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Effect of the β-Lactamase Inhibitor Vaborbactam Combined with Meropenem against Serine Carbapenemase-Producing *Enterobacteriaceae*

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***Klebsiella pneumoniae* carbapenemase (KPC)-producing isolates have become increasingly prevalent worldwide, and these organisms are often multidrug resistant, limiting the therapeutic options available for treating infections. We evaluated the activity of meropenem combined with the serine β-lactamase inhibitor vaborbactam (formerly RPX7009) against 315 serine carbapenemase-producing *Enterobacteriaceae* (CPE) isolates by use of checkerboard-designed panels to assess the optimal inhibitor concentration (range tested, 0.5 to 32 μg/ml). Overall, meropenem alone (MIC_{50} and MIC_{90}, 16 and >64 μg/ml, respectively) inhibited only 2.2% of the isolates at ≤1 μg/ml (the CLSI susceptibility breakpoint) and 7.3% of the isolates at ≤2 μg/ml (the EUCAST breakpoint). Vaborbactam restored meropenem activity for 72.7 to 98.1% of CPE isolates at ≤2 μg/ml, and maximum potentiation was achieved with fixed concentrations of ≥8 μg/ml of the inhibitor (≥96.5% of isolates were inhibited at ≤2 μg/ml of meropenem-vaborbactam). Meropenem-vaborbactam with a fixed concentration of 8 μg/ml of the inhibitor (MIC_{50}, ≤0.06 μg/ml for all organisms) inhibited 93.7% of the CPE isolates displaying elevated meropenem MICs at ≤1 μg/ml. Meropenem-vaborbactam MICs were elevated for isolates producing metallo-β-lactamases (MIC, 16 to >64 μg/ml) or displaying decreased expression of OmpK37 and/or elevated expression of the AcrAB-TolC efflux system (MIC, 16 μg/ml). Vaborbactam showed no antibacterial activity alone (all MICs, >64 μg/ml). Meropenem-vaborbactam appears to be a good candidate for further development and it could increase the options for treatment of serious infections caused by carbapenemase-producing pathogens.**

Carbapenem-resistant *Enterobacteriaceae* (CRE) isolates have been detected worldwide, and their increasing prevalence is mainly due to the dissemination of isolates producing carbapenemases, such as *Klebsiella pneumoniae* carbapenemase (KPC) and metallo-β-lactamases (largely NDM but also IMP and VIM) (1). Infections caused by carbapenemase-producing *Enterobacteriaceae* (CPE) became a serious cause of concern among infectious diseases and clinical microbiology professionals worldwide because these infections are difficult to manage (2–4). CPE isolates are resistant to all or nearly all β-lactam agents, and these organisms may also be resistant to other antimicrobial classes, limiting the therapeutic options available for the treatment of CPE infections (3, 4).

CPE isolates may be susceptible only to tigecycline and/or colistin, which have been widely used; however, there are limitations to the usage of both agents (4). Low plasma peak concentrations are achieved with tigecycline, and this antimicrobial agent is not recommended for the treatment of bloodstream infections. Furthermore, colistin use can be associated with nephrotoxicity and occasionally neurotoxicity. CPE isolates can display colistin or tigecycline resistance, a problem increasingly reported worldwide (3).

The use of β-lactamase inhibitors combined with a potent β-lactam agent has been a successful strategy for overcoming β-lactamase-mediated resistances (5); however, older inhibitors such as tazobactam, sulbactam, and clavulanate are generally not effective against isolates producing various contemporary β-lactamases, including KPC serine carbapenemases (5, 6). The increasing prevalence of multidrug-resistant (MDR) organisms producing KPC enzymes and other β-lactamases that are poorly inhibited by current inhibitors suggests the need for new treatment alternatives, including broader-spectrum β-lactamase inhibitors.

