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RESEARCH PAPER

Meropenem for treating KPC-producing *Klebsiella pneumoniae* bloodstream infections: Should we get to the PK/PD root of the paradox?

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ABSTRACT

The objective of this study was to assess the achievement of pharmacokinetic/pharmacodynamic (PK/PD) targets of meropenem (MEM) in critically-ill patients with bloodstream infections (BSI) due to *Klebsiella pneumoniae*-carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) with MEM minimum inhibitory concentrations (MICs) ≥16 mg/L. Nineteen critically-ill patients with KPC-Kp BSI were given combination therapy including MEM, tigecycline, plus colistin or gentamicin (according to susceptibility testing). MEM was administered as an extended 3-hour infusion of 2 g every 8 hours, or adjusted according to renal function. MEM plasma concentrations were determined by high-performance liquid chromatography. PK/PD targets for MEM were defined as T > 40% 1×MIC and T > 40% 4×MIC. Possible synergisms between MEM and coadministered agents were assessed by time-kill assays based on plasma levels for MEM and on fixed plasma concentrations for the other agents. In none of 19 patients MEM reached any PK/PD target. The actual MEM MICs were 256, 512, and 1024 mg/L in 1, 3, and 15 isolates, respectively. However, theoretically, the PK/PD target of T > 40% 1×MIC could have been achieved in 95%, 68%, 32% and 0% of the isolates for MIC equal to 8, 16, 32, and 64 mg/L, respectively. No synergisms were observed between MEM and coadministered agents. In conclusion, high-dose MEM failed to reach PK/PD targets in 19 patients with BSI due to KPC-Kp with very high MEM MICs. On a theoretical basis, our results suggest a possible usefulness of MEM against resistant blood isolates with MICs up to 32 mg/L.

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Introduction

Managing life-threatening infections due to *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* (KPC-Kp) has become a tough challenge in recent years.[1-12] Indeed, these infections are often caused by isolates with multidrug-resistant phenotypes, and are associated with high mortality (30–70%).[13-17] Dependable therapeutic options often rely on a few antimicrobial drugs administered in combinations, due to a survival benefit over monotherapy reported in multicenter observational studies.[13-15,17]

Somewhat paradoxically, meropenem (MEM) is almost invariably included in such combinations, since the above cited studies showed that high-dose MEM was able to improve survival from KPC-Kp severe infections, despite in vitro nonsusceptibility (minimum inhibitory concentration [MIC] > 2 mg/L).[13-18] However, this effect was clearly detectable only when the resistant KPC-Kp isolate had a MEM MIC ≤ 8 mg/L, while the existence of any benefit above this MIC threshold has still to be confirmed.[15-17] Therefore, there is uncertainty about the usefulness of including MEM in combination regimens when the MEM MIC of KPC-Kp strains is ≥ 16 mg/L. Under this light, pharmacokinetic/pharmacodynamic (PK/PD) studies could anticipate valuable theoretical evidence in favor or against this approach, nowadays widely used in the clinical practice, especially in Southern Europe.[15,17]

Aim of the present study was to assess the achievement of PK/PD targets of high-dose MEM in critically-ill patients with bloodstream infections (BSI) caused by KPC-Kp with high MEM MICs. The possible synergism between MEM and coadministered antibacterials was also investigated.

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Patients and methods

From July 2013 to May 2014, a prospective study was conducted at the University of Genoa IRCCS AOU San Martino-IST, a 1,300-bed teaching hospital in Genoa, Italy. The study was approved by the local ethics committee and all enrolled patients or their relatives signed an informed consent to participate in the study according to local regulations. During the study period, all adult patients with a monomicrobial KPC-Kp BSI fulfilling the following criteria were included in the study: (i) at least one blood culture positive for a KPC-Kp isolate with MEM MIC $\geq$ 16 mg/L by routine susceptibility testing; (ii) signs and symptoms of sepsis, severe sepsis, or septic shock, according to standard definitions;[19] (iii) estimated creatinine clearance (CrCl) > 10 mL/min using Cockcroft-Gault formula;[20] (iv) no receipt of any renal replacement therapy.

The primary study endpoint was the achievement of PK/PD targets of high-dose MEM in critically-ill patients with bloodstream infections (BSI) caused by KPC-Kp resistant to MEM (MIC $\geq$16 mg/L) with respect to their actual MEM MICs. The secondary endpoint was synergism between MEM and coadministered antibacterials.

