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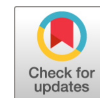

Substantial Impact of Altered Pharmacokinetics in Critically Ill Patients on the Antibacterial Effects of Meropenem Evaluated via the Dynamic Hollow-Fiber Infection Model

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ABSTRACT Critically ill patients frequently have substantially altered pharmacokinetics compared to non-critically ill patients. We investigated the impact of pharmacokinetic alterations on bacterial killing and resistance for commonly used meropenem dosing regimens. A *Pseudomonas aeruginosa* isolate ($MIC_{meropenem}$ 0.25 mg/liter) was studied in the hollow-fiber infection model (inoculum $\sim 10^{7.5}$ CFU/ml; 10 days). Pharmacokinetic profiles representing critically ill patients with augmented renal clearance (ARC), normal, or impaired renal function (creatinine clearances of 285, 120, or ~10 ml/min, respectively) were generated for three meropenem regimens (2, 1, and 0.5 g administered as 8-hourly 30-min infusions), plus 1 g given 12 hourly with impaired renal function. The time course of total and less-susceptible populations and MICs were determined. Mechanism-based modeling (MBM) was performed using S-ADAPT. All dosing regimens across all renal functions produced similar initial bacterial killing ($\leq \sim 2.5 \log_{10}$). For all regimens subjected to ARC, regrowth occurred after 7 h. For normal and impaired renal function, bacterial killing continued until 23 to 47 h; regrowth then occurred with 0.5- and 1-g regimens with normal renal function ($fT_{>5\times MIC}$ = 56 and 69%, fC_{min}/MIC < 2); the emergence of less-susceptible populations (≥32-fold increases in MIC) accompanied all regrowth. Bacterial counts remained suppressed across 10 days with normal (2-g 8-hourly regimen) and impaired (all regimens) renal function ($fT_{>5\times MIC} \geq 82\%$, $fC_{min}/MIC \geq 2$). The MBM successfully described bacterial killing and regrowth for all renal functions and regimens simultaneously. Optimized dosing regimens, including extended infusions and/or combinations, supported by MBM and Monte Carlo simulations, should be evaluated in the context of ARC to maximize bacterial killing and suppress resistance emergence.

KEYWORDS augmented renal clearance, pharmacodynamic modeling, critically ill, pharmacokinetics

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Effective antibiotic therapy is a crucial determinant of survival for patients with serious infections in an intensive care unit (ICU) (1, 2). Serious infections caused by *Pseudomonas aeruginosa* are increasing in prevalence in ICUs and present a substantial problem (3–5). Mortality rates exceed 50% for specific patient groups such as those with septic shock (6), with early administration of effective antibiotic therapy substantially improving survival (1, 7). Meropenem is a common treatment for *P. aeruginosa* infections (5, 8). It is well recognized that for β-lactam antibiotics such as meropenem the fraction of a dosing interval where unbound concentrations exceed 1× MIC of the infecting pathogen ($fT_{>MIC}$) should be optimized to achieve near-maximal bacterial killing (9, 10). Furthermore, it has become evident that higher exposures, e.g., unbound concentrations remaining above 4× or 5× MIC throughout the dosing interval or a minimum unbound concentration (fC_{min}) of ≥4× MIC is required for the suppression of the emergence of resistance (11–14). The emergence of antibiotic resistance in patients occurs in ~10% of antibiotic courses (15), with suboptimal dosing an important contributor (16).

Attainment of pharmacokinetic/pharmacodynamic (PK/PD) targets is challenging in critically ill patients who can have significantly altered antibiotic pharmacokinetics compared to non-critically ill patients (17, 18). In particular, patients with augmented renal clearance (ARC) have been demonstrated to exhibit substantially increased meropenem clearances and thereby decreased meropenem exposures (17, 19–21). This results in a significant risk of subtherapeutic antibiotic concentrations. However, the impact of a wide range of renal functions, including ARC, on bacterial killing and resistance emergence has not been quantified for meropenem. At present, clinicians still rely predominantly on the product information when selecting dosing regimens instead of adjusting the doses for patients with ARC (22, 23).

The hollow-fiber infection model (HFIM) can simulate the time course of antibiotic concentrations with a specific elimination half-life at high inocula over long durations, both of which are typically not possible with *in vivo* models. Results from the HFIM are also well correlated with clinical endpoints for bacterial killing and time course of emergence of resistance (24). This study used the HFIM to characterize the effect of different meropenem exposures, administered as standard short-term infusions, likely to occur in critically ill patients on the bacterial killing and emergence of resistance for *P. aeruginosa*. Specifically, we generated unbound concentration-time profiles consistent with those observed in patients with augmented renal clearance (ARC), and those with normal and impaired renal function following intravenous (i.v.) administration of four commonly used dosing regimens. We additionally developed a mechanism-based mathematical model (MBM) to quantitatively characterize the relationships between meropenem concentrations, bacterial killing, and regrowth over time for a wide range of studied renal functions and dosing regimens.

(Parts of this study were presented at the American Society for Microbiology Microbe and Interscience Conference on Antimicrobial Agents and Chemotherapy Congress, Boston, MA, 16 to 20 June 2016, and the Australasian Society of Clinical and Experimental Pharmacologists and Toxicologists and Molecular Pharmacology of GPCRs meeting, Melbourne, Australia, 27 to 30 November 2016.)

RESULTS

Typical examples of observed, targeted, and fitted concentration-time profiles are shown in Fig. S1 in the supplemental material. Measured meropenem concentrations adequately matched the target profiles produced in the *in silico* design simulations (Fig. 1; see also Fig. S1). The fitted meropenem concentration-time profiles were used in the MBM. Changes in total and less-susceptible bacterial populations are shown in Fig. 2 and 3. Changes in mutation frequencies and MICs are presented in Tables 1 and 2. The $\log_{10}$ mutation frequency ($\log_{10}$MF) before treatment (0 h) was −5.56 to <−6.88 on agar containing 5× and 10× the meropenem MIC (Table 1). Very few colonies grew on antibiotic-containing agar prior to treatment (Fig. 3).