Vaborbactam (formerly RPX7009) is a cyclic boronic acid pharmacophore β-lactamase inhibitor that displays potent inhibition of KPC enzymes and other Ambler class A and C enzymes (7). This inhibitor is in phase 3 clinical development in combination with meropenem. In this study, we evaluated the use of this combination against 315 *Enterobacteriaceae* strains producing serine carbapenemases collected worldwide, including 308 KPC-producing strains.

MATERIALS AND METHODS

Bacterial isolates. A total of 315 *Enterobacteriaceae* clinical isolates producing serine carbapenemases were selected and tested. This selection included isolates from multiple countries and years to provide genetic diversity, and only one strain from a clonal outbreak was included. The isolates were cultured during 2000 to 2013, mainly during 2013 (150 isolates), 2010 (48 isolates), and 2007 (45 isolates), and included 12 *Citro-*

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bacter freundii isolates, 4 *Enterobacter aerogenes* isolates, 39 *Enterobacter cloacae* isolates, 1 *Enterobacter gergoviae* isolate, 21 *Escherichia coli* isolates, 14 *Klebsiella oxytoca* isolates, 208 *Klebsiella pneumoniae* isolates, 1 *Raoultella ornithinolytica* isolate, 2 *Raoultella planticola* isolates, and 13 *Serratia marcescens* isolates.

The isolates carried genes encoding KPC enzymes (308 isolates in total; 145 for KPC-2, 117 for KPC-3, 1 for KPC-4, 1 for KPC-20, and 44 for KPC-like), SME-2 and -3 (6 isolates), and NMC-A (1 isolate). Genes encoding serine carbapenemases and other β-lactamases were previously identified using various PCR and sequencing strategies or using a microarray-based assay (8, 9). Not all isolates were screened for additional β-lactamases, and 75 isolates that were screened are listed in Table S1 in the supplemental material.

Checkerboard susceptibility testing. MICs were determined using the Clinical and Laboratory Standards Institute (CLSI) broth microdilution methodology described in the M07-A10 guideline (10). Meropenem (concentration range, 0.06 to 64 μg/ml) and the β-lactamase inhibitor vaborbactam (concentration range, 0.5 to 32 μg/ml) were tested in a checkerboard configuration (11) using cation-adjusted Mueller-Hinton broth. The quality control (QC) ranges were those published in CLSI M100-S26 (12); the tested QC strains included *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and KPC-producing *K. pneumoniae* BAA-1705. All QC results were within published ranges (12). The categorical interpretations for all antimicrobials were those found in M100-S26 (12) and on the EUCAST website (http://www.eucast.org/clinical_breakpoints/).

Expression analysis of efflux pumps and OMPs. The expression of *acrA* (AcrAB-TolC), *ompK35*, *ompK36*, and *ompK37* was determined by quantitative real-time PCR (qRT-PCR) using DNA-free RNA preparations for three *K. pneumoniae* isolates displaying MICs of 16 μg/ml for meropenem-vaborbactam with a fixed concentration of 8 μg/ml of the inhibitor. Total RNA was extracted from mid-log-phase bacterial cultures (cell density at an optical density at 600 nm [OD_{600}], 0.3 to 0.5) using RNAprotect reagent and the RNeasy minikit (Qiagen, Hilden, Germany) in the QIAcube workstation (Qiagen), and residual DNA was eliminated with RNase-free DNase (Promega, Fitchburg, WI, USA). Quantification of mRNA and sample quality were assessed using the RNA 6000 Pico kit on the Agilent 2100 bioanalyzer (Agilent Technologies, Santa Clara, CA, USA) according to the manufacturer's instructions. Only preparations with an RNA integrity number (RIN) of ≥7 that showed no visual degradation were used for experiments. Relative quantification of target genes was performed in triplicate by normalization to an endogenous reference gene (*gyrA*) on the StepOne Plus instrument (Life Technologies, Carlsbad, CA, USA), using custom-designed primers showing >93.0% efficiency. Transcription levels were compared to those of *K. pneumoniae* ATCC 13383 (13). Transcription levels were considered significantly different if a ≥10-fold difference (higher for *acrA* and lower for the outer membrane proteins [OMPs]) compared with the level for the control isolates was noted.