Antibiotic therapy

MEM was administered as an extended 3-hour infusion, and it was included in 3-drug combinations with tigecycline/gentamicin or tigecycline/colistin according to colistin resistance or susceptibility of KPC-Kp isolates. For patients with CrCl $\geq$ 40 mL/min, MEM was administered without loading dose at the dosage of 2 g every 8 hours, while for patients with CrCl between 39 and 10 mL/min the given dose was 2 g every 12 hours. Gentamicin was administered at 5–7 mg/kg once-daily, and colistin at 9 million international units (IU) of colistimethate sodium as a loading dose, then 4.5 million IU every 12 hours as a maintenance dose. Standard dose adjustments for decreased renal function were applied for gentamicin and colistin.[21,22] Tigecycline was administered at 100 mg as a loading dose, then 50 mg every 12 hours as a maintenance dose.

MEM concentration determination

MEM plasma concentrations were evaluated by validated high-performance liquid chromatography assay.[23] MEM plasma concentrations were determined for each patient, after at least 3 infusions of the drug (on the second day of therapy). Blood samples were collected according to the following schemes: (1) immediately after the end of infusion, 1, 3, and 5 hours after the end of infusion when MEM was given every 8 hours; (2) immediately after the end of infusion, 1, 3, 5, and 9 hours after the end of infusion when MEM was given every 12 hours. For each sample, an aliquot of 4 mL of blood was drawn into heparinized tubes, which were centrifuged at 1000 g for 10 min; the resulting plasma was stored at −80°C. Clinical samples, drug-free plasma and calibration standards were extracted as reported elsewhere.[23] Fresh human plasma samples were obtained from healthy volunteers for standard samples. Standard solutions for the calibration curves construction were obtained by diluting a stock solution of MEM at a concentration of 1 mg/mL in water. Calibration samples were prepared in pooled samples of blank human plasma and were prepared at 7 different concentrations ranging from 0.5 to 50 μg/ml. The results obtained from the analysis of the calibration points were examined by linear regression. In order to assess whether a calibration point could be accepted, it was back-calculated on the basis of the equation of the corresponding calibration curve; a calibration curve was rejected if more than 2 concentrations or 2 adjacent concentrations deviated more than 20% from the nominal value for the Lower Limit Of Quantification (LLOQ) and by more than 15% for the other concentrations (outliers). The precision and accuracy of the method were determined by performing replicate analyses of quality control plasma samples (1, 5, 25 μg/mL) and LLOQ (0.5 μg/mL). Two replicates of each QC/LLOQ were analyzed on 3 different days and subjected to within- and between-run analysis. Samples with concentrations higher than the upper limit of the calibration were reanalyzed by dilution of the sample. The precision (relative standard deviation of replicate analysis) was calculated using the ANOVA test. The accuracy of the method was calculated by the formula, BIAS = (mean–nominal concentration)/(nominal concentration X100).[24,25]

MEM noncompartmental pharmacokinetic analysis

The individual plasma concentration profile of MEM was obtained with a PK software program (Modkine version 1.2; 2001 – Biosoft®, Cambridge UK). The main pharmacokinetic parameters calculated for each patient were maximum plasma concentration (Cmax), area under the curve of plasma concentration vs. time extrapolated to infinity ($AUC_{0-\infty}$), elimination rate constant (Kel), mean residence time (MRT), Volume of distribution (Vd), Clearance (CL) and half-life. Cmax was determined by visual inspection of each plasma concentration time plot. $AUC_{0-\infty}$ was calculated on log-transformed values using the trapezoidal rule and extrapolated to infinity by dividing the last measurable plasma concentration value by Kel. The latter was estimated from the

points of the terminal phase of the plasma concentration curve by log-linear regression. The total clearance (CL) was calculated as dose/$AUC_{0-\infty}$, and the volume of distribution (Vd) was calculated as CL*MRT.

