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Strategies for the treatment of polymyxin B-resistant *Acinetobacter baumannii* infections

Thatiany Cevallos Menegucci[a], James Albiero[a], Letícia Busato Migliorini[a], Janio Leal Borges Alves[a], Giselle Fukita Viana[a], Josmar Mazucheli[a], Floristher Elaine Carrara-Marroni[b], Celso Luiz Cardoso[a], Maria Cristina Bronharo Tognim[a,*]

[a] Universidade Estadual de Maringá, Avenida Colombo 5790, CEP 87020-900 Maringá, Paraná, Brazil
[b] Universidade Estadual de Londrina, Rodovia Celso Garcia Cid, Pr 445 Km 380, CEP 86.057-970 Londrina, Paraná, Brazil

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ABSTRACT

In this study, the activity of meropenem (MEM), fosfomycin (FOF) and polymyxin B (PMB), alone and in combination, was analysed. In addition, optimisation of the pharmacodynamic index of MEM and FOF against six isolates of OXA-23-producing *Acinetobacter baumannii* (including three resistant to PMB) that were not clonally related was assessed. Antimicrobial combinations were evaluated by chequerboard analysis and were considered synergistic when the fractional inhibitory concentration index (FICI) was ≤0.5. Pharmacodynamic analyses of the MEM and FOF dosing schemes were performed by Monte Carlo simulation. The target pharmacodynamic index ($\%fT_{>MIC}$) for MEM and FOF was ≥40% and ≥70%, respectively, and a probability of target attainment (PTA) ≥0.9 was considered adequate. Among the PMB-resistant isolates, combinations of PMB + MEM and PMB + FOF + MEM showed the highest synergistic activity (FICI ≤0.125); isolates that were previously PMB-resistant were included in the susceptible category using CLSI interpretive criteria. Pharmacodynamic evaluation found that for a FOF minimum inhibitory concentration (MIC) of ≤16 μg/mL, treatment both by bolus dosing and prolonged infusion achieved adequate PTA, whilst for MIC = 32 μg/mL only infusion achieved adequate PTA. For a MEM MIC of 4 μg/mL, only the bolus treatment scheme with 1.5 g q6h and the infusion schemes with 1.0 g q8h, 1.5 g q6h and 2.0 g q8h achieved PTA ≥0.9. Results of antimicrobial and pharmacodynamic analyses can assist in treating infections caused by multidrug-resistant *A. baumannii*. However, in vivo clinical studies are essential to evaluate the true role of these compounds, including intravenous antimicrobial FOF therapy.

1. Introduction

Acinetobacter baumannii is a Gram-negative bacillus (GNB) that can cause serious infections, is difficult to treat and occurs mainly in intensive care unit (ICU) patients [1]. Carbapenems were once considered the drugs of choice for the treatment of infections caused by *A. baumannii*, but with the spread of carbapenemase enzymes, especially oxacillinases, this drug has lost its effectiveness over the years [2].

Increased bacterial resistance together with the low number of new antimicrobials entering the market have stimulated a return to formerly used drugs such as fosfomycin (FOF) and the polymyxins [3,4].

The mechanism responsible for the activity of FOF against *A. baumannii* isolates remains unclear [3]. In vitro and in vivo studies suggest that FOF acts in synergy with other antimicrobial agents, such as polymyxins and carbapenems [3,5,6].

Polymyxin monotherapy favours the emergence of resistant isolates; however, the global rates of polymyxin resistance remain low (<10%) [4]. Clinical use of polymyxin B (PMB) combined with other antibiotics (e.g. carbapenems, tigecycline, rifampicin and sulbactam) has been recommended for the treatment of infections such as pneumonia and bacteraemia caused by multidrug-resistant (MDR) *A. baumannii* [7].

The effectiveness of antimicrobial combinations against PMB-resistant isolates of *A. baumannii*, particularly with the presence

* Corresponding author. Present address: Laboratório de Microbiologia, Departamento de Ciências Básicas da Saúde, Universidade Estadual de Maringá, Avenida Colombo 5790, CEP 87020-900 Maringá, Paraná, Brazil. Tel.: +55 44 3011 4952; fax: +55 44 3011 4860.
E-mail addresses: mcbtognim@uem.br, cristinatognim@gmail.com (M.C.B. Tognim).

