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Pharmacodynamic Evaluation of the Potential Clinical Utility of Fosfomycin and Meropenem in Combination Therapy against KPC-2-Producing *Klebsiella pneumoniae*

James Albiero,[a] Sherwin K. B. Sy,[b] Josmar Mazucheli,[b] Silvana Martins Caparroz-Assef,[c] Bruno Buranello Costa,[a] Janio Leal Borges Alves,[a] Ana Cristina Gales,[d] Maria Cristina Bronharo Tognim[a]

Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá, Maringá, PR, Brazil[a]; Programa de Pós-Graduação em Bioestatística, Departamento de Estatística, Universidade Estadual de Maringá, Maringá, PR, Brazil[b]; Departmento de Farmacologia e Terapêutica, Universidade Estadual de Maringá, Maringá, PR, Brazil[c]; Laboratório Alerta, Divisão de Doenças Infecciosas, Departamento de Medicina Interna, Escola Paulista de Medicina, Universidade Federal de São Paulo, São Paulo, SP, Brazil[d]

KPC-producing *Klebsiella pneumoniae* causes serious infections associated with high death rates worldwide. Combination therapy consisting of fosfomycin and a carbapenem is better than monotherapy to combat multidrug-resistant microorganisms, but no dosages for the combination have been defined. The MICs of meropenem and fosfomycin were evaluated against 18 clinical isolates of KPC-2-producing *K. pneumoniae*. The activities of combination antimicrobials were also determined by the checkerboard method. The MIC_{50} and MIC_{90} of each agent alone and in combination were challenged against short (1.5-h) or prolonged (3-h) infusion regimens of meropenem (1 g every 8 h [q8h], 1.5 g q6h, 2 g q8h) and fosfomycin (4 g q8h, 6 g q6h, 8 g q8h) by Monte Carlo simulation to evaluate the time above the MIC of the free drug concentration as a percentage of the dosing interval (*f*T>MIC). The monotherapy MIC_{50}s and MIC_{90}s were 32 and 256 mg/liter for meropenem and 64 and 512 mg/liter for fosfomycin, respectively. Antimicrobial combination increased bacterial susceptibility to 1/4 the MIC_{50}s and to 1/8 to 1/16 the MIC_{90}s of monotherapy. The antimicrobial combination demonstrated a synergistic effect for at least two-thirds of the isolates. In combination therapy, fosfomycin regimens of 6 g q6h and 8 g q8h as a 3-h infusion against the MIC_{50} and MIC_{90} had better chances of achieving ≥90% probability of target attainment (PTA) of 70% *f*T>MIC. Meropenem regimens of 1.5 g q6h and 2 g q8h in prolonged infusion can achieve close to 90% PTA of 40% *f*T>MIC for MIC_{50} but not MIC_{90}. The significant reduction in the MIC values and the achievement of appropriate PTA demonstrated that regimens containing fosfomycin with meropenem can be effective against KPC-2-producing *K. pneumoniae*.

In addition to other classes of antimicrobial agents, *Klebsiella pneumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* isolates are resistant to carbapenems, one of the main classes of antibiotics used against β-lactamase-producing microorganisms (1). The bacterium's high dissemination rate is a major public health problem worldwide, and the limited options of antimicrobial agents further complicate the management of infections caused by this difficult-to-treat pathogen, resulting in high morbidity and mortality rates (2, 3).

With the exception of the recently approved ceftazidime-avibactam combination, studies have shown that multidrug-resistant bacteria expressing KPC and AmpC-type β-lactamases develop secondary resistance toward β-lactamase inhibitors, including clavulanic acid, tazobactam, and sulbactam (4–6). These β-lactamase inhibitors, which were designed to combat antibiotic drug resistance, have lost their utility, exacerbating the threat to the current antimicrobial treatment option. The rediscovered "old" antibiotics, including fosfomycin and polymyxins, may offer potential treatment options against multidrug-resistant bacteria. Several clinical studies have shown that combination therapy has better success rates than monotherapy in combating multidrug-resistant infection, and a two-drug combination that included tigecycline, colistin, or meropenem was associated with lower mortality (7). However, there is no consensus on what is the best combined regimen.

Fosfomycin has broad-spectrum bactericidal activity whose mechanism of action is to prevent cell wall synthesis. It binds to the UDP-*N*-acetylglucosamine enolpyruvyl transferase, preventing the transpeptidation of peptidoglycan (8). Like other β-lactams, meropenem binds to penicillin-binding proteins (PBP); it exhibits high affinity for PBP 2, 3, and 4 and intermediate affinity for PBP 1a and 1b, which are also involved in cell wall synthesis (9). By impeding cell wall formation at different stages of peptidoglycan synthesis, meropenem and fosfomycin should theoretically result in synergy.

Just like many other countries combating multidrug-resistant bacteria, the health centers in Brazil are facing serious infection problems caused by KPC-producing *K. pneumoniae* and the Brazilian health authorities do not have defined treatment regimens for these microorganisms. Given the prohibitively high cost of developing new classes of antibiotics, the optimization of dosing regimens of existing antimicrobial agents provides a viable option to counter the immediate threat of drug-resistant microorganisms

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Address correspondence to James Albiero, jamesalbiero1@gmail.com, or Maria Cristina Bronharo Tognim, mcbtognim@uem.br.

(10). In this study, we explored the checkerboard *in vitro* susceptibility approach and utilized a pharmacodynamic surrogate index combined with Monte Carlo simulation to evaluate treatment regimens of the meropenem-fosfomycin combination against several KPC-2-producing *K. pneumoniae* isolates from several Brazilian health centers.

MATERIALS AND METHODS

Microbiological organisms. Eighteen clinical isolates of KPC-2-producing *K. pneumoniae* of different clones were obtained from different health centers located in seven Brazilian states from the northeast, midwest, south, and southeast regions of Brazil, representing a large part of the national territory. These isolates were selected from samples previously analyzed by Nicoletti et al. (11), which confirmed the presence of the bla_{KPC-2} plasmid gene by PCR (forward primer, 5′-TCGCTAAACTCGAACAGG-3′, and reverse primer, 5′-TTACTGCCCGTTGACGCCCAATC C-3′), and the resulting amplicons were sequenced in both strands (Applied Biosystems 3130 genetic analyzer). The KPC-2 amino acid sequence in each isolate was determined by a BLAST comparison of contiguous sequences against a database of known KPC-2 proteins. The genetic relationships among KPC-2-producing isolates were analyzed by multilocus sequence typing (MLST) and pulsed-field gel electrophoresis (PFGE), using SpeI as the restriction enzyme. The specific allele sequence and sequence types were verified at the *K. pneumoniae* MLST website (http://bigsdb.web.pasteur.fr/klebsiella/klebsiella.html). The *Escherichia coli* ATCC 25922 reference strain was included as a quality control. The bacterial isolates were subcultured for 3 days on Trypticase soy agar plates containing 5% sheep blood, prior to use in the experiment.