At all levels of renal function (CL_{CR}) the three growth controls were virtually

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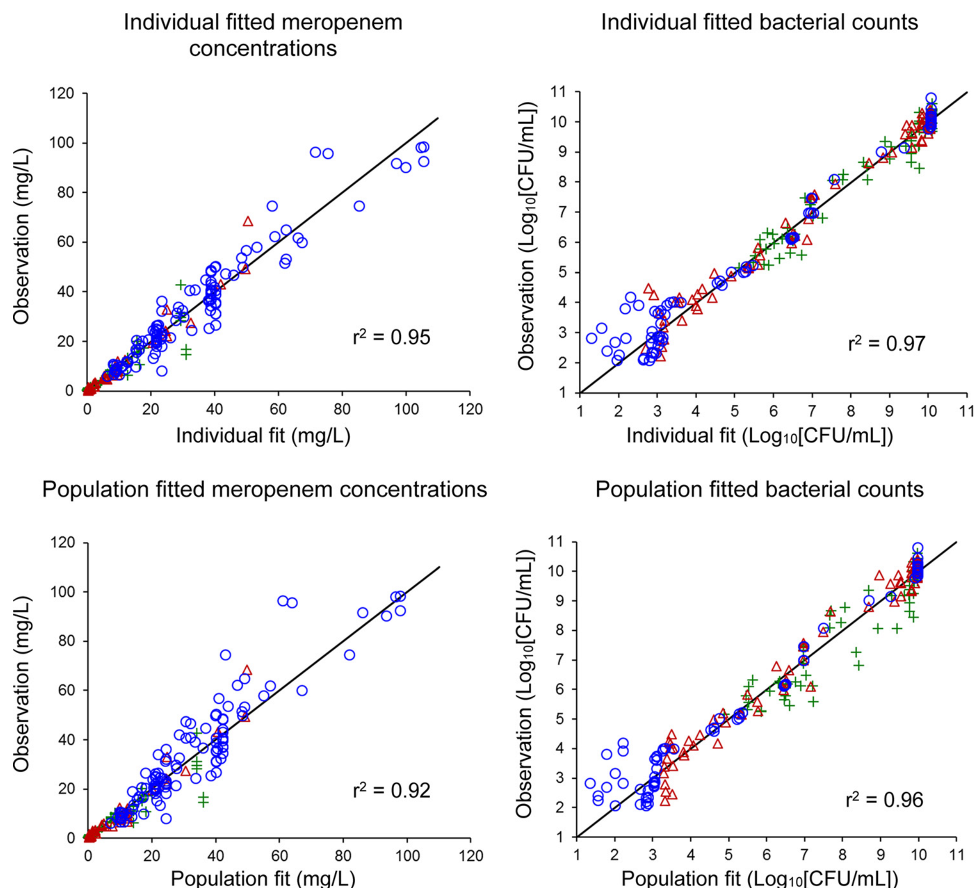

FIG 1 Observed versus individual fitted (top) and population fitted (bottom) viable counts for four meropenem dosing regimens and three different renal functions (circles, CL_{CR} ~10 ml/min; triangles, CL_{CR} 120 ml/min, plus signs, CL_{CR} 285 ml/min). Observations below the limit of counting are not shown on the graphs.

superimposable, with less-susceptible populations (i.e., those growing in the presence of meropenem at 5× and 10× MIC) plateauing at about 3 to 4 log_{10} CFU/ml by about 48 to 72 h (Fig. 3). The log_{10}MFs and MICs were also relatively stable, with MICs generally within one (occasionally two) 2-fold dilutions of the baseline when colonies were detected. At each CL_{CR}, bacterial killing over the first 7 h was similar (about 1.5 to 2.5 log_{10} killing; Fig. 2) with all meropenem-containing regimens. However, for all regimens subject to ARC, little or no further bacterial killing occurred with regrowth to within ~1.5-log_{10} of control values by 48 h (0.5- and 1-g regimens) and 96 h (2-g regimen). In each case less-susceptible populations emerged rapidly growing to within <~2 log of the total population, with MICs at each concentration (5× and 10× MIC) increasing to 16 mg/liter (0.5-g regimen) and 32 mg/liter (1- and 2-g regimens) by 191 h. For normal and impaired renal function, all regimens demonstrated essentially identical bacterial killing up to 23 h (<1-log difference between all regimens). At normal renal function, rapid regrowth with near-complete replacement of susceptible by less-susceptible bacteria (Fig. 3) and a concomitant increase in mutation frequency and MIC (Tables 1 and 2) occurred with the 0.5-g regimen after 23 h. With normal renal function, similar regrowth and resistance emergence occurred with the 1-g regimen, although regrowth was delayed until ~48 h, whereas killing continued and/or regrowth remained suppressed across 10 days with the 2-g regimen. With impaired renal function all regimens (including 1 g 12-hourly) suppressed regrowth across 10 days. In each case where regrowth was suppressed, no viable bacteria were detected on at least one occasion from 143 h onward; viable bacteria (maximum of 1.54 log_{10} CFU/ml; no less-susceptible bacteria) were detected in only three of the five regimens at 239 h.

The developed MBM was able to successfully describe simultaneously the mero-

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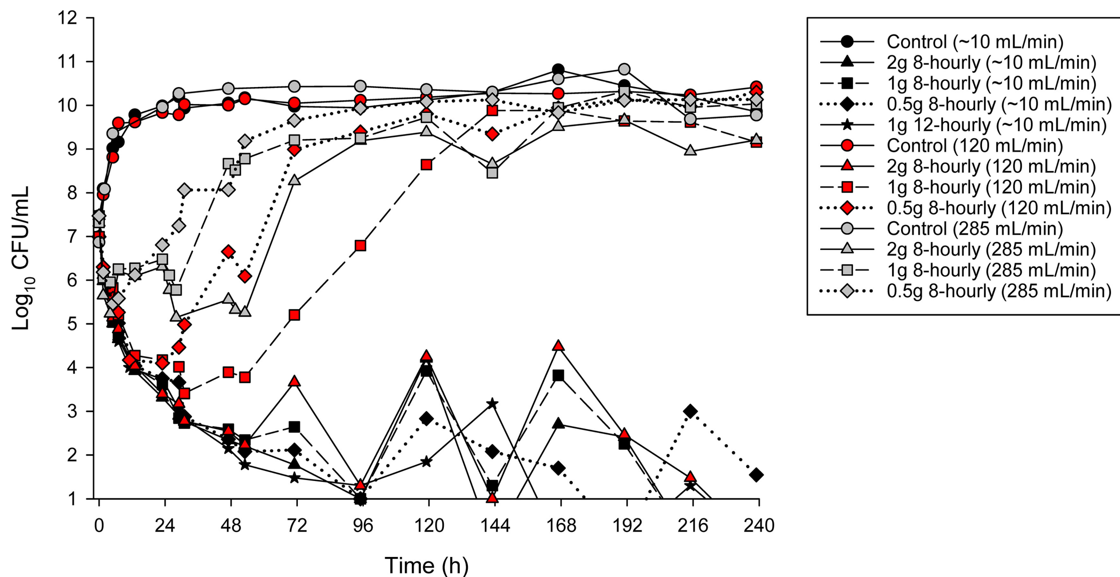

FIG 2 Time-kill curves with three different levels of renal function (CL_{CR} of 285, 120 and ~10 ml/min, corresponding to meropenem clearances of 34.0, 16.3 and 4.1 L/h, respectively) using three clinically relevant dosing regimens of meropenem (2g, 1g and 0.5g IV given 8-hourly; administered as a 0.5 h infusion). For patients with renal impairment (CL_{CR} of ~10 ml/min), a 1g IV regimen given 12-hourly was also simulated. For growth controls, flow rates for the respective renal functions were used. Samples with no colonies present on agar plates were plotted at the limit of counting (1.0 $\log_{10}$ CFU/ml).