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of oxacillinase enzymes, and the pharmacokinetics and pharmacodynamics of FOF and PMB still need to be better investigated [3,8,9].

Although laboratory data for synergism do not always correlate directly with clinical data, owing to the lack of therapeutic options results of in vitro antimicrobial combination studies can provide some support for physicians, especially in cases of multidrug resistance [10].

To the best of our knowledge, no studies have examined the pharmacokinetics and pharmacodynamics of FOF and meropenem (MEM) in PMB-resistant strains of *A. baumannii*. This study evaluated the in vitro activity of MEM, FOF and PMB, administered alone or in combination. In addition, using Monte Carlo simulation, this study also assessed optimisation of the pharmacodynamic index of MEM and FOF administered by bolus or prolonged infusion against isolates of *A. baumannii* susceptible and resistant to PMB.

2. Methods

2.1. Bacterial samples

From January 2008 to December 2012, 472 isolates of *A. baumannii* were collected consecutively from the ICUs of two university hospitals in southern Brazil. Isolate identification and analysis of the antimicrobial susceptibility profile were carried out in the laboratory using a BD Phoenix® Automated Microbiology System (Becton Dickinson & Co., Sparks, MD). Using the automated method, 31 isolates were found to be resistant to colistin. Eight isolates were found to be resistant to PMB [minimum inhibitory concentration (MIC) ≥4 μg/mL] [11] by three different methods (Etest, broth microdilution and agar dilution). These isolates were characterised genetically by the enterobacterial repetitive intergenic consensus PCR (ERIC-PCR) technique [12] and were proven to belong to three distinct clusters (Dice coefficient >0.9), with only the first isolate belonging to each cluster included in this study. For comparison purposes, three isolates susceptible to PMB, each belonging to a different cluster but obtained in the same period, were selected for the study. All six isolates used in this study were obtained from clinical specimens, as follows: tracheal aspirate (four isolates); central venous catheter (one isolate); and catheter tip (one isolate). The isolates harboured the bla_{OXA-23} gene and showed resistance to carbapenems (MIC ≥64 μg/mL).

2.2. Determination of the minimum inhibitory concentration of fosfomycin

The MIC of FOF was determined using the agar dilution technique according to the protocols for determining antimicrobial sensitivity proposed by the Clinical and Laboratory Standards Institute (CLSI) [13]. The results were interpreted according to the cut-off points established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) for Enterobacteriaceae strains [14]. *Pseudomonas aeruginosa* ATCC 27853 was used as a quality control strain.

2.3. Evaluation of the activity of antimicrobial combinations by chequerboard analysis

The activity of the antimicrobial combinations was tested in duplicate by the chequerboard method [15] using Mueller–Hinton broth with adjustment of the concentrations of Ca^{2+} and Mg^{2+} cations [13]. Combinations containing FOF were supplemented with 25 μg/mL glucose 6-phosphate [16].

Four combinations of the three antimicrobial agents were analysed: FOF + PMB, FOF + MEM, PMB + MEM, and FOF + PMB + MEM. In this last case, PMB was used at fixed concentrations of 0.5 μg/mL and 0.125 μg/mL for the samples of PMB-resistant and PMB-susceptible strains, respectively. These combinations were tested against the six *A. baumannii* isolates.

The fractional inhibitory concentration index (FICI) was calculated for each antimicrobial in each combination using the following formula: $FIC_A + FIC_B = FICI$, where FIC_A equals the MIC of drug A in combination divided by the MIC of drug A alone, and FIC_B equals the MIC of drug B in combination divided by the MIC of drug B alone. The FICIs were interpreted as follows: FICI ≤ 0.5, synergy; 0.5 < FICI < 4, indifference; and FICI ≥ 4, antagonism.

2.4. Pharmacokinetics

The steady-state pharmacokinetic data for the volume of distribution, drug clearance and protein binding of MEM and FOF were used to analyse the dosage regimens administered by bolus dosing (administration period 0.5 h for MEM and 1 h for FOF) or prolonged infusion (administration period 3 h for both antimicrobials) and were taken from population studies of healthy subjects (Table 1) [17,18]. As FOF has an insignificant degree of protein binding, this was not considered in the present study [18]. All of the pharmacokinetic parameters were assumed to have log-normal distributions and the simulated patients had a weight of 70 kg with normal renal function.