Antimicrobial agents. Meropenem (AstraZeneca, Cotia, São Paulo, Brazil) was generously donated by the State University of Maringa Hospital, and fosfomycin (Sigma-Aldrich, St. Louis, MO, USA) was purchased from LabCompany (Londrina, Paraná, Brazil). Fosfomycin was dissolved in water at 10 mg/ml and stored at 20°C (stock solution), and meropenem solutions were prepared at the same concentration on the day of experimentation.

MICs of single agents. The MICs of meropenem and fosfomycin against each isolate, after two subcultures and preparation at an 0.5 McFarland standard using a nephelometer (PhoenixSpec nephelometer; BD, Sparks, MD, USA), were determined as described in the CLSI M07-A10 approved standard (12), and MICs were interpreted according to the CLSI M100-S25 guidelines (13). Considering that there were no CLSI interpretive criteria for fosfomycin susceptibility for *K. pneumoniae*, the CLSI methodology for *E. coli* strains isolated from urinary tract infections was used to evaluate susceptibility criteria for *K. pneumoniae*. A control group was evaluated in agar containing 25 μg/ml glucose-6-phosphate without fosfomycin. The concentration ranges of meropenem and fosfomycin tested were 0.015 to 2,048 and 0.5 to 2,048 mg/liter, respectively.

Evaluation of the antimicrobial activity of the combinations of antibiotics. Meropenem and fosfomycin alone and in combination were evaluated by the checkerboard method in 96-well microtiter plates (Inlab, São Paulo, Brazil) (14). The inoculum of each bacterial isolate was prepared in cation-adjusted Mueller-Hinton broth at a 0.5 McFarland standard and added to the wells at a final concentration of 5×10^5 CFU/ml; the test was conducted in triplicate. After incubation at 37°C for 16 to 20 h, the modal MICs for each antibiotic and for the antibiotic combinations for each individual isolate were determined. The Loewe additivity (15) was evaluated for the combination using the following equation:

$$1 = \frac{\mathrm{MIC}_{\text{fosfomycin in combination}}}{\mathrm{MIC}_{\text{fosfomycin monotherapy}}} + \frac{\mathrm{MIC}_{\text{meropenem in combination}}}{\mathrm{MIC}_{\text{meropenem monotherapy}}} + \alpha$$

where α is the Loewe additivity index, which is used to classify the effects of the combination therapy. If α is 0, the activity of the combination is considered additive; if α is >0, the combined activity is synergistic; and if α is <0, the two drugs are antagonistic. The overall effect of the combination on the population of KPC-expressing bacterial isolates is considered synergistic if 80% of the estimated α value is >0.

A second measure for classifying the activity of the combination was the fractional inhibitory concentration index (FICI), which was calculated using the following equation: $\mathrm{FICI} = \mathrm{FIC}_A + \mathrm{FIC}_B$, where FIC_A is the MIC of drug A in combination divided by the MIC of drug A alone and FIC_B is the MIC of drug B in combination divided by the MIC of drug B alone. The classification of the effects of combination therapy was based on the following categories: synergism, FICI ≤ 0.5; indifferent, 0.5 < FICI < 4; antagonism, FICI ≥ 4. The MIC_{50} and MIC_{90} (of monotherapy and of combinations of two antimicrobial agents) were the MICs for the median (MIC required to inhibit 50% of the isolates) and the 90th percentile of the 18 isolates.

Simulation of meropenem and fosfomycin pharmacokinetics in critically ill patients. The population pharmacokinetic (PK) parameters of meropenem and fosfomycin in a critically ill patient population were used to simulate concentration-time profiles for estimating an individual's pharmacodynamic (PD) surrogate index. For both meropenem and fosfomycin, the PD surrogate index was best described by the time above the MIC of the free drug concentration as a percentage of the dosing interval (*f*T>MIC).

The demographics of 20,000 virtual patients were first simulated in a 50/50 ratio of males and females. Height was assumed to be normally distributed, with the height of males being $176.3 \pm 0.17\sqrt{4{,}482}$ cm (mean ± SD) and the height of females being $162.2 \pm 0.16\sqrt{4{,}857}$ cm (16). The weight-height relationship was based on the following equations: $\mathrm{WT}_{\mathrm{male}} = \exp(3.28 + 1.92 \log \mathrm{HT}_{\mathrm{male}})$ and $\mathrm{WT}_{\mathrm{female}} = \exp(3.49 + 1.45 \log \mathrm{HT}_{\mathrm{female}})$, for males and females, respectively (17), where WT refers to weight and HT refers to height. An exponential interindividual variable was incorporated into the weight-height relationship equations such that $\mathrm{WT}_i = \mathrm{WT}_{\mathrm{exp}}(\eta_i)$, wherein η is normally distributed with a mean of 0 and standard deviations (SD) of 0.14 and 0.17, for males and females, respectively, and *i* represents an individual (18).

The age of the population was uniformly distributed between 50 and 90 years of age. Serum creatinine (S_{CR}) levels in critically ill patients with normal renal function were 0.7 ± 0.05 and 0.6 ± 0.05 mg/dl for males and females, respectively, whereas S_{CR} levels in patients with impaired renal function were 1.5 ± 0.15 and 1.2 ± 0.15 mg/dl for males and females, respectively. Creatinine clearance (CL_{CR}) was computed based on the modification of renal disease (MDRD) equation (19): $\mathrm{CL}_{CR} = 186 \times S_{CR}^{-1.154} \times \mathrm{age}^{-0.203}$ (× 0.742 if the patient is female).

The population pharmacokinetic model for meropenem was a one-compartment model parameterized on clearance (CL) and volume of central compartment (V_C). The meropenem pharmacokinetic model of Muro et al. (20) was chosen because it was shown to best predict free meropenem concentrations in critically ill patients (21). The following covariate relationship was associated with its clearance: CL (liters/h) = $11.1 \times (S_{CR}0.7)^{-1}$. The mean volume of distribution (V) was 33.6 liters. Interindividual variability for CL was assumed to be log-normally distributed with 52.1% coefficient of variation (CV). No interindividual variability was assigned to the volume of distribution. Protein binding of 2% was assumed to determine the free meropenem concentrations (21–23).

The population pharmacokinetic model for fosfomycin was a two-compartment model parameterized on CL, V_C, volume of peripheral compartment (V_P), and intercompartmental clearance (Q). The population pharmacokinetic model of fosfomycin in critically ill patients from the report of Parker et al. (24) was used to simulate 20,000 virtual patient profiles. Their model reported seven interoccasion CL parameters. For the purpose of simulation, the highest CL value was used. This approach veered on the conservative side to not overpredict the fosfomycin concentration in plasma. Both creatinine clearance and body weight were influential covariates. The equations for the population CL and V_C incorporated these two covariates: CL (liters/h) = $5.57 \times (\mathrm{CL}_{CR}/90)$, and V_C (liters) = $26.5 \times (\mathrm{WT}/70)^{0.75}$. V_P and Q were 22.3 liters and 19.8 liters/h, respectively. Interindividual variability was incorporated into CL and V_C, assuming log-normal distribution of both parameters with CVs of 91.9%

TABLE 1 MICs of meropenem and fosfomycin against 18 KPC-2-producing *K. pneumoniae* isolates in both monotherapy and combination therapy by checkerboard test[a]

