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In Vitro Activity of Polymyxin B plus Imipenem, Meropenem, or Tigecycline against KPC-2-Producing *Enterobacteriaceae* with High MICs for These Antimicrobials

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We evaluated the *in vitro* activity of polymyxin B plus imipenem, meropenem, or tigecycline against six KPC-2-producing *Enterobacteriaceae* strains with high MICs for these antimicrobial agents. Polymyxin B with carbapenems, especially meropenem, were the most active combinations for *Klebsiella pneumoniae* and *Enterobacter cloacae* regardless of the polymyxin B concentration used in the time-kill assay. This combination was also synergistic against two *Serratia marcescens* strains that are intrinsically resistant to polymyxins. Polymyxin B and tigecycline also presented synergistic activity in most experiments.

KPC-2-producing members of the family *Enterobacteriaceae* have emerged as a major cause of hospital-acquired infections worldwide, and antimicrobial therapy is frequently restricted to polymyxins (1). Some preclinical and clinical studies have suggested that colistin or polymyxin B (PMB) in combination with another antimicrobial agent, particularly with carbapenems against isolates presenting low-level resistance to these agents, may be superior to monotherapy (2, 3). However, the benefit of combining a carbapenem for isolates with high-level resistance to these antibiotics is less clear (2, 3).

Because of pharmacokinetic characteristics of polymyxins, treatment of KPC-2-producing *Enterobacteriaceae* is even more challenging when MICs for these antibiotics are elevated (2 mg/liter) but still within the susceptibility range, or higher, characterizing resistance (4). Finally, there has been some debate regarding the efficacy of tigecycline (TGC), another agent commonly used in combination with polymyxins, using standard dose regimes, when the MIC is near (1 mg/liter) the FDA breakpoint (2 mg/liter) or above (2, 5).

In the presence of these "adverse" profiles, bacterial clearance could mostly rely on the efficacy of antimicrobial combinations. Thus, to provide some support for therapeutic decisions, we evaluated the *in vitro* activity of PMB in combination with imipenem (IPM), meropenem (MEM), and TGC against KPC-2-producing *Enterobacteriaceae* isolates with high MICs for PMB, carbapenems, and TGC.

Six strains recovered from clinical specimens (urine, blood, and respiratory secretions), including two *Klebsiella pneumoniae* strains, two *Enterobacter cloacae* strains, and two *Serratia marcescens* strains, previously characterized as KPC-2-producing isolates by gene sequencing and belonging to unrelated clones by pulsed-field gel electrophoresis (PFGE) (6), were selected. Isolates with MICs of ≤2 mg/liter determined by broth microdilution were considered susceptible to polymyxin B (7).

A time-kill assay (TKA) was performed by inoculating 5×10^6 CFU/ml of the organisms into 10 ml of fresh cation-adjusted Mueller-Hinton broth, and the results are displayed in Table 1.

We evaluated nine combinations with PMB against six genetically unrelated strains of KPC-2-producing *Enterobacteriaceae* with "unfavorable" antibiotic susceptibility profile, i.e., decreased susceptibility or resistance to PMB and/or TGC and high-level resistance to carbapenems. PMB with carbapenems were the most active combination for *K. pneumoniae* and *E. cloacae* isolates, regardless of the PMB concentration used in the TKA, demonstrating a bactericidal effect against all isolates. Combinations with TGC also showed bactericidal effect in some TKAs; nonetheless, the reduction in colony count was lower than those of carbapenems, and no synergism was observed in the two *E. cloacae* strains with a PMB concentration of 0.5 mg/liter and in one strain with a concentration of 1.0 mg/liter, regardless of the TGC MIC of 1 mg/liter for both strains.

Regarding the *S. marcescens* strains that were intrinsically resistant to polymyxins, PMB at any concentration in combination with MEM showed the most promising results, since these combinations were synergistic and bactericidal for both strains. The combination with IPM also demonstrated interesting results, since synergism was observed in all experiments, but bactericidal activity was noted in only one strain against PMB concentrations of 1 and 2 mg/liter. Although the combinations with TGC were less active, synergistic activity was achieved in one strain with all PMB concentrations, including a bactericidal effect at concentrations of 2 mg/liter, but it was synergistic only with a PMB concentration of 2 mg/liter in the second strain. Noteworthy, the TGC MIC of this second strain was 4 mg/liter. Interestingly, using checkerboard microdilution, antagonism between colistin and

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TABLE 1 Activity of polymyxin B in different concentrations in combination with imipenem, meropenem, or tigecycline in time-kill assays