penem concentrations and viable bacterial counts for all renal functions and dosing regimens that were evaluated in the HFIM (Fig. 1 and 4; see also Fig. S1 in the supplemental material). Three subpopulations were required to fit the observed bacterial counts, with the intermediate and resistant subpopulations growing more slowly than the susceptible subpopulation (k_{12}^{-1}; Table 3). A model incorporating inhibition of successful replication of all three bacterial subpopulations by meropenem best described the antibacterial effect. No additional functions or model complexities were necessary. The meropenem concentration required for half-maximal inhibition of successful bacterial replication of the intermediate ($IC_{50\ Rep,I}$) and resistant ($IC50_{Rep,R}$) populations was ~5- and 10-fold higher than for the susceptible population ($IC_{50\ Rep,S}$; Table 3). In this model, the resistant bacterial population (CFU_R) was responsible for driving the bacterial regrowth, which was observed with all dosing regimens subjected to ARC and with the 0.5- and 1-g 8-hourly dosing regimens at normal renal functions.

DISCUSSION

This study demonstrated the impact of altered meropenem pharmacokinetics as observed in critically ill patients on the likely antibacterial effects. Although the level of bacterial killing with all regimens at all renal functions was initially similar despite an ~36-fold range in average steady-state unbound concentrations, subsequent differences with repeated dosing were substantial. In the presence of ARC, all meropenem dosing regimens quickly failed with a rapid and substantial emergence of less-susceptible populations ~1,000-fold greater than that observed with control regimens, with accompanying increases in MICs and mutation frequencies. Such rapid regrowth of less-susceptible bacteria after minimal initial killing even with the 2-g dose is very concerning, given that ARC (defined as a $CL_{CR} > 130$ ml/min) can occur in up to 65% of critically ill patients, many with a CL_{CR} of 200 to 300 ml/min (25). The failure of these traditional dosing regimens is particularly worrying given the low meropenem MIC of the studied clinical isolate.

At normal renal function, rapid regrowth and extensive emergence of less-susceptible populations was observed with the 0.5- and 1-g 8-hourly regimens, although this occurred about 24 to 48 h later than with ARC and followed a greater initial killing of about 3 to 4 $\log_{10}$ CFU/ml. Drusano et al. (26) demonstrated for *P.*

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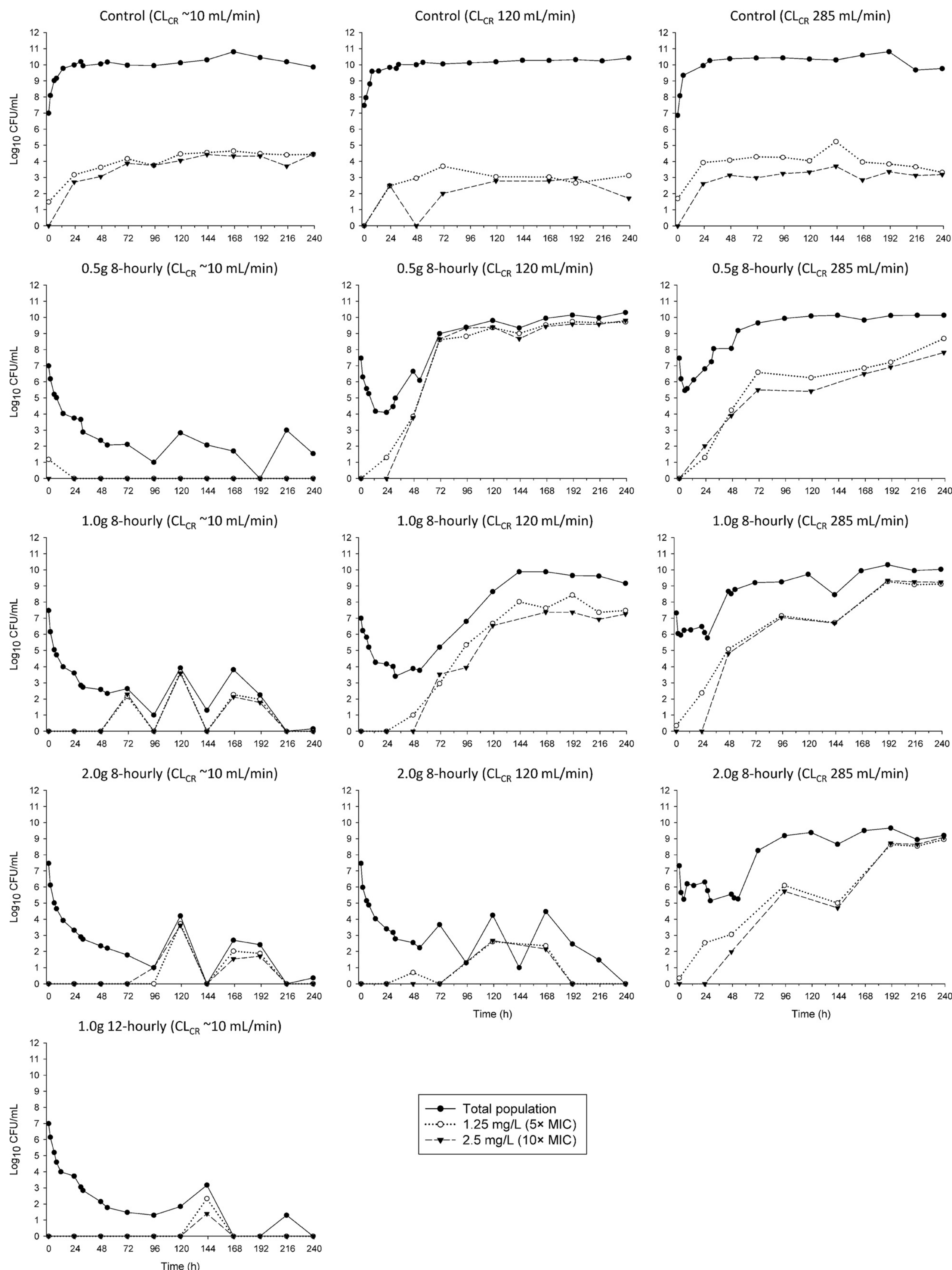

FIG 3 Effect of each level of renal function (CL_{CR} of 285, 120 and ~10 ml/min) with each dosing regimen on the total bacterial population and less-susceptible populations (i.e., able to grow in the presence of 5× or 10× the meropenem MIC). For growth controls, flow rates for the respective renal functions were used.