T > 40% 1×MIC and T > 40% 4×MIC were defined as PK/PD targets. For each patients and for different MIC thresholds, the time during which MEM concentrations were higher than MIC (T> 1×MIC and 4×MIC) was calculated as follows:[26]

$$\%T > 1\times\mathrm{MIC} = \left\{[T_{inf}/ln(C_{3h}/C_{0h})] + (1/K)\right\} \times \ln\,[C_{3h}/(1\times\mathrm{MIC})]\times(100/\tau)$$

$$\%T > 4\times\mathrm{MIC} = \left\{[T_{inf}/ln(C_{3h}/C_{0h})] + (1/\mathrm{K})\right\} \times \ln\,[C_{3h}/(4\times\mathrm{MIC})]\times(100/\tau)$$

Strain identification and antibiotic susceptibility testing

Routine identification and antibiotic susceptibility testing were carried out using the Vitek-2 AES automated system (BioMérieux, Marcy-l'Etoile, France). In addition, *in vitro* activity of meropenem, colistin, tigecycline, and gentamicin were confirmed by the broth microdilution method and interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (Breakpoint for interpretation of MIC and zone diameters. Version 5.0, 2015; www.eucast.org). Identification of KPC carbapenemase-encoding gene (bla_{KPC-}) was achieved by PCR and sequencing using primers and conditions reported elsewhere.[27] Multilocus sequence typing (MLST) was performed for all isolates as previously described.[27]

Time-kill experiments

The activities of the antimicrobial combinations were determined in triplicate for each isolates with the time-kill methods. Antimicrobial combinations to be tested were selected for each strain on the basis of the therapy received by patients. Thirteen strains were tested against meropenem plus tigecycline, meropenem plus gentamicin, tigecycline plus gentamicin, and meropenem plus tigecycline plus gentamicin. Six strains were tested against meropenem plus tigecycline, meropenem plus colistin, tigecycline plus colistin, and colistin plus tigecycline plus meropenem

Time-kill experiments were performed by adding the antibiotics to log-phase bacterial cultures diluted to 10^6-10^7 CFU/mL growing in 250 ml flasks at 37°C. Just before the antibiotics were added and at 2, 6, and 24 h thereafter, bacterial counts were carried out. Survivors were evaluated by determining CFU on agar plates. The antibiotics were tested alone and in combination. MEM was used at specific concentrations obtained for each patients on the basis of the measured area under the concentration-time curve (AUC_{0-8}/8 h for patients receiving MEM every 8 hours and AUC_{0-12}/12 h for patients receiving MEM every 12 hours), while tigecycline, gentamicin and colistin were used at fixed concentrations, according to standard plasma levels (0.125, 2, and 12 mg\L for tigecycline, colistin, and gentamicin, respectively).[28,29]

Antibiotic interactions were interpreted as synergism if the combinations, compared with the most effective single antibiotic, caused at least a ≥2 $\log_{10}$ decrease in colony count. Antagonism was defined as a ≥2 $\log_{10}$ increase in colony count, while indifference was defined as a <2 $\log_{10}$ change (increase or decrease). When a combination of 3 antibiotics was analyzed, interactions were interpreted as above comparing the combination with both the most effective single antibiotics and the most effective combination of 2 antibiotics.

Results

Nineteen patients were enrolled in the study. Their mean age was 62 y (SD ± 13), 12/19 were males (63%). The clinical characteristics and outcome of patients are reported in Table 1. Table 2 shows MEM PK parameters in the study population. Most patients (15/19) received 2 g of MEM every 8 hours, while 3/19 were treated with 2 g of MEM every 12 hours because of impaired renal function. A morbidly obese patient (body mass index [BMI] > 40 kg/m^2) with normal renal function was given 3 g of MEM every 8 hours.

According to routine Vitek-2 results, all isolates exhibited a MEM MICs > 16 mg/L. By broth microdilution method, actual MEM MICs of isolates turned out to be 256, 512, and 1024 mg/L in 1, 3, and 15 of the 19 isolates, respectively. Two out of 19 isolates were resistant to gentamicin (11%, MICs range: 2–32 mg/L), 13/19 were resistant to colistin (68%, MICs range: 0.25 – 64 mg/L), and 3/19 were resistant to tigecycline (18%, MICs range: 1–32 mg/L). Gentamicin-resistant strains were susceptible to colistin and colistin-resistant strains were susceptible to gentamicin. All strains were KPC-Kp. In particular, 16 of them produced KPC-3 (84%) and 3 produced KPC-2 (16%). Most isolates (18/19) belonged to sequence type 258 (ST258), and one belonged to ST307.

MEM did not achieve the PK/PD targets of T > 40% 1×MIC and T > 40% 4×MIC in any of the enrolled patients. However, interestingly, on the basis of measured levels, T > 40% 1×MIC (but not T > 40%

Table 1. Characteristics and outcome of 19 patients with bloodstream infection due to *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumonia* (KPC-Kp).

