

Modeling Meropenem Treatment, Alone and in Combination with Daptomycin, for KPC-Producing *Klebsiella pneumoniae* Strains with Unusually Low Carbapenem MICs

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***Klebsiella pneumoniae* strains producing *K. pneumoniae* carbapenemase (KPC) cause serious infections in debilitated and immunocompromised patients and are associated with prolonged hospital stays and increased mortality rates. Daptomycin is a lipopeptide used against *Staphylococcus aureus* infection and considered inactive against Gram-negative bacteria. We investigated the effectiveness of a daptomycin-meropenem combination by synergy kill curve and a pharmacokinetic/pharmacodynamic model. The combination may represent a novel therapeutic strategy against infections caused by KPC-producing *K. pneumoniae* strains.**

The management of infections due to *Klebsiella pneumoniae* has been complicated by emerging antimicrobial resistance, with 20% to 80% of *K. pneumoniae* strains resistant to first-line antibiotics, including cephalosporins, fluoroquinolones, and carbapenems (1). Dissemination of carbapenem-resistant *Enterobacteriaceae* can be largely attributed to organisms producing the *K. pneumoniae* carbapenemase (KPC), a β -lactamase enzyme that confers resistance to carbapenem antimicrobials (1). According to the recent WHO Report, KPC-producing *K. pneumoniae* has spread worldwide, representing a major cause of hospital-acquired infections, including pneumonia, bloodstream infections, and infections in neonatal and intensive care units (2). These multidrug-resistant organisms cause infections associated with mortality ranging between 23% and 75% due to a lack of active antimicrobial agents and underlying comorbidities, as the therapeutic options for the treatment of KPC-producing *K. pneumoniae* infections are limited (3). The standard therapy for *K. pneumoniae* infection is monotherapy with tigecycline or colistin (4, 5). However, these options are complicated by drug toxicity and failure rates comparable to those observed in patients who receive inappropriate therapy (4, 5). For this reason, these and other agents, such as fosfomycin, selected aminoglycosides, rifampin, and carbapenems (meropenem [MEM]), have been given as part of combination treatments, with various degrees of success against KPC-producing *K. pneumoniae* (3, 6). In addition to the lack of defined therapies, some KPC-producing *K. pneumoniae* isolates, regardless of carbapenemase production, display carbapenem suscepti-

bility, according to CLSI or EUCAST standards (7). Recent exploration of antimicrobial combinations has shown that antibiotics with limited or no activity can result in antimicrobial enhancement of primary agents, even in a setting where resistance to primary agents is present. Previously, we showed that daptomycin (DAP) constitutes an option for treatment of refractory methicillin-resistant *Staphylococcus aureus* (MRSA) infection when administered in combination with β -lactams (8). DAP, a cyclic anionic lipopeptide, is considered inactive against Gram-negative bacteria, in part because of the large size of the molecule, which impairs penetration of the outer membrane (9). We evaluated the effectiveness of combining DAP with MEM against KPC-producing *K. pneumoniae*. Three KPC-2-producing *K. pneumoniae* clinical isolates obtained from the repository of the Laboratory of

Received 19 January 2016 Returned for modification 4 March 2016

Accepted 19 May 2016

Accepted manuscript posted online 23 May 2016

Citation Gagetti P, Pasteran F, Martinez MP, Fatouraei M, Gu J, Fernandez R, Paz L, Rose WE, Corso A, Rosato AE. 2016. Modeling meropenem treatment, alone and in combination with daptomycin, for KPC-producing *Klebsiella pneumoniae* strains with unusually low carbapenem MICs. *Antimicrob Agents Chemother* 60:5047–5050. doi:10.1128/AAC.00168-16.

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TABLE 1 Strains used in the study and MICs for MEM and DAP

Strain	Genotype-acquired β -lactamases	Multilocus sequence type	MIC ($\mu\text{g/ml}$) ^a						
			MEM		IPM		EPM		DAP
			BMD	Etest	BMD	Etest	BMD	Etest	Etest
Kp1 (M13403)	KPC-2	ST2008	0.25	0.38	0.25	0.38	0.25	0.12	≥ 256
Kp2 (M15075)	KPC-2, CTX-M-2	ST147	0.25	0.19	0.5	0.38	0.25	0.19	≥ 256
Kp3 (M9885)	KPC-2	ST258	32	16	32	16	>32	>32	≥ 256
Kp4 (ATCC 700603)	SHV-18	NA ^b	0.25	0.25	0.12	0.12			≥ 256

^a IPM, imipenem; EPM, ertapenem; BMD, broth microdilution.