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TABLE 1 Log_{10} mutation frequencies at 1.25 and 2.5 mg/liter (5× and 10× the baseline MIC) at various time points for each dosing regimen and CL_{CR} simulated in the HFIM[a]

Dose (mg/liter)	Time (h)	CL_{CR}, 285 ml/min ($t_{1/2}$, 0.6 h)				CL_{CR}, 120 ml/min ($t_{1/2}$, 1.1 h)				CL_{CR}, ~10 ml/min ($t_{1/2}$, 4 h)				
		Control	2 g, 8 h	1 g, 8 h	0.5 g, 8 h	Control	2 g, 8 h	1 g, 8 h	0.5 g, 8 h	Control	2 g, 8 h	1 g, 8 h	0.5 g, 8 h	1 g, 12 h
1.25 (5× MIC)	0	−5.89	<−6.66[b]	<−6.66	<−6.66	<−6.88	<−6.88	<−6.88	<−6.88	−5.56	−5.56	<−6.76	−5.77	−5.77
	23	−6.31	<−5.34	<−6.33	−5.51	−7.36	<−2.7	<−3.47	−2.80	−6.82	<−2.62	<−2.91	<−3.05	<−3.03
	47	−6.39	−3.54	−2.55	−3.84	−7.05	<−1.85	−2.89	−2.81	−6.43	<−1.64	<−1.89	<−1.66	<−1.45
	71	−6.37	−3.20	−2.24	−3.06	−6.35	<−2.96	−2.25	−0.38	−5.81	<−1.08	−0.48	<−1.41	<−0.78
	95	−6.38						−1.45	−0.56	−6.20	<−0.30	<−0.30	<−0.30	<−0.60
	119	−6.74	−3.52	−2.86	−3.83	−7.14	−1.64	−1.97	−0.44	−5.67	−0.49	−0.25	<−2.13	<−1.15
	143	−5.61						−2.48	−0.34	−5.76	NC	< −0.6	<−1.38	−0.84
	167	−6.94	−1.06	−2.96	−2.99	−7.24	−2.12	−2.26	−0.41	−6.16	−0.68	−1.55	<−1.00	NC
	191	−6.98	−0.08	−2.80	−2.90	−7.66	<−1.76	−1.21	−0.41	−5.97	−0.54	−0.28	NC	NC
	215	−6.02						−2.25	−0.31	−5.78	NC	NC	<−2.13	<−0.60
	239	−6.45	−1.70	−1.82	−1.44	−7.30	NC[c]	−1.68	−0.59	−5.43	<−0.30	<−0.30	<0.70	NC
2.5 (10× MIC)	0	<−6.59	< −6.66	<−6.66	<−6.66	<−6.88	<−6.88	<−6.88	<−6.88	<−6.34	<−6.34	<−6.76	<−6.25	<−6.25
	23	−7.64	< −5.34	<−6.33	−4.78	−7.33	<−2.7	<−3.47	<−3.4	−7.27	<−2.62	<−2.91	<−3.05	<−3.03
	47	−7.32	−5.17	−3.76	−4.17	<−9.3	<−1.85	<−3.19	−2.89	−7.00	<−1.64	<−1.89	<−1.66	<−1.45
	71	−7.68	−3.63	−2.97	−4.15	−8.05	<−2.96	−1.68	−0.34	−6.10	<−1.08	−0.34	<−1.41	<−0.78
	95	−7.39						−2.85	−0.05	−6.20	0.00	<−0.30	<−0.30	<−0.60
	119	−7.44	−4.51	−3.35	−4.67	−7.40	−1.57	−2.11	−0.41	−6.08	−0.58	−0.32	<−2.13	<−1.15
	143	−7.14						−2.48	−0.67	−5.88	NC	<−0.6	<−1.38	−1.77
	167	−8.06	−3.09	−2.99	−3.34	−7.49	−2.33	−2.52	−0.505	−6.48	−1.15	−1.69	<−1.00	NC
	191	−7.46	−2.94	−2.89	−3.20	−7.36	<−1.76	−2.28	−0.566	−6.12	−0.72	−0.48	NC	NC
	215	−6.55						−2.69	−0.405	−6.48	NC	NC	<−2.13	<−0.60
	239	−6.59	−2.24	−2.63	−2.31	−8.72	NC	−1.90	−0.495	−5.41	<0.34	<0.54	<−0.85	NC

[a]When no colonies were present on antibiotic-containing plates, mutation frequencies reported represent an upper limit based on the total viable count. Dosing regimens in the column subheadings are expressed as "amount, dosing interval."
[b]The log_{10} baseline mutation frequency determined at a higher bacterial density in a separate experiment was −8.05 at 5× MIC.
[c]NC, no colonies were detected on both drug-free and drug-containing agar plates.

aeruginosa and *Staphylococcus aureus* in a murine thigh infection model that, while granulocytes contribute to the elimination of bacteria up to a certain level, this effect is saturable. In the HFIM simulating normal renal function (but without an immune system) the 1-g 8-hourly regimen did not reduce bacteria to below ~3.5 log_{10} CFU/ml,

TABLE 2 MIC values from colonies obtained from drug-containing (5× and 10× the baseline MIC, equivalent to meropenem at 1.25 and 2.5 mg/liter) agar plates at various time points for each dosing regimen and CL_{CR} simulated in the HFIM

Meropenem regimen (amt, dosing interval)	Time (h)	MIC[a]					
		CL_{CR}, 285 ml/min		CL_{CR}, 120 ml/min		CL_{CR}, ~10 ml/min	
		5× MIC plates	10× MIC plates	5× MIC plates	10× MIC plates	5× MIC plates	10× MIC plates
Control	0	2/8	NC[c]	2/8	2/4	2/4	NC
	47	2/8	2/4	8/8	4/4	4/8	4/8
	191	2/4	2/4	2/4	4/4	4/8	4/8
2 g, 8 h	0	NC	NC	NC	NC	NC	NC
	47	8/8	8/16	NC	NC	NC	NC
	191	16/32	16/32	NC	NC	8/16	64/64
1 g, 8 h	0	NC	NC	NC	NC	NC	NC
	47	16/32	16/32	2/4	NC	NC	NC
	191	16/32	16/32	4/8	8/16	16/16	32/32
0.5 g, 8 h	0	NC	NC	NC	NC	2/4	NC
	47	8/8	8/8	2/4	32/32	NC	NC
	191	8/16	8/16	8/8	32/32	NC	NC
1 g, 12 h[b]	0					NC	NC
	47					NC	NC
	191					NC	NC

[a]Susceptibility and resistance to meropenem are defined as MIC ≤2 mg/liter and MIC >8 mg/liter, respectively (57). MICs were determined according to the CLSI agar dilution method (56), and readings taken at both 24 and 48 h are reported (24 h/48 h).
[b]Only performed with a CL_{CR} of ~10 ml/min.
[c]NC, no colonies detected.