Molecular typing. Seven *K. pneumoniae* isolates displaying MICs of ≥16 μg/ml for meropenem-vaborbactam were epidemiologically typed by pulsed-field gel electrophoresis (PFGE). Genomic DNA prepared in agarose blocks, digested with SpeI (New England BioLabs, Beverly, MA, USA), was resolved in the CHEF-DR II apparatus (Bio-Rad, Richmond, CA, USA). Results were analyzed by GelCompar II software (Applied Math, Kortrijk, Belgium). Percent similarities were identified on a dendrogram derived from the unweighted pair group method using arithmetic averages and based on Dice coefficients. Band position tolerance and optimization were set at 1.2% and 0.5%, respectively.

RESULTS

Effect of different concentrations of vaborbactam on meropenem MIC results. Meropenem alone (MIC_{50} and MIC_{90}, 16 and >64 μg/ml, respectively) (Table 1) had very limited activity against isolates carrying genes encoding serine carbapenemases.

TABLE 1 MIC distribution for meropenem alone and combined with vaborbactam at various concentrations against 315 *Enterobacteriaceae* strains producing serine carbapenemases

Antimicrobial agent(s)	No. (cumulative %) of strains with indicated MIC (μg/ml)[a]									MIC (μg/ml)	
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64	50%	90%
Meropenem alone	—	—	—	1 (0.3)	6 (2.2)	16 (7.3)	43 (21.0)	53 (37.8)	82 (63.8)	36 (75.2)	24 (82.9)	54 (100.0)	16	>64
Meropenem + vaborbactam at fixed concn (μg/ml) of:														
0.5	60 (19.0)	51 (35.2)	46 (49.8)	31 (59.7)	25 (67.6)	16 (72.7)	9 (75.6)	8 (78.1)	6 (80.0)	17 (85.4)	28 (94.3)	18 (100.0)	0.5	64
1	124 (39.4)	47 (54.3)	36 (65.7)	16 (70.8)	10 (74.0)	10 (77.1)	4 (78.4)	2 (79.0)	19 (85.1)	17 (90.5)	19 (96.5)	11 (100.0)	0.12	32
2	182 (57.8)	30 (67.3)	16 (72.4)	11 (75.9)	7 (78.1)	5 (79.7)	14 (84.1)	15 (88.9)	15 (93.7)	12 (97.5)	3 (98.4)	5 (100.0)	≤0.06	16
4	207 (65.7)	22 (72.7)	13 (76.8)	9 (79.7)	18 (85.4)	15 (90.2)	15 (94.9)	5 (96.5)	7 (98.7)	1 (99.0)	1 (99.4)	2 (100.0)	≤0.06	2
8	224 (71.1)	20 (77.5)	18 (83.2)	21 (89.8)	12 (93.7)	9 (96.5)	4 (97.8)	0 (97.8)	6 (99.7)	0 (99.7)	0 (99.7)	1 (100.0)	≤0.06	1
16	233 (74.0)	21 (80.6)	29 (89.8)	17 (95.2)	5 (96.8)	4 (98.1)	0 (98.1)	1 (98.4)	4 (99.7)	0 (99.7)	0 (99.7)	1 (100.0)	≤0.06	0.5
32	236 (74.9)	30 (84.4)	26 (92.7)	13 (96.8)	1 (97.1)	3 (98.1)	1 (98.4)	0 (98.4)	4 (99.7)	0 (99.7)	0 (99.7)	1 (100.0)	≤0.06	0.25
Vaborbactam alone	—	—	—	—	—	—	—	—	—	—	—	315 (100.0)	>64	>64

[a] —, dilution not tested or no isolates displaying MIC values at this concentration.

This carbapenem inhibited only 2.2% of the CPE strains at the current CLSI susceptibility breakpoint (≤1 μg/ml) and 7.3% of the isolates at the EUCAST susceptibility breakpoint (≤2 μg/ml) (Table 1). By the CLSI (MIC, ≥4 μg/ml) and EUCAST (MIC, ≥8 μg/ml) resistance interpretative category criteria, 92.7 and 79.0% of the isolates, respectively, were resistant to meropenem (Table 1).

