MHB) using a peristaltic pump (Ismatec; Bennett & Co., Weston-Super-Mare, England) at a flow rate of 231 ml/h for doripenem. The temperature was maintained at 37°C, and the broth in the central chamber was agitated by a stirrer.

Medium. Fifty percent MHB in sterile water was used in all experiments. Nutrient agar plates (Thermo Fisher, Basingstoke, England) were used to recover *P. aeruginosa* and *A. baumannii* strains from the *in vitro* model. Ten microliters of β-lactamase/ml was used to neutralize doripenem. The β-lactamase neutralized doripenem up to 100 μg/ml. Doripenem was added to nutrient agar plates in studies of emergence of resistance containing 1×, 2×, 4×, and 8× MIC multiples of the test strain.

Strains. Three clinical strains of *P. aeruginosa* (strain 38475 [doripenem MIC, 0.24 mg/liter], strain 39135 [doripenem MIC, 0.75 mg/liter], strain 17286 [doripenem MIC, 3.0 mg/liter]) and three clinical strains of *A. baumannii* (strain 33980 [doripenem MIC, 0.45 mg/liter], strain 28893 [doripenem MIC, 0.75 mg/liter], strain 7186 [doripenem MIC, 3.0 mg/liter]) were used. The strains were selected, as they had a range of MIC values of <4 mg/liter.

Antibiotics. Doripenem was supplied by Johnson & Johnson Pharmaceutical Research and Development, LLC, Raritan, NJ. Fresh stock solutions were prepared daily according to British Society for Antimicrobial Chemotherapy Guidelines (3).

Pharmacokinetics. The doripenem concentration at 1 h was 23 mg/liter, with a half-life ($t_{1/2}$) of 1.0 h, when performing dosing simulations of doripenem at 500 mg every 8 h (2, 4). In addition, between 6 and 12 doses were simulated per strain in dose-ranging experiments designed to achieve a doripenem T_{MIC} of 0 to 100% for each strain by changing the amount of drug given. In experiments to define the T_{MIC} -antibacterial effect relationship in dose-fractionation experiments, T_{MIC} targets of 12.5%, 25%, and 37.5% were achieved using six doses in 48 h, two doses in 48 h, and a single dose in 48 h. Human dose simulations of 500 mg, 1,000 mg, 2,000 mg, and 3,000 mg every 8 h were also performed over 96 h.

Doripenem concentrations were determined using a modification of a validated in-house high-pressure liquid chromatography method for ertapenem (16). Chromatography was performed using a Concept II pump, a 310-nm detector, and a Hypersil 5 octyldecyl silane column (10 by 4.6 mm). The mobile phase was composed of 12.5% methanol, 1% phosphoric acid, 86.5% water (vol/vol) and was pumped at a flow rate of 2

TABLE 2 Relationship between T_{MIC} and log change in viable count of *P. aeruginosa* after 24 and 48 h of exposure to doripenem

Effect	% T_{MIC} for the indicated strains at the following times:							
	24 h				48 h			
	38475 (0.24) ^a	39135 (0.75)	17286 (3.0)	Mean ± SD	38475 (0.24)	39135 (0.75)	17286 (3.0)	Mean ± SD
Static	37	24	14	25 ± 11	41	31	30	34 ± 6
–1 log drop	42	29	19	30 ± 11	62	34	— ^b	—
–2 log drop	48	34	23	35 ± 13	—	35	—	—
–3 log drop	62	41	28	44 ± 17	—	37	—	—
R ² for model	0.92	0.94	0.96		0.96	0.99	0.99	

^a Data in parentheses are MICs (in mg/liter).

^b —, endpoint not achieved.

TABLE 3 Relationship between T_{MIC} and log change in viable count of *Acinetobacter baumannii* after 24 and 48 h of exposure to doripenem

Effect	% T_{MIC} for the indicated strains at the following times:							
	24 h				48 h			
	33980 (0.45) ^a	28893 (0.75)	7186 (3.0)	Mean $\pm$ SD	33980 (0.45)	28893 (0.75)	7186 (3.0)	Mean $\pm$ SD
Static	30	21	9	20 $\pm$ 11	30	22	14	22 $\pm$ 8
–1 log drop	32	30	14	25 $\pm$ 10	32	36	21	30 $\pm$ 7
–2 log drop	37	43	20	33 $\pm$ 12	36	56	29	40 $\pm$ 14
–3 log drop	— ^b	77	27	—	—	—	39	—
R^2 for model	0.95	0.95	0.96		0.99	0.98	0.97	