K. pneumoniae isolate	ST	MIC of monotherapy antimicrobial (mg/liter)		MIC (mg/liter) of Mero-Fosfo combination	MIC fold reduction (Mero/Fosfo)	FICI	S or I based on FICI	Loewe additivity index	S or A based on Loewe index
		Mero	Fosfo						
Kp-3	70	4	16	4/1	0/16	1.006	I	−0.0625	A
Kp-4	70	8	64	2/8	4/8	0.375	S	0.625	S
Kp-5	437	1	512	0.02/16	50/32	0.046	S	0.95	S
Kp-8	437	16	32	8/4	2/8	0.625	I	0.375	S
Kp-9	133	16	128	1/16	16/8	0.185	S	0.812	S
Kp-10	437	64	128	2/32	32/4	0.280	S	0.719	S
Kp-11	11	64	128	4/16	16/8	0.185	S	0.812	S
Kp-12	437	128	128	8/16	16/8	0.185	S	0.812	S
Kp-15	11	16	32	8/2	2/16	0.560	I	0.437	S
Kp-19	437	64	64	4/8	16/8	0.131	S	0.812	S
Kp-28	617	1	16	1/16	0/0	2.000	I	−1	A
Kp-38	437	32	64	2/16	16/4	0.310	S	0.687	S
Kp-39	11	64	64	8/16	8/4	0.375	S	0.625	S
Kp-40	11	256	1,024	64/512	4/2	0.750	I	0.25	S
Kp-43	340	16	16	16/16	0/0	2.000	I	−1	A
Kp-44	11	512	256	32/32	16/8	0.185	S	0.812	S
Kp-46	17	32	128	4/32	8/4	0.375	S	0.625	S
Kp-55	11	32	32	4/2	8/16	0.131	S	0.812	S
MIC_{50}		32	64	4/16	8/4				
MIC_{90}		256	512	32/32	8/16				

[a] ST, sequence type; Mero, meropenem; Fosfo, fosfomycin; FICI, fractional inhibitory concentration index; S, synergy; I, indifferent; A, antagonism.

and 39%, respectively. Fosfomycin has negligible plasma protein binding (25, 26).

Pharmacodynamics. The pharmacodynamic (PD) surrogate indices for both meropenem and fosfomycin were previously characterized by time above MIC of the free drug concentration as a percentage of the dosing interval (*f*T>MIC). The PD surrogate indices for meropenem and fosfomycin were 40% and 70%, respectively (22, 23, 26, 27). Pharmacodynamic analyses of antimicrobial regimens in both monotherapy and combination therapy as 0.5-h and 3-h infusions at the MIC_{50} or MIC_{90} against this isolate population were conducted to evaluate *f*T>MIC for each dosage regimen. The following dosage regimens were evaluated: meropenem, 1 g every 8 h (q8h) and 2 g q8h; fosfomycin, 4 g q8h and 8 g q8h. The potential importance of infusion time was evaluated for all regimens, including the short infusion of 0.5 h and the prolonged infusion of 3 h for both fosfomycin and meropenem. These regimens were chosen based on the most common regimens used in the countries in which they were registered. The dosage regimens of 1.5 g meropenem q6h and 6 g fosfomycin q6h were included considering the time-dependent action of these antimicrobials, since daily regimens including more divided doses may provide better results. These two shorter dosing intervals with more frequent dosing are less practiced in the clinic; they still provide the recommended maximum daily dosage for meropenem, 6 g a day, and for fosfomycin, 24 g a day.

Monte Carlo simulation. Monte Carlo simulation was carried out to generate 20,000 virtual profiles with representative demographical distributions in R v.3.1.1. The plasma meropenem and fosfomycin profiles for the virtual patients were generated using NONMEM v.7.2 (ICON, Ellicott City, MD) with Advan 1 and Advan 3 subroutines, respectively. The times in both the ascending and descending phases of the time-concentration profiles in which the concentration is at the MIC were estimated by a linear interpolation algorithm in R. The duration above the MIC was determined as the difference between the two time points. The percentage of the duration above the MIC over the dosing interval was determined for each individual's profile. Probability of target attainment (PTA) for each regimen was evaluated to determine the percentage of the simulated profiles that achieved or exceeded the pharmacodynamic surrogate indices for meropenem and fosfomycin of ≥40% and ≥70% *f*T>MIC at increasing MICs, respectively. It was considered successful when 90% of the population reached the target values (28, 29). The results of the simulations were used to compute the cumulative fraction of response (CFR) for each dosing regimen at 40% and 70% *f*T>MIC of meropenem and fosfomycin, respectively. The summation of the density or percentage of bacteria at each MIC across the distribution multiplied by the PTA at the MIC is the CFR for the regimen.

Some investigators have suggested that using MIC metrics may not be sufficient in resistance suppression (30, 31). The mutant selection window (MSW) is a range of drug concentrations between the MIC for the susceptible bacteria and the mutant prevention concentration that fosters the emergence of resistant mutants (32). Firsov and colleagues have shown that time within the MSW is a suitable predictor of resistance development in *Staphylococcus aureus* following exposure to fluoroquinolones and that an fT_{MSW} of >20% of the dosing interval is a useful target (33). The upper mutant prevention concentrations of the MSW were previously assumed to be 4- to 6-fold the MIC (34). Tam and colleagues, on the other hand, showed that resistance suppression in a dense *Pseudomonas aeruginosa* population can be achieved by maintaining trough meropenem concentrations in excess of a minimum drug concentration (C_{min})/MIC ratio of 1.7 (35). A hypothetical C_{min}/MIC ratio of 2 was used to evaluate resistance suppression, and the percentage of the population that achieved or exceeded this ratio was determined for the dosage regimens of meropenem and fosfomycin described above.

RESULTS

***In vitro* susceptibility.** All 18 *K. pneumoniae* clinical isolates harbored the plasmid bla_{KPC-2} gene, which was confirmed by PCR