^b NA, not assigned.

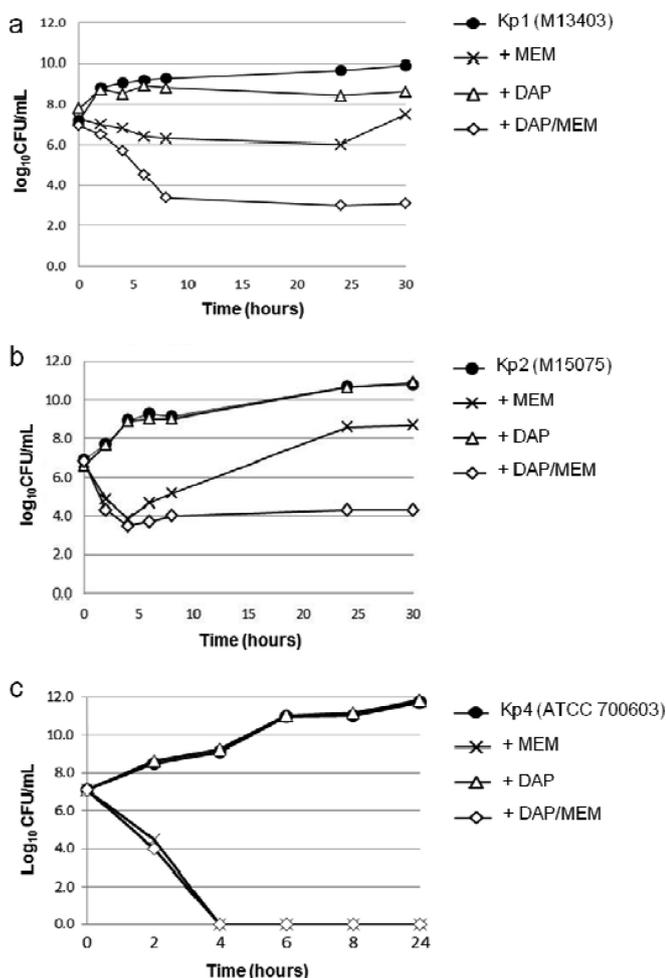


FIG 1 Analysis of MEM, DAP, and DAP-MEM antibacterial efficacy against Kp1 (M13403) (a), Kp2 (M15075) (b), and Kp4 (ATCC 700603) (c). Time-kill analyses were performed with a 10^6 CFU/ml inoculum at 0, 2, 4, 8, 24, and 30 h and the specified concentrations of antibiotics: DAP, 10 μ g/ml; MEM, 6 μ g/ml. A minimum of three independent experiments were performed.

Reference at ANLIS (Buenos Aires, Argentina) were included: Kp1 (M13403) and Kp2 (M15075), both susceptible to carbapenems, and Kp3 (M9885), a carbapenem-resistant strain (Table 1). Strains Kp4 (ATCC 700603) and Kp5 (ATCC 13833) were used as carbapenemase nonproducer controls.

Susceptibilities to MEM and DAP were determined by Etest (bioMérieux) and in-house microdilution according to manufacturer and CLSI guidelines, respectively. *bla*_{KPC} genes were characterized by PCR and DNA sequencing (7). MLST was performed according to Pasteur Institute recommendations (www.pasteur.fr) (Table 1). The *in vitro* effectiveness of the combination DAP-MEM against strains Kp1 (Fig. 1a) and Kp2 (Fig. 1b) was analyzed by time-kill curves performed in Mueller-Hinton (MH) broth supplemented with 50 μ g/ml of Ca^{2+} for DAP. Subcultures were performed at 0, 2, 4, 6, 8, 24, and 30 h (initial inoculum, 1×10^7 CFU/ml). At concentrations of 6 μ g/ml MEM and 10 μ g/ml DAP tested alone, DAP did not display any effect on growth, in agreement with the intrinsic resistance to this agent, while MEM, despite having tested as susceptible (isolates Kp1 and Kp2), resulted in regrowth of cells after 24 h without significant bactericidal effects (Fig. 1). MEM alone was active against the carbapenemase