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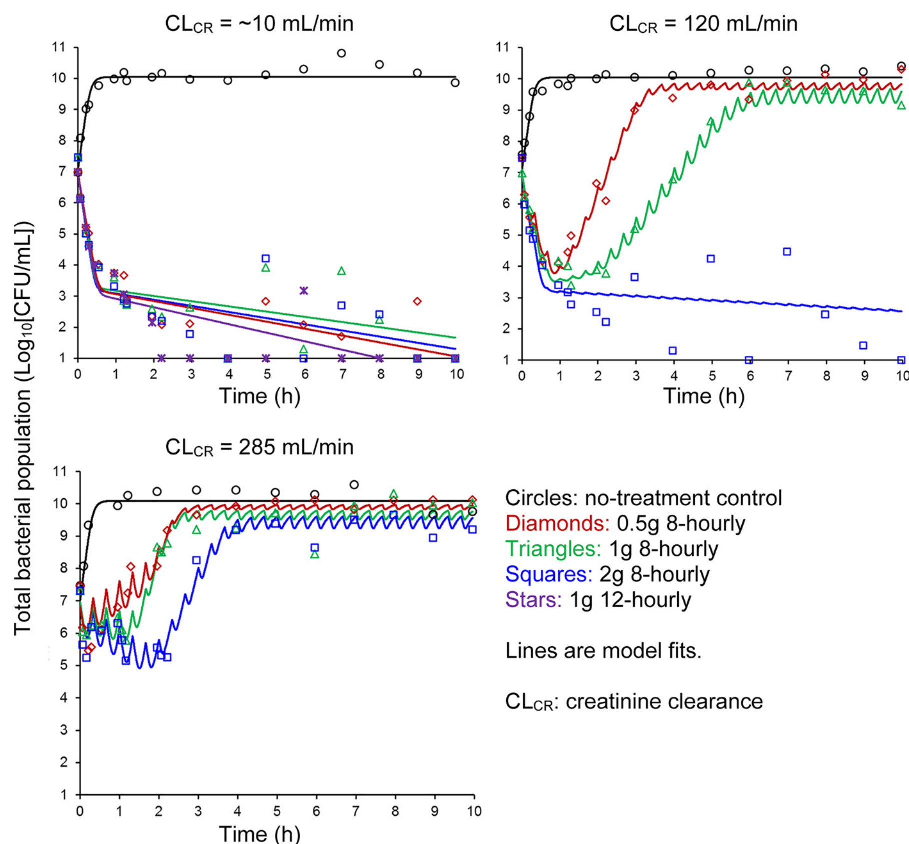

FIG 4 Model fits to the observed bacterial count profiles over time, grouped by simulated CL_{CR}. Samples with no colonies present on agar plates were plotted at the limit of counting (1.0 $\log_{10}$ CFU/ml).

with replacement of susceptible by less-susceptible bacteria occurring. Therefore, treatment failure with the 1-g 8-hourly regimen in patients with normal renal function is a distinct (and concerning) possibility even considering the potential added effect of the immune system. Replacement of susceptible by less-susceptible bacteria also has potential long-term implications for both individual patients and infection control. In contrast, the 2-g dosing regimen at normal renal function and all regimens (including the 1-g 12-hourly regimen) evaluated in impaired renal function resulted in complete or near-complete bacterial killing. We have previously reported similar results for piperacillin-tazobactam against *P. aeruginosa* (14), although the emergence of resistant subpopulations in the presence of ARC was greater for meropenem.

Results from studies examining β-lactams, including meropenem, undertaken in the HFIM correlate well with those from neutropenic mouse models and predicted emergence of resistance in patients (27–30). However, of the few studies examining meropenem against a variety of pathogens in the HFIM, none have included a half-life representative of ARC (31–33). In addition, very few have examined the emergence of resistance to meropenem, which is known to occur primarily via downregulation of the gene for the outer membrane porin OprD (which allows entry of carbapenems into the cell) and overexpression of efflux pumps MexAB-OprM, MexXY-OprM, and MexCD-OprJ (34–40). Louie et al. (13) used a *P. aeruginosa* wild-type strain (MIC 0.5 mg/liter) and its MexAB pump-overexpressed mutant (MIC = 2 mg/liter) to evaluate meropenem (1-, 2-, and 3 g, 8-hourly regimen via a 1-h [wild-type] or 4-h [pump-overexpressed mutant] infusion; $t_{1/2}$ = 1 h) over 14 days. All regimens for the wild-type had a $fT_{>MIC}$ of 100%. For both isolates, monotherapy with 1- and 2-g regimens produced approximately 2- and 4-$\log_{10}$ killing over the first 48 h, respectively, followed by rapid regrowth due to amplification of less-susceptible subpopulations (able to grow at 5× MIC). In contrast, the 3-g regimen suppressed regrowth (wild-type) or eradicated the bacteria (mutant).

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TABLE 3 Population parameter estimates for meropenem against *P. aeruginosa* 1280

Parameter	Term	U	Estimate (SE)[d]
Growth parameters			
Log_{10} initial inoculum	Log_{CFU0}	Log_{10} CFU/ml	6.97 (1.5)
Mean generation time			
Susceptible population	$k_{12,S}^{-1}$	min	49.3 (9.5)
Intermediate population	$k_{12,I}^{-1}$	min	683 (19)
Resistant population	$k_{12,R}^{-1}$	min	78.5 (10.4)
Replication rate constant	k_{21}	h^{-1}	50 (fixed)[a]
Log_{10} (maximum population size)	Log_{CFUmax}	Log_{10} CFU/ml	9.98 (0.9)
Log_{10} (mutation frequencies)			
Intermediate population	$LogMF_I$		−3.66 (5.2)
Resistant population	$LogMF_R$		−6.28 (6.9)
Inhibition of probability of successful replication by meropenem			
Maximum inhibition			
Susceptible population	$I_{max\ Rep,S}$		1.0 (fixed)[b]
Intermediate population	$I_{max\ Rep,I}$		0.673 (56.3)
Resistant population	$I_{max\ Rep,R}$		0.956 (25.0)
Concn causing 50% of inhibition			
Susceptible population	$IC_{50\ Rep,S}$	mg/liter	0.648 (39.8)
Intermediate population	$IC_{50\ Rep,I}$	mg/liter	2.96 (45.3)
Resistant population	$IC_{50\ Rep,R}$	mg/liter	6.09 (23.3)
Hill coefficient			
Susceptible population	Hill,S		1.0 (fixed)[c]
Intermediate population	Hill,I		7.14 (23.9)
Resistant population	Hill,R		2.19 (62.3)
SD of additive residual error on log_{10} scale	SD_{CFU}	Log_{10} CFU/ml	0.493

[a]Bacterial replication was assumed to be fast, as previously described (14, 54, 67).
[b]The $I_{max\ Rep,S}$ was estimated at 0.999 and therefore was eventually fixed to 1.0.
[c]Hill,S was estimated at 1.03 and therefore was eventually fixed to 1.0.
[d]Standard errors (in parentheses) are expressed as percentages.