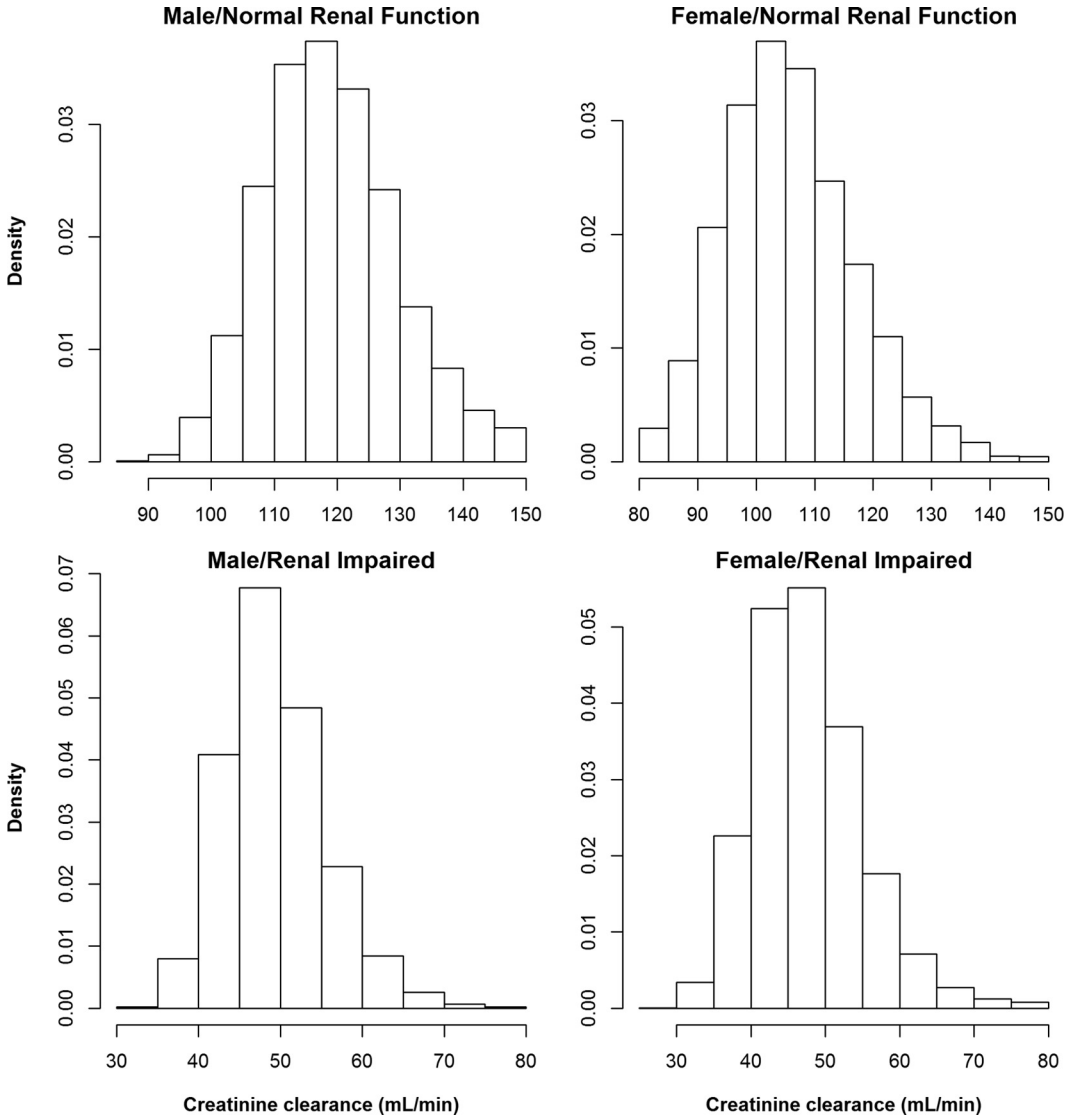

FIG 1 Histograms showing the distribution of creatinine clearance computed using the modification of renal disease (MDRD) equation in virtual critically ill male (left) and female (right) patients with normal renal function (top) and renal impairment (bottom).

and sequencing. Table 1 presents the antimicrobial susceptibility profile of 18 KPC-2-producing microorganisms to meropenem and fosfomycin as both monotherapy and combination therapy. The ranges of meropenem and fosfomycin MICs in monotherapy were 1 to 512 and 16 to 1,024 mg/liter; the MIC_{50}s for this collection of isolates were 32 and 64 mg/liter, respectively. MIC_{90}s were 8-fold higher. Only 2 (11%) and 10 (56%) isolates were susceptible to meropenem and fosfomycin, based on CLSI breakpoint values of ≤1 and ≤64 mg/liter, respectively.

For combination of the two antimicrobials, the majority of the MICs were markedly lower than the MICs of the antimicrobials in monotherapy and the resulting MIC values in the combination against these microorganisms were well within the susceptibility level of either one of the two antimicrobial agents. The MIC_{50}s were decreased to 1/4, and for the MIC_{90s}, there were reductions to 1/8 and 1/16 of the values in the monotherapy setting. In two-thirds of the isolates, the effect of the combination was considered synergistic by FICI scores of ≤0.5, and 83% of the isolates exhibited synergistic activities in the combination therapy based on Loewe additivity criteria. Only six isolates showed FICI values classified as indifferent ($0.5 < FICI \leq 4$), and none of the isolates had FICIs of ≥4. Of the resistant isolates for which the antimicrobial monotherapy MICs were greater than the breakpoints, when they were tested against meropenem and fosfomycin in combination, the meropenem MIC for one isolate (Kp-9) and the fosfomycin MICs for seven isolates (Kp-5, Kp-9, Kp-10, Kp-11, Kp-12, Kp-44, Kp-46) were lower than the breakpoints.

Pharmacokinetic-pharmacodynamic simulations. The population pharmacokinetic parameters of meropenem and fosfomycin used in the simulation were obtained from critically ill patients previously described in the literature (20, 24) and were used for simulations of pharmacodynamic surrogate indices of 20,000 virtual patients subdivided into 10,000 patients with normal renal function and 10,000 with mild to moderate renal impairment. Figure 1 shows the distribution of creatinine clearance based on the MDRD computation for male and female patients with nor-