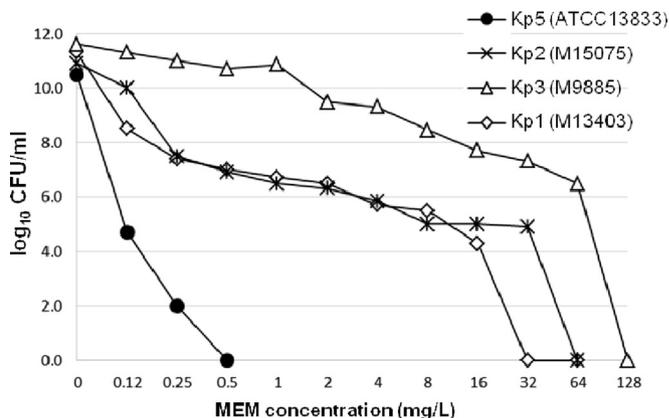


FIG 2 Population analysis profile (PAP) of MEM resistance of KPC-producing *K. pneumoniae* strains. Phenotypic expression of MEM resistance was measured from overnight cultures from Kp1 (M13403), Kp2 (M15075), Kp3 (M9885), and Kp5 (ATCC 13833) strains plated at various dilutions on MH plates containing a series of concentrations of MEM and incubated at 37°C for 48 h, after which the bacterial colonies were counted. The results of a representative experiment are shown. Two other experiments gave similar results.

nonproducer Kp4 strain, displaying a decrease of 7 \log_{10} CFU/ml at 4 h (Fig. 1c). In contrast, MEM alone was not active against the KPC-2-producing Kp1 and Kp2 strains, despite having similar MEM MICs. The combination DAP-MEM was highly synergistic, as demonstrated by cell killing of $\geq 5 \log_{10}$ CFU/ml at 24 h compared with that achieved with either agent alone or the initial inoculum (Fig. 1).

The observation that these strains manifested recurrent growth even during exposures above their MEM MIC values led us to speculate the existence of a heteroresistant phenotype. We investigated the existence of subpopulations of resistant cells expressing different levels of resistance to MEM by population analysis profile (PAP) (10). PAPs were performed by spreading $\sim 10^8$ bacterial CFU on MH agar plates with MEM in serial dilutions (concentrations ranging from 0.25 to 256 μ g/ml) and incubating for 48 h (10). The frequency of heteroresistant subpopulations at the highest drug concentration was calculated by dividing the number of colonies grown on antibiotic-containing plates by the colony counts from the same bacterial inoculum plated onto antibiotic-free plates. MIC stability was evaluated after seven daily subcultures in antibiotic-free medium. As shown in Fig. 2, all three KPC-producing *K. pneumoniae* strains (Kp1, Kp2, and Kp3) were able to grow in concentrations of 16 to 64 μ g/ml with a serially diluted inoculum (10^1 to 10^8). Consistent MIC increases from parent to derivative variants occurred, while no effect was observed in control strain Kp5 (Table 2). These observations explain the survival of Kp1, Kp2, and Kp3 at bactericidal concentrations of MEM despite displaying a susceptible phenotype. This corroborates a recent study by Nodari et al. (11), which showed that heteroresis-

TABLE 2 Frequency of KPC-producing *K. pneumoniae* heteroresistance to MEM detected by population analysis profile

Strain	MEM MIC (μ g/ml)	MEM concn of highest growth (μ g/ml)	Heteroresistance frequency
Kp1 (M13403)	0.25	32	1×10^{-7}
Kp2 (M15075)	0.25	64	3.75×10^{-7}
Kp3 (M9885)	16	128	7.50×10^{-6}
Kp5 (ATCC 13833)	0.25	0.5	Nondetectable

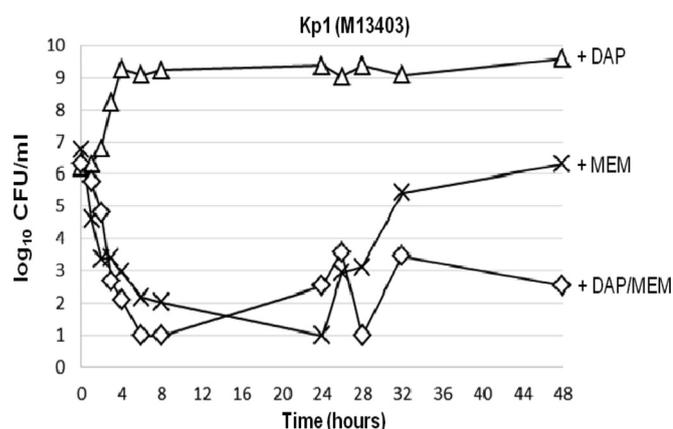


FIG 3 Activity of single and combination antibiotic simulations in the PK/PD model against Kp1 (M13403). MEM (1,000 mg every 8 h) and DAP (10 mg/kg every 24 h) were modeled alone and in combination over 48 h. Two independent replicates for each regimen were performed.