Tam et al. (11) investigated varying the meropenem half-life (to alter C_{min}) while keeping the C_{max} at 64 mg/liter using a *P. aeruginosa* reference strain and its stably depressed AmpC β-lactamase-producing isogenic mutant (MIC = 1 mg/liter for both). Bolus doses were administered 8 hourly for 5 days with half-lives ranging from about 1 to 3 h (i.e., not representative of patients with ARC); $fT_{>MIC}$ was 75% (one regimen) and 100% (three regimens). For both strains at the shortest half-lives (approximately 1 and 1.6 h), regrowth and amplification of less-susceptible subpopulations (growing at 3× MIC) followed moderate initial killing. At the longer half-lives (about 2.4 and 3 h), regrowth was suppressed without amplification of less-susceptible subpopulations. These results broadly match those of the present study for normal renal function and renal impairment, although in the present study with a more susceptible isolate, the 2-g 8-hourly regimen with normal renal function suppressed regrowth. We have also shown the rapid failure and emergence of resistance of commonly used regimens even at the highest approved daily dose in the presence of ARC.

The $fT_{>MIC}$ required for optimal bactericidal activity of carbapenems, derived from *in vitro* studies and animal *in vivo* studies, is generally reported to be 40% (10, 41). However, it is also important to consider the emergence of resistance. In our study, the $fT_{>MIC}$ of all failed regimens exceeded 40% (Table 4). However, as also observed in the studies described above (11, 13), treatment failure with rapid resistance emergence nonetheless occurred even in some situations where $fT_{>MIC}$ was at or close to 100%. Such *in vitro* findings plus data from recent retrospective clinical studies supporting a longer $fT_{>MIC}$ in critically ill patients (42–45) challenge the traditional PK/PD targets. With maximal bactericidal activity of the β-lactams occurring at 4× to 5× MIC (46, 47) and regrowth often observed as soon as concentrations fall below the MIC (48–50), other targets, e.g., 100% $fT_{>4-5\times MIC}$ (14, 51, 52) and fC_{min}/MIC, have been investigated.

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TABLE 4 Meropenem dosing regimens and pharmacokinetic parameters for three different simulated levels of renal function used in the HFIM against *P. aeruginosa* 1280[a]

Meropenem regimen (amt, dosing interval)[b]	CL_{CR}[c] (ml/min)	$CL_{meropenem}$ (liters/h)	$t_{1/2}$[d] (h)	fC_{max} (mg/liter)	fC_{min} (mg/liter)	fC_{min}/MIC	$fC_{ss,avg}$ (mg/liter)	$\%fT_{>MIC}$	$\%fT_{>5\times MIC}$	$fAUC_{24}$ (mg·h/liter)	Outcome[e]
2 g, 8 h	285	34.0	0.6	51.3	0.01	0.04	7.1	62	45	170.7	RR
1 g, 8 h	285	34.0	0.6	25.7	0.00	0.00	3.6	54	38	85.3	RR
0.5 g, 8 h	285	34.0	0.6	12.8	0.00	0.00	1.8	47	30	42.7	RR
2 g, 8 h	120	16.3	1.1	70.1	0.50	2.0	15.5	100	82	372.4	SR
1 g, 8 h	120	16.3	1.1	35.0	0.25	1.0	7.8	100	69	186.2	RR
0.5 g, 8 h	120	16.3	1.1	17.5	0.12	0.48	3.9	87	56	93.1	RR
2 g, 8	~10	4.1	4.0	114.5	31.5	126.0	64.9	100	100	1556.9	SR
1 g, 8 h	~10	4.1	4.0	57.2	15.7	62.8	32.4	100	100	778.5	SR
0.5 g, 8 h	~10	4.1	4.0	28.6	7.9	31.6	16.2	100	100	389.2	SR
1 g, 12 h	~10	4.1	4.0	49.1	6.8	27.2	21.6	100	100	519.3	SR

[a]For *P. aeruginosa* 1280, the meropenem MIC is 0.25 mg/liter. All values presented are concentrations simulated at steady state. Abbreviations: $t_{1/2}$, half-life; fC_{max}, unbound maximal concentration at steady state; fC_{min}, unbound minimal concentration; $fC_{ss,avg}$, unbound average concentration at steady state; $\%fT_{>MIC}$, the percentage of time that unbound concentrations exceeded the MIC; AUC_{24}, the area under the unbound piperacillin concentration-versus-time curves over 24 h.

[b]The infusion time for all regimens was 0.5 h.

[c]A creatinine clearance (CL_{CR}) of 285 ml/min represents patients with augmented renal clearance, a CL_{CR} of 120 ml/min represents patients with normal renal clearance, and a CL_{CR} of ~10 ml/min represents patients with renal impairment.

[d]Samples for viable counting were collected at 0, 1.5, 5, 7, 13, 23, 29, 31, 47, 53, 71, 95, 119, 143, 167, 191, 215, and 239 h. Samples for pharmacokinetic determinations were collected at 0.66, 1, 2, 3.5, 5, 7, 8.66, 13, 23, 24.66, 47, 48.66, 49, 50, 51.5, 53, 55, 71, 72.66, 95, 119, 143, and 167 h for the CL_{CR} of 285 ml/min. For the CL_{CR} at 120 and ~10 ml/min, samples were collected as for the 285-ml/min CL_{CR}, as well as at 29 and 31 h. For growth controls, the flow rates for the respective renal functions were maintained.

[e]RR, regrowth, with resistance; SR, suppression of regrowth.

Both Tam and Louie et al. examined the fC_{min}/MIC value in relation to the suppression of the emergence of resistance (11–13). Regimens where the fC_{min}/MIC was ≤~2 failed with rapid regrowth and emergence of resistance, whereas a value of >3 (or, for Tam et al., of >6, the next lowest fC_{min}/MIC examined) prevented regrowth and suppressed resistance. Tam et al. (12) subsequently utilized mathematical modeling to determine a value of ≥4 was required. A retrospective clinical study utilizing population pharmacokinetic modeling to estimate meropenem pharmacokinetic parameters for lower respiratory tract infections found that an $fC_{min}/MIC > 5$ was the most significant predictor of successful clinical and microbiological response, although *P. aeruginosa* was only one of a range of pathogens included and resistance emergence was not examined (43). In our study, all regimens with a $fC_{min}/MIC \leq 1$ failed, with resistance emerging, whereas those with a $fC_{min}/MIC \geq 2$ suppressed regrowth. Also, both regimens that failed despite a $fT_{>MIC}$ at or close to 100% had an $fT_{>5\times MIC}$ well below 100% (0.5- and 1-g regimens with normal renal function, i.e., $fT_{>5\times MIC}$ values of 56 and 69%, respectively; Table 4). All regimens achieving 100% $fT_{>5\times MIC}$ were successful, as was the 2-g regimen at normal renal function where concentrations exceeded 5× MIC for 82% of the dosing interval. Such findings are consistent with our earlier study utilizing piperacillin-tazobactam against *P. aeruginosa*, where all regimens achieving 100% $fT_{>5\times MIC}$ suppressed regrowth and the emergence of resistance. Clearly, such targets cannot be achieved in the presence of ARC with current commonly used short-term infusion regimens, even at the highest approved daily dose.