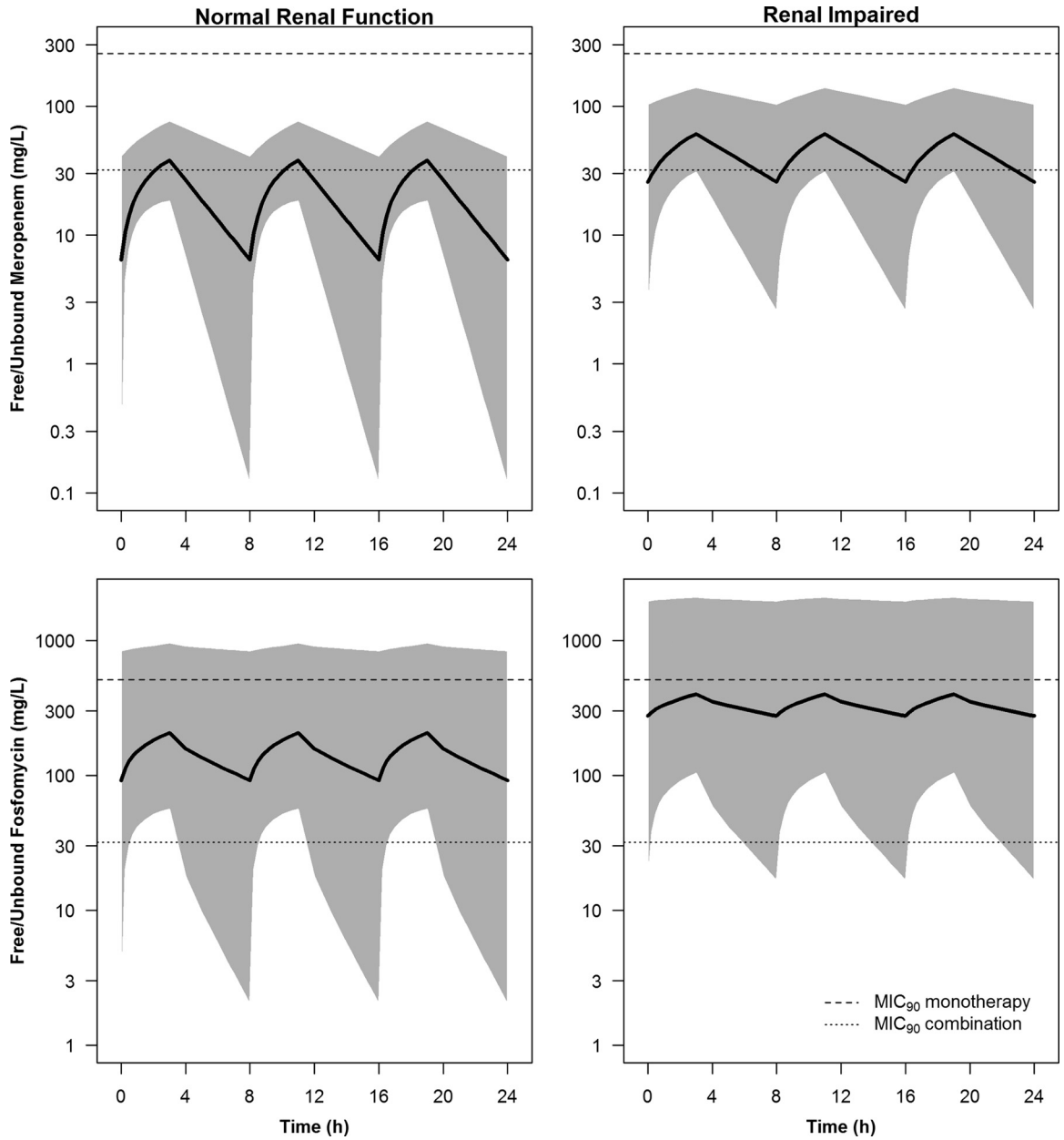

FIG 2 Simulated median and 95% prediction interval of steady-state free meropenem (top) and fosfomycin (bottom) concentrations in plasma in critically ill virtual patients with normal renal function (left) and renal impairment (right). The MIC_{90}s in monotherapy and combination therapy are shown by dashed and dotted lines.

mal renal function and mild to moderate renal impairment. For the purpose of simulation, the range of creatinine clearance for the normal renal function group is 80 to 150 ml/min and the range for the group with mild to moderate renal impairment is 30 to 80 ml/min. The upper bound for creatinine clearance is 150 ml/min, and values greater than 150 ml/min were set to 150 ml/min. The mean values and ranges for serum creatinine were selected so that the estimated creatinine clearance resulted in modal values of approximately 100 to 120 ml/min for the normal renal function group and 40 to 55 ml/min for the impaired renal function group, taking into account the weight and age distributions as well. The simulated ranges of creatinine clearance in both the normal renal function population and the impaired renal function population are well within the reported creatinine clearance range in patients treated for hospital-acquired and ventilator-associated bacterial infections (36). Creatinine clearances of <30 ml/min were not simulated, since patients with end stage renal disease (ESRD) require hemodialysis and estimations of drug clearance based on covariate relationships for ESRD patients are often inaccurate. In addition, pharmacokinetic simulations in hemodialysis patients are done differently, since hemodialysis removes drugs rapidly during the process (37).

Renal function has a significant impact on the pharmacokinetics of both fosfomycin and meropenem. Figure 2 shows the sim-

TABLE 2 PTAs at targeted pharmacodynamic surrogate indices ($fT>MIC$) for meropenem at 40% and fosfomycin at 70% for dosing regimens by infusion duration and renal function in monotherapy and combination therapy against KPC-2-producing *K. pneumoniae* isolates[a]

Patient renal function and antimicrobial regimen	PTA (%)							
	MIC_{50}				MIC_{90}			
	Monotherapy		Combination		Monotherapy		Combination	
	0.5 h	3 h	0.5 h	3 h	0.5 h	3 h	0.5 h	3 h
Normal renal function								
Meropenem								
1 g q8h	0.8	1	54	70	0	0	0.8	1
1.5 g q6h	10	14	**85**	97	0	0	10	14
2 g q8h	7	11	74	**88**	0	0	7	11
Fosfomycin								
4 g q8h	44	48	**80**	**86**	1.3	1.4	65	71
6 g q6h	68	74	91	96	7	8	**83**	**89**
8 g q8h	65	71	**89**	93	7	8	**80**	**86**
Renal impairment								
Meropenem								
1 g q8h	14	17	92	97	0	0	14	17
1.5 g q6h	53	62	99	99	0	0	53	62
2 g q8h	48	56	97	99	0	0	48	56
Fosfomycin								
4 g q8h	77	**80**	96	97	10	11	90	93
6 g q6h	91	93	99	100	29	30	97	98
8 g q8h	90	93	98	99	28	30	96	97

[a] PTA, probability of target attainment; $fT \geq MIC$, percentage of the dosing interval that free antimicrobial concentrations remain above the MIC of the bacteria. Gray shading indicates ≥90% PTA, and boldface indicates 80% to <90% PTA.

ulated median and 95% prediction interval of steady-state free meropenem and fosfomycin concentrations in patients with normal renal function and those with renal impairment after the highest doses of 2 g meropenem and 8 g fosfomycin q8h were administered as a 3-h infusion. The MIC_{90}s in monotherapy and combination therapy are also shown. The meropenem monotherapy MIC_{90} against KPC-producing *K. pneumoniae* was unattainable in all patients, while the MIC_{90} in the fosfomycin monotherapy situation was unattainable in the majority of the patients. The lower MIC_{90} for fosfomycin in the combination (32 mg/liter) was attainable in a majority of the patients, regardless of their renal function, whereas in the case of meropenem, the MIC_{90} was attainable in the majority of patients with renal impairment and in a lower percentage of the patients with normal renal function. Table 2 shows the probabilities of target attainment (PTA) of 40% and 70% $fT>MIC$ for meropenem and fosfomycin, respectively, in various dosing regimens as 0.5-h and 3-h infusions. Figure 3 shows the PTA of 70% $fT>MIC$ of several fosfomycin dosing regimens and the MIC frequency in fosfomycin monotherapy as well as in combination with meropenem. For the 0.5-h infusion, fosfomycin monotherapy regimens did not achieve 90% PTA of >70% $fT>MIC$ against the MIC_{50} or MIC_{90} in patients with normal renal function. In patients with renal impairment, the higher doses of 6 g q6h and 8g q8h achieved 90% PTA. All fosfomycin regimens in combination with meropenem achieved ≥90% PTA against the MIC_{90} in patients with renal impairment but not in patients with normal renal function. Even though the prolonged infusion of 3 h improves the PTA over that of a 0.5-h infusion, fosfomycin monotherapy regimens did not reach 90% PTA against either the MIC_{50} or the MIC_{90} in patients with normal renal function. In combination with meropenem, fosfomycin in a dosing regimen of 6 g q6h as a 3-h infusion followed by 8 g q8h in an infusion of the same duration had the best chance of achieving PTA.