Patient characteristics	
Age, years, mean ± SD	62 ± 13
Male gender	12 (63)
BMI, mean ± SD	26 ± 10
BSA, m^2, mean ± SD	1.89 ± 0.38
Type of patient	
Surgical	12 (63)
Medical	7 (37)
Mechanical ventilation	10 (53)
Diabetes Mellitus	9 (47)
COPD	4 (21)
Chronic renal failure*	2 (11)
Solid cancer	3 (16)
Hematological malignancies	6 (32)
ANC < 500/mm^3	2 (11)
Charlson score, median (IQR)	2 (1–3)
Renal failure at BSI onset*	3 (16)
Serum creatinine, mg/dl, median (IQR)	0.90 (0.65–1.35)
Albumin, g/liter, median (IQR)	22.2 (18.4–25.9)
Total bilirubin, mg/dl, median (IQR)	0.61 (0.46–1.30)
Clinical presentation	
Sepsis	9 (47)
Severe sepsis	8 (42)
Septic shock	(11)
APACHE II score, median (IQR)	11 (8–13)
Treatment	
Colistin-based regimen	6 (32)
Gentamicin-based regimen	613 (68)
Treatment failure** (missing = 5)	5 (36)
30-day mortality	3 (16)

Notes. Values are reported as number (%) unless otherwise indicated; SD, standard deviation; BMI, Body Mass Index; BSA, Body Surface Area; COPD, chronic obstructive pulmonary disease; ANC, absolute neutrophil count; IQR, interquartile range; BSI, bloodstream infection; APACHE, Acute Physiology and Chronic Health Evaluation.

*Creatinine clearance < 40 mL/min.

**Defined as persistence of blood cultures positive for KPC-Kp after at least 72 h of combination therapy. Data available for 14/19 patients.

4×MIC) could have been attained in 68%, and 32% of patients for hypothetical MEM MICs of 16, and 32 mg/L, respectively. The relationship between T > 40% 1×MIC and either actual or hypothetical MEM MICs is further detailed in Figure 1.

Additionally, possible synergisms between MEM and coadministered antibacterials were evaluated. Results concerning the activities of administered combinations are detailed in Table 3. Although in 18/19 cases (95%) 3-drug combinations determined a further reduction in colony counts when compared with the most effective double-antibiotic combinations, no synergism was detected. Similarly, in subgroup analyses the 2-drug combinations of tigecycline plus colistin and meropenem plus gentamicin seemed to perform better than their counterparts (meropenem plus colistin and tigecycline plus gentamicin, respectively), but the overall effect was indifferent.

Discussion

High-dose MEM administered as an extended 3-hour infusion did not achieve any of the predefined PK/PD targets in 19 critically-ill patients with BSI due KPC-Kp with high MEM MICs, ranging from 256 to 1024 mg/L. In addition, no synergism was found between MEM and coadministered agents by time-kill assays.

MEM has been reported to improve survival from infections caused by KPC-Kp with slightly increased MEM MICs, if administered in combination with one or more active agents and despite in vitro nonsusceptibility

Table 2. Pharmacokinetic parameters of high-dose meropenem in 19 patients with bloodstream infection due to *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae*.

Patient no.	Dose	Cmax (mg/L)	$AUC_{0-\infty}$ (mg/L*h)	$t_{1/2}$ (min)	Kel	MRT (min)	CL (L/min)	Vd (L)
1	**2 g tid**	14.55	42.36	126.00	0.006	181.82	0.76	138.87
2	**2 g tid**	50.16	118.53	94.93	0.007	136.99	0.22	30.34
3	**3 g tid**	77.06	282.00	150.65	0.005	217.39	0.22	47.02
4	**2 g tid**	42.03	78.36	72.19	0.010	104.17	0.26	27.53
5	**2 g bid**	41.09	290.06	288.75	0.002	416.67	0.27	112.68
6	**2 g tid**	78.77	114.15	42.00	0.017	60.61	0.14	8.55
7	**2 g tid**	113.36	293.28	113.61	0.006	163.93	0.10	16.07
8	**2 g tid**	63.95	107.62	96.25	0.007	138.89	0.17	24.13
9	**2 g tid**	37.92	91.90	94.93	0.007	136.99	0.29	40.14
10	**2 g tid**	37.45	308.02	385.00	0.002	555.56	0.30	164.83
11	**2 g tid**	29.47	67.20	108.28	0.006	156.25	0.38	58.91
12	**2 g bid**	39.08	185.29	182.37	0.004	263.16	0.28	74.82
13	**2 g tid**	62.88	107.65	63.00	0.011	90.91	0.18	16.06
14	**2 g tid**	48.30	78.95	51.33	0.014	74.07	0.23	17.04
15	**2 g tid**	111.79	258.54	106.62	0.007	153.85	0.10	15.29
16	**2 g tid**	75.65	89.24	43.86	0.016	63.29	0.15	9.30
17	**2 g bid**	66.81	542.47	364.74	0.002	526.32	0.17	87.53
18	**2 g tid**	48.16	284.09	238.97	0.003	344.83	0.23	79.56
19	**2 g tid**	83.95	310.89	130.75	0.005	188.68	0.13	24.97