The percentage of the dosing interval that the free drug concentration remained above the MIC of the bacteria ($\%fT_{>MIC}$) for the dosage regimens was calculated using a monocompartmental model and its value was obtained through model equations for administration by bolus [19] or prolonged infusion [17].

2.5. Pharmacodynamics

Pharmacodynamic analyses for FOF and MEM were performed using Monte Carlo simulation methodology with 10 000 iterations generated in the R language (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria, v.3.1.1, 2014-07-10) [20].

The dosages tested for MEM were 0.5 g every 8 h (q8h), 1.0 g q8h, 1.5 g every 6 h (q6h) and 2.0 g q8h, and those for FOF were 4.0 g of

Table 1
Steady-state pharmacokinetic (PK) data (mean ± standard deviation) for meropenem and fosfomycin administered by bolus dosing or prolonged infusion obtained from published studies in healthy volunteers.

PK parameter	Meropenem			Fosfomycin[a]
	500 mg	1000/1500 mg	2000 mg	
V_d (L/kg)	0.258 ± 0.032	0.266 ± 0.043	0.272 ± 0.019	0.32 ± 0.08
CL (L/h)	15.58 ± 1.66	14.40 ± 1.80	15.24 ± 3.79	8.74 ± 1.89
PB (%)	8.00	8.00	8.00	–

V_d, volume of distribution; CL, drug clearance; PB, protein binding.
[a] There was no difference in the PK data for the different dosage regimens for fosfomycin.
Bolus: administration period of 0.5 h for meropenem and 1 h for fosfomycin. Prolonged infusion: administration period of 3 h for both antimicrobials.

Table 2
Minimum inhibitory concentrations (MICs in μg/mL) and effects obtained from in vitro combinations of antimicrobials, identified by chequerboard analysis, in six isolates of *Acinetobacter baumannii*.

Isolate	MIC			MIC–FICI (interpretation of FICI[a])			
	FOF	MEM	PMB	FOF + MEM	FOF + PMB	MEM + PMB	FOF + MEM + PMB[b]
Ac576	64	64	16	32/32 – 1 (I)	32/16 – 1.5 (I)	0.5/0.5 – 0.039 (S)	2/0.25 – 0.035 (S)
Ac680	128	64	16	64/32 – 1 (I)	32/8 – 0.7 (I)	0.5/0.5 – 0.039 (S)	8/4 – 0.125 (S)
Ac-Cl	512	64	4	256/32 – 1 (I)	32/2 – 1 (I)	4/0.5 – 0.125 (S)	16/1 – 0.046 (S)
Ac-S22	64	128	0.5	32/32 – 0.75 (I)	32/0.5 – 1.5 (I)	64/0.25 – 1 (I)	32/64 – 1 (I)
Ac53	512	64	0.5	32/2 – 0.093 (S)	128/0.125 – 0.5 (S)	32/0.25 – 1 (I)	64/0.5 – 0.132 (S)
Ac68	512	64	0.5	256/32 – 1 (I)	256/0.25 – 1 (I)	32/0.25 – 1 (I)	64/0.5 – 0.132 (S)

FICI, fractional inhibitory concentration index; FOF, fosfomycin; MEM, meropenem; PMB, polymyxin B.
[a] FICI ≤ 0.5, synergism (S); 0.5 < FICI < 4.0, indifferent (I).
[b] PMB at a fixed concentration of 0.125 μg/mL for susceptible isolates and 0.5 μg/mL for resistant isolates.
EUCAST 2015 Enterobacteriaceae cut-off points: FOF: susceptible (S), ≤32 μg/mL; resistant (R), >32 μg/mL. CLSI 2015 *Acinetobacter baumannii* cut-off points: MEM: S, ≤2 μg/mL; intermediate, 4 μg/mL; R, ≥8 μg/mL; and PMB: S, ≤2 μg/mL; R, ≥4 μg/mL.

q8h, 6.0 g of q6h and 8.0 g of q8h. These doses were tested both for bolus and prolonged infusion against different MIC values (MEM, 2, 4 and 8 μg/mL; and FOF, 16, 32 and 64 μg/mL).