None of the meropenem dosing regimens achieved ≥90% PTA against the MIC_{90}, regardless of renal function. Patients with renal impairment had a better chance of attaining 40% $fT>MIC$ with meropenem at the MIC_{50} of the combination than patients with normal renal function. A meropenem dosing regimen of 1.5 g q6h followed by 2 g q8h as a 3-h infusion was the preferred regimen for achieving PTA. Figure 4 displays the PTA of meropenem regimens to achieve target pharmacodynamic indices in both monotherapy and combination therapy and also the MIC frequency in meropenem monotherapy and in combination therapy with fosfomycin. Against a MIC_{50} of 32 mg/liter in meropenem monotherapy, none of the dosing regimens achieve 90% PTA of 40% $fT>MIC$, whereas combination therapy resulted in ≥90% PTA of at least 40% $fT>MIC$ for the regimens of 1.5 g q6h as a 3-h infusion. A meropenem dosing regimen of 2 g q8h as a 3-h infusion had 88% probability at the MIC_{50}. Higher-dose combination therapy consisting of meropenem and fosfomycin and prolonged infusion demonstrated significant improvement in achieving 90% PTA against both the MIC_{50} and the MIC_{90}. Fractional dosing further improves this probability.

Table 3 summarizes the cumulative fraction of response (CFR) for each dosing regimen of meropenem and fosfomycin for the KPC-2-producing *K. pneumoniae* clinical isolates from various regions of Brazil. Overall, there is greater than 80% CFR for the regimens with higher doses of fosfomycin in combination with meropenem. The higher meropenem dosage regimens in combination with fosfomycin in patients with renal impairment have

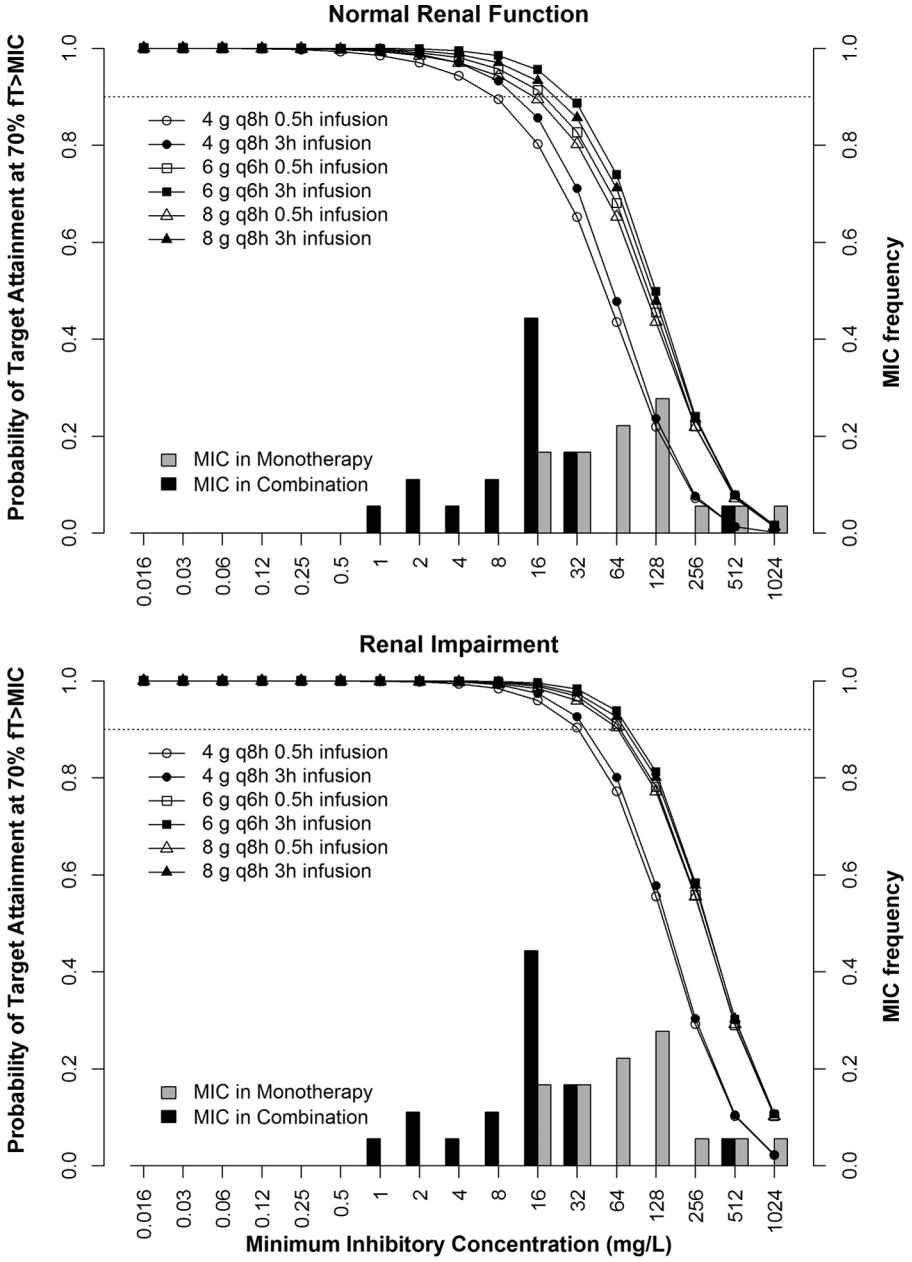

FIG 3 MIC frequency of 18 KPC-2-producing *Klebsiella pneumoniae* clinical isolates that were susceptible at fosfomycin MICs in monotherapy and in combination with meropenem and probability of target attainment of 70% *f*T>MIC for the fosfomycin dosing regimens of 4 g q8h, 6 g q6h, and 8 g q8h in critically ill virtual patients with normal renal function (top) and renal impairment (bottom). Open symbols represent a 0.5-h infusion, and filled symbols indicate a 3-h infusion. The dotted line indicates 90% probability of target attainment.

greater than 80% CFR, whereas this value is lower in patients with normal renal function.

In this study, we also evaluated the percentage of the virtual population for which the C_{min} was 2-fold the MIC_{50} and MIC_{90} of meropenem and fosfomycin; the results are shown in Table 4. Meropenem dosing regimens in combination therapy are largely inadequate to attain a 90% probability that the C_{min}/MIC ratio is $\geq$2 if the MIC_{90} is used for evaluation regardless of renal function. In patients with renal impairment, the two higher doses as a 3-h infusion were able to achieve >80% probability using the MIC_{50} reference. The higher fosfomycin dosage regimens in combination therapy in patients with renal impairment fared better in achieving >80% probability that the C_{min}/MIC ratio was $\geq$2 with the MIC_{50} reference. Only 6 g q6h as a 3-h infusion achieved over 80% probability using the MIC_{90} reference.