^a Data in parentheses are MICs (in mg/liter).^b —, endpoint not achieved.

ml/min. A 5- μ l injection volume was used for samples and standards. Detection of doripenem was by determination of UV absorbance at 310 nm, with collection and storage of data performed using a Dionex Chromeleon system. Prior to chromatography, standards and samples were diluted with an equal volume of methanol at a 50:50 ratio and spun at 13,000 $\times$ g. The resultant supernatant was pipetted into an injection vial, which was capped and placed in the autoinjector. Samples were assayed within 5 h of being placed in the autoinjector. Previous studies have shown doripenem to be stable under such conditions. The injection volume was 5 μ l. A standard was included after every 6th sample. The intra-assay precision was below 5% (16).

Antibacterial effects (ABEs). All experiments were performed with an initial inoculum of 10⁶/ml, as described before (11). Samples were taken throughout the simulations for detection of viable counts. Bacteria were quantified using a spiral plater (Don Whitley Spiral Systems, West Yorkshire, England). The minimum level of detection was 10² CFU/ml. Aliquots were stored at –70°C for measurement of doripenem.

Emergence of resistance. Resistance to doripenem was assessed by population analysis profiles (10) at time zero (preexposure), and every 24 h, postexposure aliquots were plated onto agar containing no antibiotic

and antibiotic at 1 $\times$, 2 $\times$, 4 $\times$, and 8 $\times$ MIC to quantify any changes in profile. The limit of detection was 10² CFU/ml.

All pharmacokinetic simulations of human drug doses (doripenem at 500 mg, 1,000 mg, 2,000 mg, and 3,000 mg) to determine ABE and emergence of resistance were performed at least in triplicate, as were the dose-fractionation studies.

Pharmacodynamics and measurement of ABEs. The ABE of doripenem was calculated by determining the log change in viable counts between time zero and 12 h (d12), 24 h (d24), 36 h (d36), 48 h (d48), 72 h (d72), and 96 h (d96). The area under the bacterial kill curve (AUBKC; log CFU $\cdot$ h/ml) was calculated using the trapezoid rule for the areas between 0 and 24 h and 0 and 48 h. The relationship between T_{MIC} and ABE was delineated using a sigmoid maximum-effect model (GraphPad Prism software; GraphPad Software, San Diego, CA).

RESULTS

Pharmacokinetics. The target and modeled pharmacokinetics of the 500-mg, 1,000-mg, 2,000-mg, and 3,000-mg doripenem doses are illustrated in Table 1.

TABLE 4 Risk of emergence of resistance in *P. aeruginosa* and *A. baumannii* after exposure to doripenem over 48 h

Organism-exposure and % T_{MIC}	No. of experiments	Growth on 4× MIC plates		Growth on 8× MIC plates		Growth on 16× MIC plates	
		No. of occasions	Count (log CFU/ml)	No. of occasions	Count (log CFU/ml)	No. of occasions	Count (log CFU/ml)
<i>P. aeruginosa</i> at 24 h of exposure							
12.5	4	2	6.0	0	<2	0	<2
25–37.5	6	1	6.7	0	<2	0	<2
50–75	6	0	<2	0	<2	0	<2
≥85	5	0	<2	0	<2	0	<2
<i>P. aeruginosa</i> at 48 h of exposure							
12.5	4	4	6.4 ± 1.0	2	8.0	0	<2
25–37.5	6	2	7.6	2	6.5	0	<2
50–75	6	3	5.6 ± 0.8	3	4.7 ± 1.6	0	<2
≥85	5	2	5.4	1	5.0	0	<2
<i>A. baumannii</i> at 24 h of exposure							
12.5	1	1	5.7	1	5.7	0	<2
25–49	6	0	<2	0	<2	0	<2
50–80	5	0	<2	0	<2	0	<2
≥85	3	0	<2	0	<2	0	<2
<i>A. baumannii</i> at 48 h of exposure							
12.5	1	1	5.7	1	5.7	1	3.9
25–49	6	2	4.6	2	4.2	7	4.8
50–80	5	1	5.0	1	4.6	0	<2
≥85	3	1	3.9	1	3.5	0	<2

FIG 1 Changes in doripenem population profiles over time after exposure to T_{MIC} target of 12.5% as a single dose or two or six doses over 48 h: dosing every 8 h (A), 24 h (B), and 48 h (C).