The antibacterial effect of meropenem was best described by a model including the inhibition of successful replication, as described for studies with other β-lactams where bacterial killing was found to be less rapid than growth (14, 53). The meropenem profiles from all regimens with ARC and the 1- and 0.5-g 8-hourly regimens at normal renal function did not suppress regrowth of the resistant population in the model. This is in agreement with a high proportion of bacteria being able to grow on meropenem-containing agar (Fig. 3, Table 1). Although some bacterial counts at 119 and 167 h were slightly underpredicted by the model, this was attributed to experimental variability at relatively low bacterial counts. Since the model well described the bacterial counts and pharmacokinetic profiles over a large range of renal functions and meropenem concentrations, it may be useful for predicting the antibacterial effects of meropenem for other than the studied renal functions and ultimately to develop optimized dosing regimens (54, 55). Although the simulation of plasma instead of tissue concentrations

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in the HFIM is a potential limitation, our study best reflects the clinical scenario of bloodstream infections. An effect of the immune system, which was absent in the HFIM, may in the future be incorporated into our MBM using data derived from animal studies (26).

In conclusion, this study is the first to examine the effects of altered pharmacokinetics, including ARC, in critically ill patients on bacterial killing and emergence of resistance by meropenem using a HFIM. When subjected to widely varying renal functions and thus meropenem clearances, substantial differences in bacterial killing and the emergence of less-susceptible populations occurred between commonly used dosing regimens against a susceptible *P. aeruginosa* isolate with a low meropenem MIC. Although all regimens in the presence of renal impairment achieved substantial bacterial killing (i.e., with no viable bacteria detected on ≥1 occasion) and the suppression of regrowth, minimal killing with rapid regrowth and substantial increases in less-susceptible populations occurred with all regimens in the presence of ARC. Worryingly, regrowth and large increases in less-susceptible populations similarly occurred with 0.5- and 1-g regimens with normal renal function, with only the 2-g regimen resulting in substantial and prolonged bacterial killing and resistance suppression. Individualized dosing regimens accounting for altered pharmacokinetics and aiming for higher than standard antibiotic exposures are necessary to maximize bacterial killing (100% $fT_{>5\times MIC}$) and suppress resistance emergence (fC_{min}/MIC ≥ 2). Such exposures will be difficult to achieve using standard short-term infusions in the presence of ARC. Optimized dosing regimens, including extended infusions and/or combinations, supported by MBM and Monte Carlo simulations, should be evaluated in the context of ARC.

MATERIALS AND METHODS

Antibiotic, media, bacterial isolate, and susceptibility testing. Meropenem (lot MAUS1025; Fresenius Kabi, New South Wales, Australia) stock solutions were prepared as described previously (14). Viable counting was performed on cation-adjusted Mueller-Hinton agar (CAMHA; 25 mg/liter Ca^{2+} and 12.5 mg/liter Mg^{2+}; University of Melbourne, Melbourne, Australia). Drug-containing agar plates were prepared by adding meropenem stock solution to CAMHA (lot 5030699; BD, Sparks, MD). HFIM studies were performed using cation-adjusted Mueller-Hinton broth (CAMHB; lot 3322206; BD) containing 20 to 25 mg/liter Ca^{2+} and 10 to 12.5 mg/liter Mg^{2+}. Clinical isolate *P. aeruginosa* 1280 (meropenem MIC, 0.25 mg/liter; Royal Brisbane and Women's Hospital, Australia) was obtained from a critically ill patient with a soft tissue infection. MICs prior to and following drug exposure were determined using the Clinical and Laboratory Standards Institute agar dilution method (56). Susceptibility and resistance were defined as MICs of ≤2 and >8 mg/liter, respectively (57).

HFIM. A hollow-fiber *in vitro* infection model (HFIM; 36°C) was used to evaluate changes in bacterial burden and the suppression of resistance with four commonly used meropenem dosing regimens. Cellulosic cartridges (batch B720170715b; FiberCell Systems, Inc., Frederick, MD) were used. An initial inoculum of $\sim10^{7.5}$ CFU/ml was prepared as described previously (14). Experiments were conducted over 10 days with periodic sampling (1.0 to 1.5 ml) for viable counting (Table 4). Samples were twice centrifuged (4,000 × *g*, 5 min), resuspended in saline to reduce antibiotic carryover, and manually plated on CAMHA containing no drug (100 μl plated; limit of counting, 1.0 $\log_{10}$ CFU/ml) and meropenem at 5× and 10× MIC (see "Mutation frequency and emergence of resistance studies" below).

Experimental design and simulated meropenem regimens. The free (non-protein-bound) plasma concentration-time profiles based on a population pharmacokinetic model for meropenem in critically ill patients (protein binding 2%) (20) and a fraction excreted unchanged in urine of 79% of a meropenem dose in subjects with normal renal function (58) were first simulated using Berkeley Madonna (v8.3.18). Profiles were simulated for patients with different drug clearances due to altered renal function, namely, patients with ARC (creatinine clearance [CL_{CR}], ~285 ml/min; meropenem clearance, 34.0 liters/h; half-life [$t_{1/2}$], 0.6 h), normal renal clearance (CL_{CR}, 120 ml/min; meropenem clearance, 16.3 liters/h; $t_{1/2}$, 1.1 h), and renal impairment (CL_{CR}, ~10 ml/min; meropenem clearance, 4.1 liters/h; $t_{1/2}$, 4 h) (Table 4). For each level of renal function, the *in silico* simulations evaluated dosing regimens of 2, 1, and 0.5 g of meropenem administered via a 30-min i.v. infusion given every 8 h (Table 4) (59). A further 1-g regimen, administered via a 30-min i.v. infusion every 12 h, as used in patients with renal impairment, was also evaluated via simulation. The final simulated meropenem concentration-time profiles were then generated in the HFIM. No loading dose was administered. Untreated growth controls were included.