DISCUSSION

The dosing regimens evaluated in this study are well within the recommended daily doses used in clinical practice (23, 26). When administered as monotherapy, the meropenem and fosfomycin dosing regimens even at the maximum daily doses would not be effective against KPC-2-producing *K. pneumoniae* isolates, as

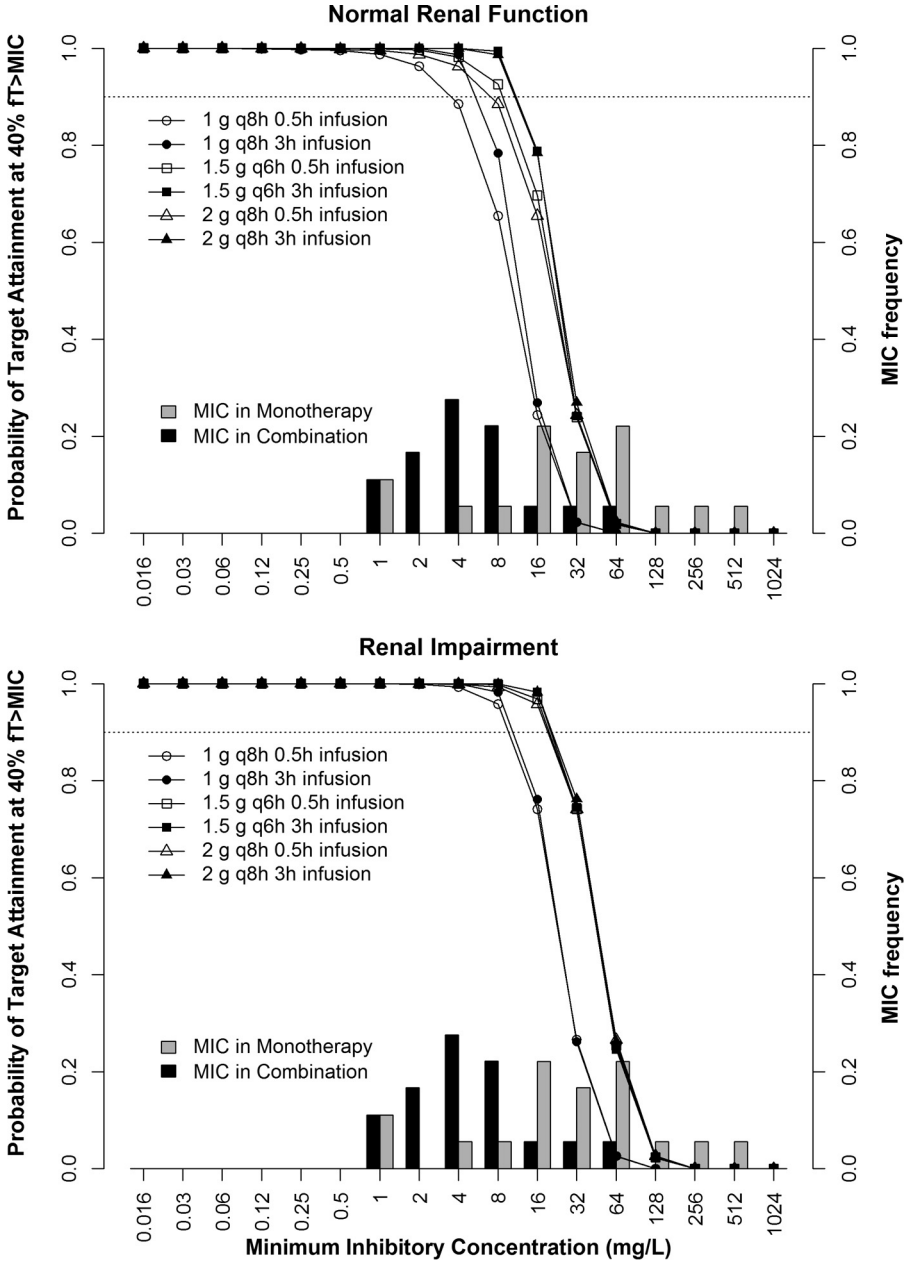

FIG 4 MIC frequency of 18 KPC-2-producing *Klebsiella pneumoniae* clinical isolates that were susceptible at meropenem MICs in monotherapy and in combination with fosfomycin and probability of target attainment of 40% *f*T>MIC for the meropenem dosing regimens of 1 g q8h, 1.5 g q6h, and 2 g q8h in critically ill virtual patients with normal renal function (top) and renal impairment (bottom). Open symbols represent a 0.5-h infusion, and filled symbols indicate a 3-h infusion. The dotted line indicates 90% probability of target attainment.

shown by the PTA falling markedly below the 90% target for meropenem and fosfomycin PD indices of 40% and 70% *f*T>MIC. When administered as a single agent, neither meropenem nor fosfomycin has any utility against multidrug-resistant Gram-negative microorganisms that carry the bla_{KPC-2} gene. It is noted that there are other resistance mechanisms that may exist in these isolates and that were not evaluated in this study.

Combination antimicrobial therapy can potentially alleviate the global crisis of prohibitively limited antimicrobial treatment options by rescuing agents that are considered obsolete (7, 38). This study has shown that combination therapy consisting of fosfomycin and meropenem increases the susceptibility of KPC-2-producing *K. pneumoniae* isolates to an acceptable level for at least one of the two antimicrobial agents. A reduction to 1/4 to 1/16 the MIC_{50} and MIC_{90} of the monotherapy antimicrobials was observed in the agents used in combination therapy against this collection of clinical isolates. In a majority of the isolates tested, the actions of fosfomycin and meropenem were synergistic. In a retrospective analysis of 41 patients infected by KPC-2-producing *K. pneumoniae*, Qureshi et al. noted that the mortality rates of patients receiving combination regimens were markedly lower than those receiving monotherapy (13.3% versus 57.8% mortality

TABLE 3 Cumulative fraction of response to fosfomycin and meropenem regimens against KPC-2-producing *K. pneumoniae* clinical isolates from various regions of Brazil

Patient renal function and antimicrobial regimen	CFR (%)[a]			
	Monotherapy		Combination	
	0.5 h	3 h	0.5 h	3 h
Normal renal function				
Meropenem[b]				
1 g q8h	16	19	44	54
1.5 g q6h	29	35	67	77
2 g q8h	25	31	59	69
Fosfomycin[c]				
4 g q8h	40	44	78	**82**
6 g q6h	59	63	**87**	**89**
8 g q8h	57	61	**86**	**89**
Renal impairment				
Meropenem[b]				
1 g q8h	34	37	74	78
1.5 g q6h	53	57	**85**	**87**
2 g q8h	50	54	**83**	**86**
Fosfomycin[c]				
4 g q8h	66	68	91	93
6 g q6h	**80**	**82**	95	96
8 g q8h	79	**81**	95	95

[a] Gray shading indicates ≥90% CFR, and boldface indicates 80% to <90% CFR.
[b] CFR calculated at 40% *f*T>MIC for meropenem.
[c] CFR calculated at 70% *f*T>MIC for fosfomycin.

rate), and combination therapy significantly improved patient survival (39). Tumbarello et al. demonstrated that combinations containing meropenem were associated with significantly higher survival rates when KPC-containing *K. pneumoniae* isolates had meropenem MICs of ≤8 mg/liter (40) and that combination therapy is effective in decreasing treatment failure (7). In 83% of the isolates in the present study, the combination with fosfomycin brought the meropenem MIC to ≤8 mg/liter, but the meropenem MIC was at or below the CLSI breakpoint of 1 mg/liter against *K. pneumoniae* for only 3 of the 18 isolates when the combination with fosfomycin was used. For this reason, we have seen that none of the recommended meropenem dosage regimens can achieve a 90% probability of target attainment.