The values for the probability of target attainment (PTA) of each drug regimen were considered to be adequate when a target of ≥0.9 was reached [17,21]. The time-dependent pharmacodynamic parameter was used both for MEM and FOF, with $\%fT_{>MIC}$ of ≥40% and ≥70%, respectively, considered appropriate [17,18,21].

3. Results

3.1. Minimum inhibitory concentration determination and chequerboard analyses

The MICs of the antimicrobials alone and in combination are given in Table 2. The MICs of MEM and FOF were ≥64 μg/mL for all isolates.

For the combinations tested, MEM + PMB and the triple combination of FOF + MEM + PMB displayed the most synergistic activity (Table 2). The combination of MEM + PMB was synergistic for all of the PMB-resistant isolates and showed significant reductions in the MIC for both antimicrobials, which were then classified as susceptible (i.e. MIC ≤ 4 μg/mL for MEM and MIC ≤ 0.5 μg/mL for PMB).

For the triple combination, only one PMB-susceptible isolate did not display synergistic activity. In addition to synergistic activity, the others displayed a reduction in the MIC of MEM and were classified as susceptible (MICs ≤ 4 μg/mL). For FOF, four initially resistant isolates were ultimately classified as susceptible (MIC ≤ 32 μg/mL).

The combinations of FOF + MEM and FOF + PMB had the lowest rates of synergism, with only one isolate (Ac53). Although it showed an indifferent result using chequerboard methodology, the PMB-resistant Ac-Cl isolate displayed a reduction in the MICs of PMB and FOF and was classified as susceptible (MICs of 2 μg/mL and 32 μg/mL, respectively).

3.2. Pharmacodynamic assessment

Fig. 1 and Table 3 show the PTA of each antimicrobial scheme administered by bolus or prolonged infusion attaining the pharmacodynamic target $\%fT_{>MIC}$ (MEM, ≥40%; FOF, ≥70%). Schemes with a PTA ≥ 0.9 (dotted line on the graphs) were considered adequate.

The treatment schemes for FOF by bolus or prolonged infusion (4.0 g q8h, 6.0 g q6h and 8.0 g q8h) against an MIC of 16 μg/mL produced adequate PTAs, whilst for an MIC of 32 μg/mL only schemes with the same dosages administered by prolonged infusion were adequate (PTA ≥ 0.9).

For the MEM treatment schemes, desirable PTAs were not achieved by bolus dosing against MIC = 8 μg/mL, whilst for lower MICs, such as MIC = 4 μg/mL, both the bolus (1.5 g q6h) and prolonged infusion (1.0 g q8h, 1.5 g q6h and 2.0 g q8h) treatment schemes were capable of achieving PTA ≥ 0.9 (Fig. 1; Table 3).

4. Discussion

Infections caused by *A. baumannii* are difficult to treat, especially when the infection profile includes extensively drug-resistant (XDR) isolates [22]. For these cases, identification of synergism between antimicrobials is an important tool because of both the lack of treatment options and the recommended use of combined therapy with certain antimicrobials, such as PMB and FOF, to achieve a more effective therapeutic response [23,24].

The present study evaluated the in vitro presence of synergism of PMB combined with MEM and FOF against XDR isolates of *A. baumannii* and, using Monte Carlo simulation, demonstrated the

Table 3
Probability of target attainment of the pharmacodynamic index ($\%fT_{>MIC}$)[a] for bolus dosing and prolonged infusion schemes against *Acinetobacter baumannii*.