Dose ranging. A range of doses ($n = 6$ to 12) was used to provide a T_{MIC} range of 0 to 100% for each strain of *P. aeruginosa* and *A. baumannii*. ABEs were measured by d24 and AUBKC at 24 h and related to T_{MIC} . Using d24 as the primary ABE measure, the T_{MIC} for a static effect for *P. aeruginosa* was $25\% \pm 11\%$ and that for *A. baumannii* was $20\% \pm 11\%$. The T_{MIC} for a 2-log-unit drop in the *P. aeruginosa* viable count was $35\% \pm 13\%$, and that for *A. baumannii* was $33\% \pm 12\%$ (Tables 2 and 3). Growth on $4\times$ MIC plates was noted on 2/4 occasions with a T_{MIC} of 12.5% and 1/6 occasions with a T_{MIC} of 25 to 37.5% with *P. aeruginosa* after 24 h (Table 4). In none of the experiments prior to doripenem exposure were *P. aeruginosa* colonies able to grow on $4\times$ MIC-containing medium. Growth on $4\times$ MIC plates was also noted in the single experiment performed at a free T_{MIC} of 12.5% with *A. baumannii* (Table 4). With *P. aeruginosa* and *A. baumannii*, the maximum response at 24 h occurred at a T_{MIC} of 40 to 50%. At 48 h of doripenem exposure, the T_{MIC} for a static effect against *P. aeruginosa* was $34\% \pm 6\%$, and that for *A. baumannii* was $22\% \pm 8\%$ (Tables 2 and 3). Maximum eradication from the model occurred at 40 to 50% for both species, but for *P. aeruginosa*, only 2 of 3 strains were killed to a -1 -log-unit reduction in count at 48 h, and only for one strain there was a 2-log-unit kill (Table 2). Changes in population analysis profiles, using data from all three *P. aeruginosa* strains and all three *A. baumannii* strains, were much more marked at 48 h than 24 h, with more growth on $8\times$ MIC recovery plates across a wide range of T_{MIC} exposures (Table 4).

FIG 2 Changes in doripenem population profiles over time after exposure to T_{MIC} target of 25% as a single dose or two or six doses over 48 h: dosing every 8 h (A), 24 h (B), and 48 h (C).

Dose fractionation. *P. aeruginosa* strain 38475 was exposed to a T_{MIC} of doripenem targeted at 12.5%, 25%, and 37.5% but divided into six dose exposures over 48 h (dosing every 8 h), two dose exposures over 48 h (dosing every 24 h), and a single dose exposure (dosing every 48 h). Population profiles before and after 24 h and 48 h of exposure were performed. When the T_{MIC} was 12.5%, the regimen of dosing every 8 h produced greater changes in the population profile than the other two regimens, as evidenced by higher bacterial counts on $4\times$ MIC, $8\times$ MIC, and $16\times$ MIC plates. None of the regimens were effective at reducing total bacterial counts (Fig. 1). A similar pattern was noted with T_{MIC} targets of 25%, with none of the regimens producing a reduction in total viable counts, the regimen of dosing every 8 h producing the greatest changes in population profile, and the highest counts of bacteria occurring on $8\times$ MIC, $16\times$ MIC, and $32\times$ MIC plates (Fig. 2). In contrast, the simulation with a regimen of dosing every 8 h and a free T_{MIC} of 37.5% produced the most marked reduction in viable counts and also suppressed changes in population profiles compared to those for the regimens of dosing every 24 h or 48 h (Fig. 3).