Notes. tid, *tris in die* (every 8 h); bid, *bis in die* (every 12 h); Cmax, maximum plasma concentration; $AUC_{0-\infty}$, area under the curve concentration-time to infinity; $t_{1/2}$, terminal elimination half-life; Kel, elimination rate constant; MRT, mean residence time; CL, clearance; Vd volume of distribution.

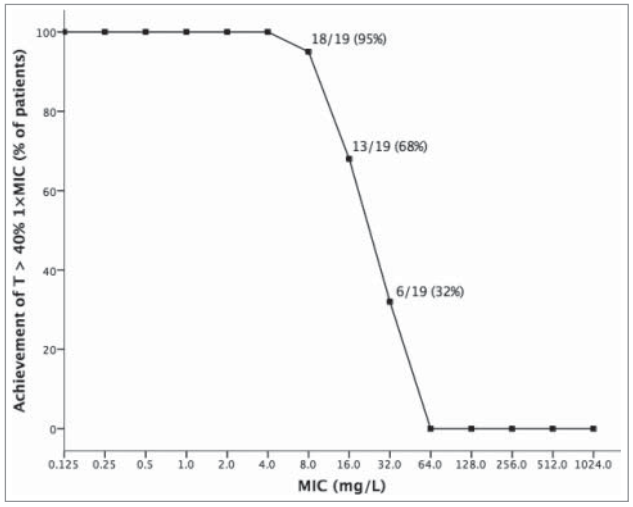

Figure 1. Achievement of T > 40% 1×MIC by measured meropenem levels in 19 critically-ill patients with BSI due to KPC-Kp, according to different hypothetical meropenem MICs. Actual meropenem MICs of all 19 isolates turned out to be far higher than those compatible with the achievement of such a PK/PD target, ranging from 256 to 1024 mg/L.

(MICs > 2 mg/L).[13-15,17,18] On the basis of a literature review, Daikos et al. first suggested that this approach could improve survival of patients in case of isolates with MEM MICs of 4 mg/L.[18] Then, Tumbarello et al. confirmed these findings in an Italian multicenter cohort of 125 patients with KPC-Kp BSI.[14] Recently, results from the same cohort have been updated.[17] Among 661 patients with KPC-Kp infections, mostly BSI, MEM-based combinations significantly improved survival when MEM MICs were > 2 and ≤ 8 mg/L.[17] Similar results were found in a Greek study of 167 patients with KPC-Kp BSI.[15] However, it should be noted that in all these studies no effort was made to evaluate the actual MEM MIC of isolates with MIC above 8 mg/L, since higher values were lumped together. In reality, MEM MICs > 8 mg/L might include several MIC levels ranging from 16 mg/L to likely much higher values. This possibly prevented the authors from observing a beneficial effect on survival for MEM MICs slightly exceeding 8 mg/L, and the issue remains controversial. Although only large dedicated studies, preferably randomized clinical trials, can definitely address this issue, valuable theoretical evidence in support or against the use of MEM in such a gray zone could be provided by targeted PK/PD analyses.

In our selected population of critically-ill patients with KPC-Kp BSI, high-dose MEM did not achieve either T > 40% 1×MIC or T > 40% 4×MIC in any of the enrolled subjects. Since MEM plasma levels were far below those necessary for the achievement of these targets, the possibility of any activity appears very unlikely for KPC-Kp with MEM MICs as high as 256-1024 mg/L, even by taking into account the high inter-patient variability of PK parameters in critically-ill subjects.[30,31] Nonetheless, it is worth noting that T > 40% 1×MIC could have been theoretically attained in a fairly large patient proportion of our patients for hypothetical MEM MICs of 8 and 16 mg/L (95% and 68%, respectively) and still in 32% of

Table 3. *In vitro* interactions of administered antibacterials by time-kill methods against blood *Klebsiella pneumoniae* carbapenemase-producing *Klebsiella pneumoniae* isolates of 19 critically-ill patients.