tance in KPC-producing *K. pneumoniae* strains is directly associated with the presence of carbapenemase, a phenomenon that was not observed in the non-KPC group of strains analyzed.

We previously demonstrated that the pharmacokinetic/pharmacodynamic (PK/PD) model is a valuable tool with which to evaluate the effectiveness of novel antimicrobial combinations (12–14) by simulating one-compartment antibiotic exposures of single and combined antibiotic therapy (12, 15). Experiments were performed in duplicate over 48 h using overnight cultures of Kp1 (M13403) adjusted to 0.5 McFarland and diluted to obtain a starting inoculum of $\sim 10^6$ CFU/ml. The following antibiotic regimens were evaluated: MEM at 1,000 mg every 8 h (targeted maximum free drug concentration [fC_{max}], 49 $\mu\text{g/ml}$; half-life, 1 h) and DAP at 10 mg/kg every 24 h (fC_{max} , 11.3 $\mu\text{g/ml}$; half-life, 8 h) (12). Modeling of antibiotics in combination with two different elimination rates was performed according to the methods described by Blaser (16), where supplemental doses of the more slowly cleared antibiotic were administered to account for drug loss from the more rapid flow rate of the combination. Samples were retrieved at prespecified time points from the model for bacterial quantification. Antibiotic activity (MEM versus DAP-MEM) was evaluated by the extent and rate of bacterial killing over the duration of the simulation by *t* test. As shown in Fig. 3, MEM alone was highly active in the first 3 doses up to 24 h but failed to suppress growth thereafter. Consistent with this regrowth, Kp1 cultured from the 48-h time point with MEM alone resulted in a 64-fold-less-susceptible organism prior to the experiment (MEM MIC, 32 versus 0.5 $\mu\text{g/ml}$), in concordance with the results of the PAP assay. DAP alone had no antimicrobial activity. However, the DAP-MEM combination resulted in a faster time to kill to the limit of detection (6 h with combination versus 24 h with MEM alone) and a significantly lower bacterial burden at the end of treatment (2.5 ± 0.4 versus 6.3 ± 0.2 log₁₀ CFU/ml, respectively; $P = 0.007$) (Fig. 3). Importantly, the DAP-MEM regimen prevented the development of MEM resistance (MIC, 0.5 $\mu\text{g/ml}$ at 48 h).

Our results showed the following. First, MEM alone was inactive against KPC-producing *K. pneumoniae* strains displaying MEM MICs considered susceptible by both CLSI and EUCAST guidelines, indicating that MIC values may not be sufficient to predict a successful treatment outcome, as revealed by recent sur-

veys showing that only 14% to 20% of the carbapenemase-producing isolates were susceptible to MEM in a large-scale surveillance program (17). Second, DAP-MEM may represent a novel therapeutic strategy against infections caused by KPC-producing *K. pneumoniae* isolates. Third, although a limited number of strains were tested, the heteroresistance phenotype in carbapenem-susceptible KPC producers is a new finding in *K. pneumoniae*. Finally, the fact that DAP may extend its usefulness to Gram-negative infections in combination with MEM, and perhaps other carbapenems, makes this strategy attractive as a broad-spectrum option. The molecular bases and mechanisms involved in DAP-MEM interactions against KPC-producing *K. pneumoniae* strains remain unknown, and further studies are in progress.

ACKNOWLEDGMENTS

W.E.R. and A.E.R. received grant support from Merck unrelated to this work.

We have no relevant financial interests to report.

FUNDING INFORMATION

This work, including the efforts of A. E. Rosato, was funded by HHS | National Institutes of Health (NIH) (1R56AI102503-0A1).

Work at ANLIS was supported by the regular federal budget of the Ministry of Health of Argentina.

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