Treatment scheme	Bolus	Infusion	Bolus	Infusion	Bolus	Infusion
Fosfomycin	MIC = 16 μg/mL		MIC = 32 μg/mL		MIC 64 = μg/mL	
4.0 g q8h	0.93	0.99	0.59	0.97	0.01	0.40
6.0 g q6h	0.97	1.0	0.85	1	0.27	0.87
8.0 g q8h	0.9	0.99	0.65	0.96	0.16	0.71
Meropenem	MIC = 2 μg/mL		MIC = 4 μg/mL		MIC = 8 μg/mL	
0.5 g q8h	0.26	1	0	0.85	0	0
1.0 g q8h	0.94	1	0.54	1	0.02	0.94
1.5 g q6h	1	1	1	1	0.88	1
2.0 g q8h	0.96	1	0.83	1	0.5	1

$\%fT_{>MIC}$, percentage of the doing interval that the free drug concentration remains above the MIC of the bacterium; MIC, minimum inhibitory concentration; q8h, every 8 h; q6h, every 6 h.
Pharmacodynamic target: $\%fT_{>MIC} \geq 70\%$ for fosfomycin and $\%fT_{>MIC} \geq 40\%$ for meropenem.
Bolus: intravenous infusion for 0.5 h for meropenem or 1.0 h for fosfomycin.
Prolonged infusion: intravenous infusion for 3 h for both antimicrobials.