Human dose simulations. Three human dose simulations were tested against *P. aeruginosa* strain 38475 (doripenem MIC, 0.24 mg/liter): 500 mg every 8 h, 1,000 mg every 8 h, and 2,000 mg every 8 h over a 96-h period. Population profiles were assessed

FIG 3 Changes in doripenem population profiles over time after exposure to T_{MIC} target of 37.5% as a single dose or two or six doses over 48 h: dosing every 8 h (A), 24 h (B), and 48 h (C).

every 24 h. The antibacterial effect against *P. aeruginosa* increased with increasing dose, with regrowth occurring from 6 h with the 500-mg simulation, 12 h with the 1,000-mg simulation, and 36 h with the 2,000-mg simulation (Fig. 4). Population profiles were largely unchanged over 96 h with the 1,000-mg and 2,000-mg simulations: there was no growth on 4× MIC recovery medium. With the simulation of 500 mg every 8 h, colony counts were 2.9 ± 1.0 log CFU/ml at 96 h (Table 5). The findings with *A. baumannii* strain 33980 (doripenem MIC, 0.45 mg/liter) were less consistent; however, the 2,000-mg and 3,000-mg simulations tended to be more active than the 500-mg and 1,000-mg simulations (Fig. 5). In contrast to the results for *P. aeruginosa*, *A. baumannii* grew on 4× MIC recovery medium with all simulations, especially from 48 h (Table 5).

DISCUSSION

These data derived from an *in vitro* pharmacokinetic model of infection are in excellent agreement with data from animal models studying the pharmacodynamics of doripenem against *P. aeruginosa*. We have shown that a T_{MIC} of $25\% \pm 11\%$ is required for a 24-h bacteriostatic effect and a T_{MIC} of $35\% \pm 13\%$ is required for a –2-log-unit reduction in viable count. The maximum antibacterial effect in our model occurred at a T_{MIC} of $\geq 40\%$. Previous data from neutropenic murine thigh models showed that for Gram-negative rods, including *P. aeruginosa*, the T_{MIC} required

FIG 4 Antibacterial effect of doripenem at 500 mg (■), 1,000 mg (□), and 2,000 mg (○) every 8 h against *P. aeruginosa* strain 38475.

for a 24-h bacteriostatic effect was $29\% \pm 5\%$ and that required for a 2-log-unit reduction in count was $44\% \pm 7\%$. The maximum effect occurred at a T_{MIC} of $\geq 40\%$ (1, 9). There are no published T_{MIC} -response relationships for doripenem and *A. baumannii*, but our 24-h static T_{MIC} exposure of $20\% \pm 11\%$ and maximal response at 50% T_{MIC} are in keeping with the observations made for other Gram-negative rods (1). The T_{MIC} s required for bactericidal activity at 48 h are not usually presented from animal models; our data imply that for both *P. aeruginosa* and *A. baumannii*, T_{MIC} exposures required for 48-h bacteriostatic and maximal effects were similar or modestly increased over the values at 24 h.

Our previous work studying the T_{MIC} relationship to changes in *Enterobacteriaceae* population profiles with the carbapenem razupenem produced results similar to those presented here (12). For doripenem and *P. aeruginosa* or *A. baumannii*, the changes in the bacterial population profiles occur at T_{MIC} values associated with the bacteriostatic effect at 24 h, and such changes are greater at 48 h than 24 h. Changes in population profiles with *A. baumannii* were more marked than those with *P. aeruginosa*, especially after 48 h of drug exposure. Dose fractionation of doripenem at a T_{MIC} exposure of 12.5 or 25%, values previously shown to pro-

TABLE 5 Population profiles of *P. aeruginosa* strain 38475 and *A. baumannii* strain 33980 before and after exposure to doripenem^a

Time (h)	Dose regimen (mg)	Viable count on recovery medium with indicated multiple of doripenem MIC					
		<i>P. aeruginosa</i>			<i>A. baumannii</i>		
		0×	4×	8×	0×	4×	8×
0	500	6.2 ± 0.1	<2	<2	6.1 ± 0.1	<2	<2
	1,000	6.2 ± 0.2	<2	<2	6.5 ± 0.1	<2	<2
	2,000	6.3 ± 0.1	<2	<2	6.1 ± 0.1	<2	<2
	3,000				6.1 ± 0.1	<2	<2
48	500	4.6 ± 1.7	<2	<2	4.7 ± 0.3	4.7 ± 0.4	<2
	1,000	3.6 ± 0.5	<2	<2	4.9 ± 0.4	4.6 ± 0.3	<2
	2,000	4.4 ± 1.4	<2	<2	2.9 ± 1.0	2.7 ± 0.7	<2
	3,000				4.0 ± 0.3	3.7 ± 0.2	<2
96	500	6.6 ± 1.4	2.9 ± 1.0	<2	3.9 ± 0.8	3.8 ± 0.8	<2
	1,000	5.3 ± 0.3	<2	<2	5.0 ± 0.9	4.7 ± 0.6	<2
	2,000	4.4 ± 1.6	<2	<2	3.5 ± 1.5	3.5 ± 1.5	<2
	3,000				4.2 ± 0.2	3.9 ± 0.2	<2

^a Dose simulations of 500 mg, 1,000 mg, 2,000 mg, or 3,000 mg every 8 h over 96 h were used.