Mutation frequency and emergence of resistance studies. Mutation frequencies were determined at baseline (0 h) and at 23, 47, 71, 95, 119, 143, 167, 191, 215, and 239 h to quantify less-susceptible subpopulations. A 200-μl sample of appropriately diluted log-phase growth suspension was manually plated onto CAMHA containing no antibiotic and meropenem at 5× and 10× MIC (i.e., 1.25 and 2.5 mg/liter meropenem). Given the potential for a low number of less-susceptible bacteria in samples from untreated controls and some treatment regimens, 200 μl was used to increase sensitivity (limit of

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counting, 0.7 $\log_{10}$ CFU/ml). For determination of total and less-susceptible counts at baseline, log-phase growth suspension was used, and enumeration was performed manually after 24 h of incubation at 36°C. The $\log_{10}$ (mutation frequency) ($\log_{10}$MF) was determined as follows: $\log_{10}$MF = $\log_{10}$(CFU/ml on antibiotic-containing agar) − $\log_{10}$(CFU/ml on antibiotic-free agar).

Although some viable counts were too low to quantify colonies on antibiotic-containing agar, these samples provided information on the upper limit of the $\log_{10}$MF. To confirm changes in MIC from baseline, MICs were determined at 47 and 191 h using a subset of at least three colonies from antibiotic-free and antibiotic-containing plates.

Measurement of meropenem concentrations. Duplicate (1-ml) samples were collected periodically (Table 4) in cryovials and stored at −80°C. Concentrations were measured at ambient temperature using a validated high-performance liquid chromatography-tandem mass spectrometry (HPLC-MS/MS) method. The HPLC-MS/MS system comprised two Shimadzu Nexera2 LC-30AD liquid chromatographs, a SIL-30ACMP autosampler, and an 8030+ triple-quadrupole detector (Shimadzu, Tokyo, Japan). To a 2.5-μl sample, 10 μl of 0.5 μg/ml meropenem-d6 (internal standard) and 30 μl of acetonitrile were added before centrifugation (5 min, 13,000 × *g*). A 30-μl volume of supernatant was removed to an autosampler vial, and 0.5 μl was injected onto a SeQuant ZIC-HILIC 5.0-μm column (2.1 by 50 mm; Merck, Darmstadt, Germany). The mobile phase consisted of 80% acetonitrile, 20% water, and 0.1% formic acid delivered isocratically at 0.3 ml/min. Meropenem was monitored by positive-mode electrospray at multiple reaction monitoring of 383.50→68.15, 383.50→114.15, and 383.50→141.10. Meropenem-d6 was monitored in positive mode at 390.10→147.10 and 390.10→73.90. The run time was 3.2 min, and the assay range was 0.2 to 100 mg/liter; samples were diluted when the expected concentrations exceeded the upper limit of quantification. Unknown samples were assayed in batches alongside calibration and quality control samples, and the results were subjected to batch acceptance criteria. The assay method was validated for linearity, lower limit of quantification, precision, and accuracy according to both U.S. Food and Drug Administration and European Medicines Evaluation Agency criteria (60, 61). The precision was within 4.9% and the accuracy was 8.1% at the concentrations tested (0.60, 4.0, and 80 mg/liter).

Pharmacodynamic modeling. Modeling was conducted using importance sampling (pmethod = 4) in parallelized S-ADAPT with SADAPT-TRAN (62–64). All pharmacokinetic and pharmacodynamic observations were fitted simultaneously. Models were evaluated based on the S-ADAPT objective function (−1×log-likelihood), standard diagnostic plots, visual predictive checks, and plausibility of the parameter estimates (14, 54, 65). Models with one, two, and three bacterial subpopulations and which described the effect of meropenem via direct bacterial killing, the inhibition of successful bacterial replication, or both were evaluated (54, 66, 67).

The final model incorporated three subpopulations that were susceptible (CFU_S), intermediate (CFU_I) or resistant (CFU_R) to meropenem. The size of the total inoculum ($\log_{CFU0}$) and the $\log_{10}$ mutation frequencies for the intermediate ($\log MF_I$) and resistant ($\log MF_R$) subpopulations were estimated parameters. Thus, the estimated subpopulations did not directly reflect bacterial counts on meropenem-containing agar plates at 5× and 10×MIC. Each subpopulation contained bacteria in two states: those preparing for replication (CFU_1) and those immediately before replication (CFU_2) (53, 67). The total concentration of viable bacteria (CFU_{ALL}) was calculated as follows:

$$CFU_{ALL} = CFU_{S1} + CFU_{S2} + CFU_{I1} + CFU_{I2} + CFU_{R1} + CFU_{R2}$$

The initial condition of the CFU_S population was calculated as the difference between the total inoculum (CFU_0, i.e., the value of CFU_{ALL} at time = 0 h) and the initial conditions of the CFU_I and CFU_R populations. All bacteria were initialized in state 1, and the initial conditions for CFU_{S2}, CFU_{I2}, and CFU_{R2} were 0.

The concentration of meropenem-susceptible bacteria in state 1 (CFU_{S1}) was determined as follows:

$$\frac{dCFU_{S1}}{dt} = REP \cdot \left(1 - lnh_{Rep,s}\right) \cdot k_{21} \cdot CFU_{S2} - k_{12,s} \cdot CFU_{S1}$$

where k_{21} is the replication rate constant, $k_{12,S}$ is the growth rate constant, and REP is the replication factor:

$$REP = 2 \cdot \left(1 - \frac{CFU_{ALL}}{CFU_{max} + CFU_{ALL}}\right)$$

At a low CFU_{ALL}, REP approached 2, representing a 100% probability of successful replication. As CFU_{ALL} approached the maximum population size (CFU_{max}), REP approached 1, i.e., a 50% probability of successful replication with the total viable count remaining constant.

The effect of meropenem is best described as the inhibition of successful replication ($Inh_{Rep,S}$):

$$lnh_{Rep,S} = \frac{I_{max\ Rep,S} \cdot C^{Hill,S}}{IC_{50Rep,S}^{Hill,S} + C^{Hill,S}}$$

where C is the meropenem concentration in the growth medium, $I_{max\ Rep,S}$ is the maximum inhibition of successful replication, $IC_{50\ Rep,S}$ is the concentration causing 50% of the maximum inhibition effect, and Hill,S is the Hill coefficient. An $Inh_{Rep,S}$ value of 0.50 resulted in net stasis, whereas an $Inh_{Rep,S}$ value of >0.50 led to bacterial killing due to the elimination of bacteria that replicate unsuccessfully.

The concentration of meropenem-susceptible bacteria in state 2 (CFU_{S2}) was determined as follows:

$$\frac{dCFU_{S2}}{dt} = -k_{21} \cdot CFU_{S2} + k_{12,S} \cdot CFU_{S1}$$

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The bacterial concentrations of the intermediate and the resistant subpopulations were described in a similar manner. The biological variability between the viable count curves was assumed to be log-normally distributed, except for parameters constrained between 0 and 1 that were logistically transformed. Between-curve variability was set to 15% coefficient of variation during the end of the estimation (65). An additive residual error model was used to fit the viable counts. For observations <100 CFU/ml the number of colonies/plate was directly fitted via a previously developed residual error model (53).

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02642-16.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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