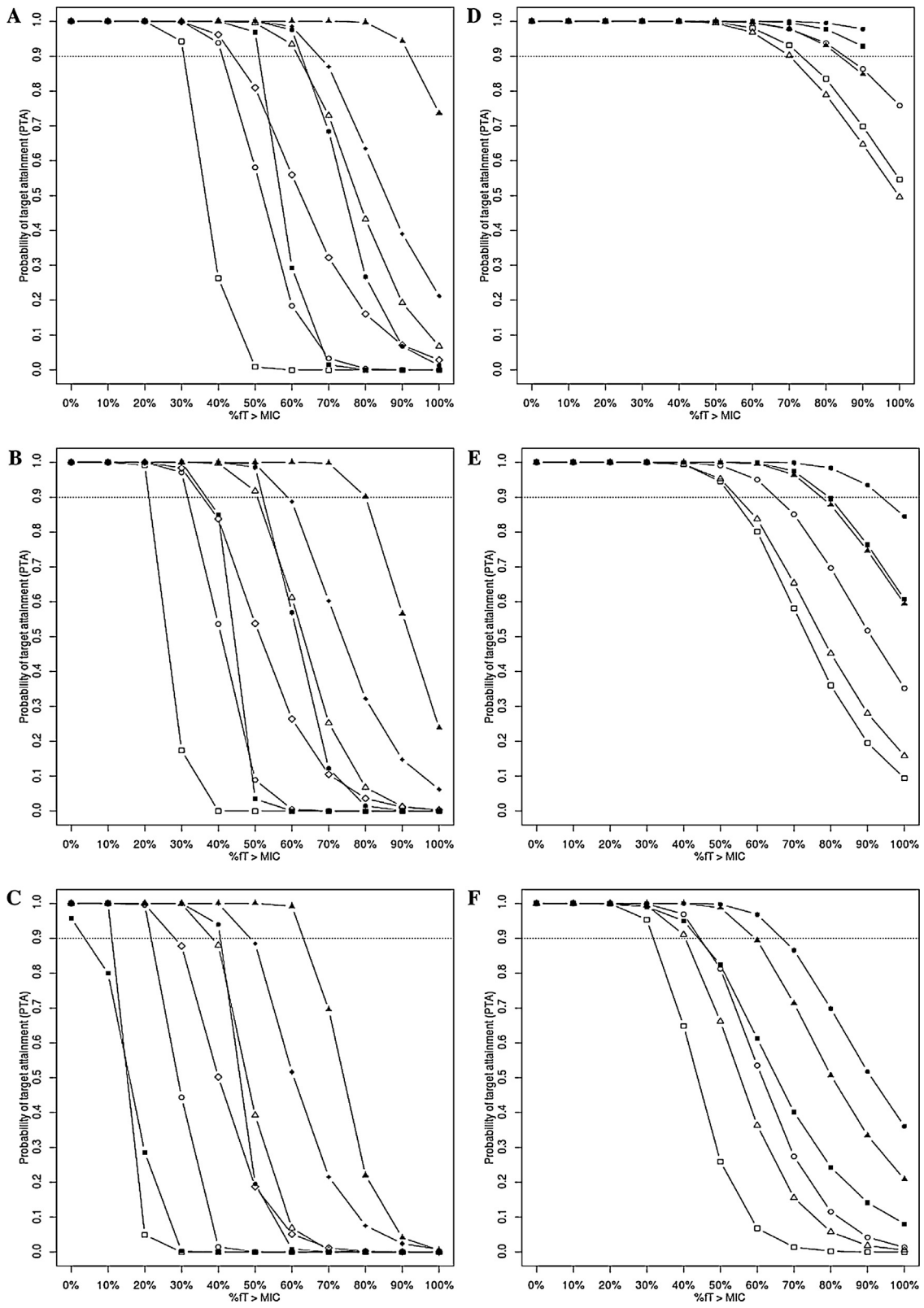

Fig. 1. Dosage schemes of meropenem (MEM) and fosfomycin (FOF). Probability of target attainment (PTA) of the pharmacodynamic index ($\%fT_{>MIC}$) for MEM and FOF against *Acinetobacter baumannii* in bolus dosing (open symbols) and prolonged infusion (closed symbols) schemes. The horizontal dotted line indicates adequate PTA (≥0.9), i.e. $\%fT_{>MIC}$ reached for 90% of the simulated population. The pharmacodynamic target ($\%fT_{>MIC}$) was ≥40% for MEM and ≥70% for FOF. (A–C) MEM at MIC = 2 μg/mL (A), MIC = 4 μg/mL (B) and MIC = 8 μg/mL (C); (D–F) FOF at MIC = 16 μg/mL (D), MIC = 32 μg/mL (E) and MIC = 64 μg/mL (F). For MEM: ❑,■, 0.5 g q8h; ❍,●, 1.0 g q8h; △,▲, 1.5 g q6h; and ◊,♦, 2.0 g q8h. For FOF: ❑,■, 4.0 g q8h; ❍,●, 6.0 g q6h; and △,▲, 8.0 g q8h. $\%fT_{>MIC}$, percentage of the dosing interval that the free drug concentration remains above the MIC of the bacteria; MIC, minimum inhibitory concentration; q8h, every 8 h; q6h, every 6 h.

pharmacodynamic possibilities of using these antimicrobials alone and in combination for the treatment of these micro-organisms.

Regardless of the resistance mechanism involved in PMB-resistant *A. baumannii*, significant synergistic activity was observed in vitro (FICI ≤ 0.5), with a reduction of the MIC in the MEM + PMB combination as well as in the triple combination of MEM + PMB + FOF for these isolates. Triple combinations of antimicrobials against isolates of *A. baumannii* and *P. aeruginosa* also displayed positive results in a study by the authors' research group using in vitro tests with PMB, MEM and gatifloxacin [25]. Recently, an in vivo study showed the efficacy of a triple combination of antimicrobials, including PMB, against isolates of KPC-2-producing *Klebsiella pneumoniae*, with a higher rate of synergism and survival for PMB combined with MEM or tigecycline [9].

According to the pharmacodynamic analysis in the present study, in the case of FOF, the reduction of MIC with the combination of three antimicrobials allowed both the bolus and prolonged infusion treatment schemes to achieve PTA ≥ 0.9, meaning that they were safe to use (Fig. 1; Table 3). FOF showed good results when combined with other antimicrobials; however, care should be taken with its use as a monotherapy due to the rapid in vitro emergence of resistance in addition to the selection of resistant micro-organisms [3,24].

In most countries, including Brazil, FOF is only used for the oral treatment of urinary tract infections. However, considering the small number of antibiotics available for the treatment of MDR-GNB infections, in vitro and in vivo experiments with FOF have been performed and have reported good activity [3,26–28]. A recent in vivo study using FOF combined with colistin found significantly higher microbial elimination over 72 h of treatment in the group of patients treated with a combination of the two drugs (90.7%) than in the group treated with colistin alone (58.1%). At the end of treatment, complete microbiological elimination occurred in 100% of patients receiving the combination therapy compared with only 81.2% of patients treated with colistin alone [5].

The dual combination of MEM + PMB and the triple combination of MEM + PMB + FOF resulted in a reduction of the MIC of MEM (≤4 μg/mL), allowing the use of the drug even when treating infections caused by *A. baumannii* that were previously resistant to MEM.