Patient no	MEM+TIG †	MEM+GEN†	TIG+GEN†	MEM+TIG+GEN†	MEM+TIG+GEN††
1	−0,17	0,64*	0,13	0,91	0,27
2	0,38	0,14*	1,3*	1,5	0,20
3	0,26	0,31	0,07	1,02	0,71
4	0,42	0,41	0,23	1,03	0,62
5	0,41	0,78	0,36	1,39	1,11
6	−0,10	0,54	0,10	0,63	0,09*
7	0,39	0,42	0,35	0,84	0,42
8	1,07	0,32	0,25	0,54	0,22
9	0,59	0,43	0,64	1,13	0,49
10	0,59	0,63	0,43	1,25	0,62
11	−0,24*	0,30	0,07*	0,36	0,06*
12	1,80	0,70	0,48	1,02	0,32
13	0,91	0,43	0,3	0,73	0,3
Patient no	**MEM+TIG†**	**MEM+COL†**	**TIG+COL†**	**MEM+TIG+COL†**	**MEM+TIG+COL††**
14	0,9	0,44	1,33	1,84	−0,49*
15	0,16	0,45	0,50	1,31	0,81
16	−0,24	0,25	0,35	1,11	0,76
17	0,27	1,23	1,07	1,45	0,22
18	0,15	0,18	0,30	0,42	0,24
19	0,14	0,07*	0,26	0,94	0,68

Notes. Values are reported as mean Δlog; I, indifferent interaction.
*Non statistically significant Δlog ($p \geq 0.05$).
†Δlog was calculated comparing the 2-drug or the 3-drug combination with the most effective single agent.
††Δlog was calculated comparing the triple combination with the most effective double-antibiotic concentration.

cases with MIC up to 32 mg/L. Although even these hypothetical MICs would have not allowed to reach T > 40% 4×MIC, which is necessary for MEM to exert an optimal bactericidal activity according to some literature data,[32,33] our results suggest that MEM might retain some bactericidal activity *per se* against KPC-Kp with MEM MICs up to 32 mg/L, albeit not at its maximum and only in some cases.

It should be noted that possible synergisms between MEM and coadministered agents could have supported the use of MEM in our patients even if PK/PD targets were not reached. This possibility is in line with previous reports of synergistic effects between MEM and either colistin or tigecycline.[34-37] However, we did not detect any synergism between MEM and coadministered agents against the isolates collected from our patients. Of note, although with the inherent limits of approximation and static model, we tried to individualize time-kill assays on the basis of PK/isolate pairs, in order to better reproduce the pharmacodynamics of antibiotic interactions and increase their clinical relevancy.

We should acknowledge that the 30-day mortality in the patients included in this study is lower than that reported in other studies, including ours,[7,14-18] and also seems incoherent with our PK/PD findings. However, this study was not designed for studying the direct effect of MEM MICs on mortality, but to provide PK/PD data as a first step in the process. The low mortality we found in this small group of patients might well be due to chance. Indeed, no related statistically or clinically significant conclusion could be obtained from a study including only 19 patients, and information about mortality was just reported for descriptive reasons. Hopefully, our PK/PD results could be an aid for designing larger clinical studies which can adequately address this critical issue.

This study has some important limitations. First, our results should be considered tentative, since the number of cases was too limited for broad generalization. Another important limitation is that our analysis was limited to plasma concentrations of MEM, and both the attainment of PK/PD targets and MEM interactions with other agents might be different in patients with foci of infection other than blood.

In conclusion, high-dose MEM failed to reach PK/PD targets in patients with BSI due to KPC-Kp with very high MEM MICs, ranging from 256 to 1024 mg/L. Our results also suggest that MEM-based combinations might not offer any benefit in terms of bactericidal activity in comparison to non MEM-based regimens in case of resistant blood isolates with MEM MIC higher than 32 mg/L. For these reasons, we think MEM use in treatment of BSI due to KPC-Kp with MEM MICs > 16 mg/L should rely on more detailed information about actual MEM MICs of locally prevalent KPC-Kp clones.

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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