Fosfomycin is an important companion drug in the combination to "rescue" meropenem's utility and is often used as an adjunct antimicrobial agent against serious infections, since fosfomycin typically demonstrates synergistic activities with other antimicrobial agents (26, 41). Fosfomycin is being utilized more frequently, particularly against multidrug-resistant bacterial infections (42), although there are reported new cases of fosfomycin resistance development (43–45). Our study shows that fosfomycin dosing regimens are more likely to achieve the PTA against the MIC_{90} of the combination, providing sufficient antimicrobial coverage even when the meropenem regimens at the maximum daily dosage fall short. We also evaluated a hypothetical trough/MIC ratio of ≥2 for resistance prevention and showed that the higher fosfomycin dosing regimens in a 3-h infusion in combination had >75% probability of achieving a trough/MIC ratio of ≥2 in patients with normal renal function and >90% in patients with renal impairment, with the MIC_{50} as a reference. With the MIC_{90} as a reference, the probability of achieving a trough/MIC ratio of ≥2 was smaller.

The evaluation of clinical and microbiological actions of antimicrobial regimens utilize pharmacokinetic and pharmacodynamic properties to understand the drug effects. This concept relates the characteristics of the drug, the patient, and the patho-

TABLE 4 Percentage of population whose trough drug concentrations are >2-fold the MIC against KPC-2-producing *K. pneumoniae* by dosing regimens, infusion duration, and renal function in monotherapy and combination therapy

Patient renal function and antimicrobial regimen	% of population with C_{min} of >2-fold the respective MIC[a]:							
	MIC_{50}				MIC_{90}			
	Monotherapy		Combination		Monotherapy		Combination	
	0.5 h	3 h	0.5 h	3 h	0.5 h	3 h	0.5 h	3 h
Normal renal function								
Meropenem								
1 g q8h	0	0	13	20	0	0	0	0
1.5 g q6h	0.34	0.63	43	60	0	0	0.34	0.63
2 g q8h	0.28	0.47	31	42	0	0	0.28	0.47
Fosfomycin								
4 g q8h	18	20	55	61	0.15	0.16	37	40
6 g q6h	39	44	75	**81**	1.5	1.6	59	66
8 g q8h	37	40	71	76	1.1	1.3	55	61
Renal impairment								
Meropenem								
1 g q8h	0.78	1.1	59	69	0	0	0.78	1.1
1.5 g q6h	10	14	**87**	94	0	0	10	14
2 g q8h	8.4	11	79	**87**	0	0	8.4	11
Fosfomycin								
4 g q8h	50	53	**85**	**88**	2.1	2.1	71	74
6 g q6h	73	77	94	96	9.8	10	**87**	90
8 g q8h	73	74	94	95	9.8	10	**87**	**88**

[a] Gray shading indicates ≥90% probability that the C_{min}/MIC ratio is ≥2, and boldface indicates 80% to <90% probability that the C_{min}/MIC ratio is ≥2.

gen to derive optimized antimicrobial regimens with higher clinical and microbiological efficacies (46, 47). However, most of these evaluations are performed primarily in a monotherapy setting, and combination antimicrobial synergy studies using this concept are scarce. Recommendations for dosages of antibiotics in combination regimens should be optimized and maximized so that they can reach their respective pharmacodynamic indices and microbiological outcomes (48, 49). Our study used this concept to assess which of the combined and optimized regimens of meropenem with fosfomycin have better probabilities of therapeutic effectiveness against KPC-2-producing *K. pneumoniae*.

Notably, factors such as the inclusion of a second antimicrobial, prolonged infusion, increased dosage, and more divided doses show important utility in increasing the probability of target attainment, of which the inclusion of a second agent has the greatest impact. This observation corroborates the results of combination therapy against bacteria containing KPC enzymes, as demonstrated by other studies (7, 39, 40).

The antimicrobial regimens tested against the MIC_{50} that showed the best results were a combination of meropenem at 1.5 g q6h and fosfomycin at 6 g q6h by prolonged infusion. Due to the higher resistance to meropenem among bacterial isolates in Brazil, these isolates are still susceptible to fosfomycin but not meropenem in the fosfomycin-meropenem combination. These regimens, at the highest recommended daily dose for both agents, are administered more frequently, thus favoring the maintenance of the plasma drug concentration above the MIC for a longer period of time. Dosing regimens with more fractional doses also allowed for reduced daily doses, bringing down the cost of antimicrobial treatment, while maintaining the same efficacy in the treatment. Kotapati et al. used a meropenem regimen of 0.5 g q6h, which yielded clinical outcomes similar to those of a regimen of 1 g q8h and reduced the daily drug acquisition costs associated with antibiotic therapy (50).

Our study has two main limitations. First, the number of isolates evaluated is not very large and may bias the MIC_{50} and MIC_{90} statistics. However, these isolates demonstrated good variability in MIC ranges for both meropenem and fosfomycin; the isolates came from different regions of Brazil, thus providing a representative map of infection in the country. Second, the isolates were primarily *K. pneumoniae*, which is easier to treat with a β-lactam/β-lactamase inhibitor combination than *P. aeruginosa* (51). The outer membrane of *P. aeruginosa* is less permeable by antibiotics and is regulated by porins, whereas loss of porins can increase resistance to antibiotics (52–54).

The pharmacokinetic parameters of meropenem and fosfomycin used for the simulation came from one- and two-compartment models, respectively, previously developed from a critically ill population (20, 24). Other studies used a two-compartment model to characterize the total concentration of meropenem in plasma (55–58). These models were shown to underpredict free meropenem concentrations in critically ill patients, and a one-compartment model had the least bias in predicting free meropenem concentration (21). The predicted C_{max} may be sensitive to the number of compartments used in the model. C_{max} is also affected by the duration of infusion The distribution phase, however, is no longer apparent when the drugs are infused for more than half an hour, since the intercompartmental clearance rates reported for the two drugs are very high (24, 57). Moreover, the V for both drugs is low, indicating that the drugs are distributed extracellularly. The small volume of distribution and rapid distribution between central and peripheral compartments would result in a negligible difference in the overall probability of achieving a time-dependent pharmacodynamic index, when determining whether to use a one- or two-compartment model to predict free meropenem exposure. The predicted peak and trough plasma meropenem concentrations in this study were consistent with values reported in the literature for septic critically ill patients (59).

In conclusion, the reduction in the MICs of meropenem and fosfomycin in combination for the majority of isolates improves attainment of the target PD index, with the dosing regimens of fosfomycin with meropenem including higher daily doses, more fractionated doses, and prolonged infusion. Our study demonstrated that the antimicrobial combination consisting of meropenem and fosfomycin can be a viable alternative to combat infections caused by KPC-2-producing *K. pneumoniae*.

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