Antimicrobials and concns (mg/liter)[a]	Log ΔCFU[b] in strain[c]:					
	EC1	EC2	KP1	KP2	SM1	SM2
PMB+IPM						
PMB (0.5) + IPM (4)	**−6.47**	**−4.74**	**−5.24**	**−4.70**	**−2.88**	**−2.51**
PMB (1) + IPM (4)	**−9.80**	**−5.64**	**−9.17**	**−4.60**	**−3.26**	**−2.62**
PMB (2) + IPM (4)	**−9.62**	**−4.54**	**−7.81**	**−4.20**	**−3.39**	**−2.65**
PMB+MEM						
PMB (0.5) + MEM (4)	**−11.60**	**−4.24**	**−3.47**	**−5.17**	**−4.98**	**−3.55**
PMB (1) + MEM (4)	**−10.14**	**−3.78**	**−8.38**	**−5.08**	**−5.78**	**−3.58**
PMB (2) + MEM (4)	**−3.07**	**−5.62**	**−8.14**	**−4.87**	**−7.93**	**−3.77**
PMB+TGC						
PMB (0.5) + TGC (1)	−1.20	−0.94	**−5.78**	**−4.69**	**−2.90**	−1.04
PMB (1) + TGC (1)	−1.32	**−2.20**	**−6.48**	**−4.60**	**−2.97**	−1.18
PMB (2) + TGC (1)	**−2.71**	**−4.54**	**−6.57**	**−4.20**	**−3.07**	**−2.51**

[a] Polymyxin B (PMB) in different concentrations in combination with imipenem (IPM), meropenem (MEM), or tigecycline (TGC).

[b] Log ΔCFU was calculated as follows: final inoculum of the combined drugs − final inoculum of the most active drug in combination ($\log_{10}$ CFU/ml). Synergy highlighted in bold type was defined as a decrease in colony count of ≥2 $\log_{10}$ units after 24 h by the combination compared with the most active single agent. Bactericidal activity was defined as a decrease in colony count of ≥3 $\log_{10}$ units after 24 h.

[c] The two *E. cloacae* strains are EC1 and EC2. The two *K. pneumoniae* strains are KP1 and KP2. The two *S. marcescens* strains are SM1 and SM2. The MICs (in milligrams per liter) of PMB, IPM, MEM, and TGC, respectively, of each strain follow (MICs highlighted in bold indicate resistance): for strain EC1, 2, **64**, **128**, and 1; for EC2, 2, **64**, **32**, and 1; for KP1, 2, **32**, **32**, and **4**; for KP2, 2, **8**, **32**, and 1; for SM1, **64**, **128**, **32**, and 1; and for SM2, **>64**, **256**, **64**, and **4**.

TGC in carbapenem-resistant *S. marcescens* isolates has been demonstrated (8).This fact highlights the strain-to-strain variations in the response to antimicrobial combinations.

Synergistic activity between colistin and carbapenems has been found in other studies either by checkerboard or by time-kill assay, although *Acinetobacter baumannii* and *Pseudomonas aeruginosa* have been the most common bacteria evaluated, with less *in vitro* data for *Enterobacteriaceae* (9). Furthermore, most studies assessed isolates with low MICs for polymyxins (9). Despite the relatively low number of isolates, our study evaluated only "difficult to treat" KPC-2-producing strains, owing to the high MICs for the antibiotics assessed. Additionally, although not providing the pharmacokinetic/pharmacodynamic evaluation of the antibiotic combinations, as expected for TKAs, our experiments provided more robust evidence of synergism because the fixed concentrations of carbapenems used (4 mg/liter, the former CLSI breakpoint for both agents [10]; after publication of the 2010 CLSI guidelines, the breakpoint was 1 mg/liter [11]) were considerably lower than the MICs of the strains tested. Even though the TGC concentrations used in TKAs were lower than the MIC in only two strains, the finding of synergism and even bactericidal effect against *S. marcescens* strains should be highlighted, since carbapenem-resistant *S. marcescens* isolates are extremely difficult to treat and the polymyxin B-tigecycline (PMB+TGC) combination is frequently neglected owing to the intrinsic resistance of the species to polymyxins. Finally, it must be acknowledged that *in vitro* synergistic activity may not predict *in vivo* synergism and does not predict clinical outcomes.

In summary, the combination of PMB with both IPM and MEM were very active against KPC-2-producing *K. pneumoniae*, *E. cloacae*, and *S. marcescens* strains with high-level resistance to carbapenems, and MEM seemed to be superior to IPM for *S. marcescens*. PMB combinations with TGC were less active against some strains but demonstrated synergistic activity even against *S. marcescens* strains. Further *in vitro* investigations with these combinations with a larger number of isolates and pharmacokinetic/pharmacodynamic studies assessing the potential synergism of these combinations are warranted, since strains with this "unfavorable" phenotype are emerging and challenge current antimicrobial therapy.

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