FIG 5 Antibacterial effect of doripenem 500 mg (■), 1,000 mg (□), 2,000 mg (○), and 3,000 mg (●) every 8 h against *A. baumannii* strain 33980.

duce significant changes in population profiles, clearly showed that dosing every 8 h produced greater changes in population profiles than dosing every 24 h or 48 h for *P. aeruginosa*. Such experiments have not been previously performed in preclinical pharmacodynamic models, but it is known from human studies that low-dose, prolonged-course β -lactam therapy increases carriage of resistant *Streptococcus pneumoniae* isolates (6). Similar to these human data, our *in vitro* data imply that repeated antibiotic exposure near the 24-h bacteriostatic pharmacodynamic index target—that is, for a doripenem T_{MIC} value of 12.5 or 25%—produces the most significant changes in population profiles. In contrast, once the T_{MIC} exposure reaches that required to produce significant killing changes in *P. aeruginosa*, population profiles were suppressed. Similar observations were made some time ago with fluoroquinolones and *P. aeruginosa* (10).

Crandon et al. (4) have used a neutropenic murine thigh model to simulate human doses of doripenem of 1,000 or 2,000 mg, showing that they were equally effective over a 24-h period for *P. aeruginosa* strains with MICs of ≤ 8 mg/liter. Our data with *P. aeruginosa* showed that simulation of 500-, 1,000-, and 2,000-mg doses of doripenem every 8 h for 96 h produced rapid clearing from the model, followed by regrowth with all dosing regimens, and by 96 h, mutants were able to grow on $4\times$ MIC recovery medium with the low-dose regimen, that is, 500 mg every 8 h. Exposure of *A. baumannii* to doripenem dose simulations of 500, 1,000, 2,000, and 3,000 mg every 8 h also showed initial bactericidal activity. However, none of the regimens were able to suppress growth over 96 h, and significant regrowth occurred, with mutants able to grow on $4\times$ MIC recovery medium emerging by 48 h with all dosing regimens. Comparing these strains of *P. aeruginosa* and *A. baumannii*, it seemed to be more difficult to prevent the emergence of resistant mutants with *A. baumannii*; the reason for this is unclear. We did not study the mechanism of resistance underlying the phenotypic population profile changes that we describe; however, others have shown with *P. aeruginosa* that doripenem-resistant single-step mutants have a loss of OprD and that with multistep mutants resistance is due to other additional mechanisms, perhaps upregulated efflux pumps (14).

In conclusion, these data show that doripenem has similar T_{MIC} exposure targets for bacteriostatic and cidal effects with *P. aeruginosa* and *A. baumannii*. Population profiles for *A. baumannii* seem to be more subject to change after exposure to doripenem at T_{MIC} s over the range 12 to 25% and after 48 h than those for *P. aeruginosa*. Dose fractionation of doripenem indicated that multidosing, which failed to produce suppression of total bacterial

counts, was most likely to produce a change in population profiles. Escalating doses of doripenem to 2,000 to 3,000 mg every 8 h and similarly dosing over 96 h failed to produce clearance of either *P. aeruginosa* or *A. baumannii*; emergence of resistance occurred more readily with *A. baumannii*. These data show how challenging treatment of *P. aeruginosa* and *A. baumannii* is, even with highly potent antibacterials such as doripenem, when periods of exposure of >48 h are simulated. Such data argue for the use of doripenem in combination with other agents to improve bactericidal activity over periods of >24 h as well as prevent emergence of resistance. Combinations of doripenem, polymyxin, and rifampin have recently been shown to be promising in this regard (19). In addition, this work further underlines the association between 24-h pharmacodynamic targets of a static effect to a -1 -log-unit drop and potential changes in target population profiles. These observations have great potential significance in terms of future clinical breakpoint setting and drug development.

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