Pharmacodynamic assessment of different dosage regimens of MEM in patients with intra-abdominal infections, community-acquired pneumonia and ventilator-associated pneumonia showed that using 1.0 g of MEM by prolonged infusion for 3 h at 8-h intervals increased the PTA in bacteria with an MIC of 4 μg/mL compared with the bolus scheme (infusion of up to 30 min) [29]. In the present study, Monte Carlo simulation (Fig. 1) demonstrated that prolonged infusion is the best method of achieving higher PTAs; however, pharmacodynamic evaluation also showed that for bacteria with MICs of 4 μg/mL, it was possible to attain a target with different dosage regimens (1.0 g q8h, 1.5 g q6h and 2.0 g q8h) by prolonged infusion and that a dose of 1.5 g q6h could achieve an adequate PTA by the bolus regimen (Fig. 1).

Whilst laboratory data for synergism are not always reproducible in vivo [10], the data from the present study are particularly encouraging as the combination of PMB + MEM, even for isolates that produce carbapenemase and are resistant to these two antimicrobials, produced a reduction in the MIC even of PMB. All of the isolates were classified as susceptible (MIC ≤ 0.5 μg/mL), allowing its clinical use. Data from the literature show that PMB dosing regimens of up to 2.5 mg/kg/day are appropriate for less severe infections or for micro-organisms with MICs ≤ 1 μg/mL and that dosing regimens with a high daily dose of PMB (e.g. 3 mg/kg/day) followed by a maintenance dose may be appropriate for critical individuals or micro-organisms with MIC ≤ 2 μg/mL [30]. According Sandri et al., the good results obtained with the triple combinations (i.e. synergism with low concentrations of PMB of 0.125 μg/mL or 0.5 μg/mL) show that the current results have the potential for clinical application, even in isolates with an MIC ≥ 2 μg/mL for PMB [30].

The present study also examined other dual combinations, such as FOF + MEM and FOF + PMB. The FOF + MEM combination did not produce good results, whilst treatment with the FOF + PMB combination reduced the MIC of FOF for three samples (MIC ≤ 32 μg/mL). This level, as shown in Fig. 1, is capable of achieving PTA ≥ 0.9.

The most notable observation of this study was that the triple combination displayed synergism in 83.3% of the isolates tested, including all of those resistant to PMB. In all cases there was a reduction in the MICs of FOF and MEM, with favourable results for the use of these drugs. For susceptible isolates, the use of PMB independent of the MICs of other antimicrobials is not discouraged, as polymyxins or tigecycline are considered the drugs of choice for samples that produce OXA-23-type carbapenemase [22].

The pharmacokinetic data presented graphically originated from healthy volunteers, which may be a limitation of the present study as the responses may differ in critically ill patients, especially in relation to creatinine clearance and volume of distribution. However, this bias can be decreased because for time-dependent pharmacodynamic agents, such as MEM and FOF evaluated here, the pharmacokinetic characteristics of the drugs in healthy volunteers (with good renal function and rapid antimicrobial elimination) can be offset by the greater volume of distribution and reduced elimination observed in critically ill patients, which could result in minor pharmacodynamic changes for these antimicrobials [31].

An inherent limitation of in vitro studies is their often limited clinical applicability [10]. However, these results can suggest the potential of these combinations for clinical use, directing future studies of in vitro dynamic synergy and in vivo studies. A recent in vivo study identified a direct correlation with an in vitro evaluation against a KPC-2-producing *K. pneumoniae* isolate, mainly for a triple combination of antimicrobials [9].

Considering the lack of options for the treatment of XDR *A. baumannii* and the urgent need for potential therapeutic options, the data obtained here indicate the possibility of treating these isolates, which are resistant to PMB, with a combination of drugs. The data also suggest that irrespective of the definition of synergism as FICI ≤ 0.5, the MICs of these antimicrobials for the isolates are reduced, allowing them to achieve adequate PTAs (≥0.9), as assessed through Monte Carlo simulation, meaning that the treatment is reliable.

The encouraging results of this study demonstrate that the use of PMB, MEM and FOF in combination may be an important alternative for the treatment of infection by XDR *A. baumannii*. Nevertheless, in vivo clinical studies are essential to evaluate the true role of these combinations, including intravenous FOF, in antimicrobial therapy for MDR-GNB.

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