When meropenem was tested in combination with vaborbactam at concentrations ranging from 0.5 to 32 μg/ml, the meropenem MIC_{50} and MIC_{90} values ranged from 0.5 to ≤0.06 μg/ml and from 64 to 0.25 μg/ml, respectively (Table 1). Totals of 67.6 to 97.1% of the isolates were inhibited at ≤1 μg/ml of meropenem (the CLSI susceptibility breakpoint for meropenem tested alone, used for comparison purposes) in the presence of 0.5 to 32 μg/ml of vaborbactam (Table 1), and 72.7 to 98.1% of the isolates were inhibited at ≤2 μg/ml. Additionally, 78.1 to 98.4% of the isolates were inhibited at ≤8 μg/ml of meropenem combined with 0.5 to 32 μg/ml of vaborbactam (Table 1).

Higher concentrations of vaborbactam resulted in the inhibition of a greater number of tested strains; meropenem combined with vaborbactam concentrations of 4, 8, 16 and 32 μg/ml inhibited 90.2, 96.5, 98.1, and 98.1% of the isolates, respectively, at ≤2 μg/ml of meropenem (Table 1). The activity of meropenem was at least 64-fold greater when this carbapenem was combined with a fixed vaborbactam concentration of 8 μg/ml (MIC_{50} and MIC_{90}, ≤0.06 and 1 μg/ml, respectively), and this concentration of inhibitor was considered optimal for further testing. Vaborbactam alone displayed no activity against these isolates, and all MIC results were >64 μg/ml (Table 1).

Activity of meropenem-vaborbactam with a fixed concentration of 8 μg/ml of the β-lactamase inhibitor. Against KPC-producing *K. pneumoniae* ($n = 208$) (Table 2), 93.3% of the isolates were inhibited at ≤1 μg/ml and 96.6% were inhibited at ≤4 and ≤8 μg/ml of meropenem-vaborbactam with a fixed concentration of 8 μg/ml of the inhibitor. Similar to the result for the overall collection of CPE (315 isolates), meropenem-vaborbactam (MIC_{50} and MIC_{90}, ≤0.06 and 1 μg/ml, respectively) was at least 64-fold more active than meropenem alone (MIC_{50} and MIC_{90}, 16 and >64 μg/ml, respectively) against KPC-producing *K. pneumoniae* (Table 2).

All *E. cloacae* isolates ($n = 39$), including KPC-2, KPC-3, NMC-A, and KPC-4 producers, were inhibited at ≤4 μg/ml by meropenem-vaborbactam with a fixed concentration of 8 μg/ml of the inhibitor, and 92.3 and 97.4% of these isolates would be categorized as susceptible by the CLSI and EUCAST breakpoints, respectively, for meropenem tested alone (Table 2).

Twelve of 14 KPC-producing *K. oxytoca* isolates were inhibited by ≤0.06 μg/ml of meropenem-vaborbactam with a fixed concentration of 8 μg/ml of the inhibitor, and all these isolates were inhibited by ≤2 μg/ml of this carbapenem-inhibitor combination (Table 2). KPC-producing *E. coli* ($n = 21$) and *C. freundii* ($n = 12$) isolates were inhibited by ≤0.06 or 0.25 μg/ml of meropenem-vaborbactam with a fixed concentration of 8 μg/ml of the inhibitor (Table 2). Additionally, SME- and KPC-producing *S. marcescens* isolates were inhibited by ≤1 μg/ml of meropenem-vaborbactam with a fixed concentration of 8 μg/ml of the inhibitor (data not shown).

Among 75 isolates carrying the genes encoding KPC and at least one additional known broad-spectrum β-lactamase, such as an extended-spectrum β-lactamase (ESBL), a transferable cephalosporinase, or a derepressed intrinsic enzyme (e.g., OXY or AmpC) (see Table S1 in the supplemental material), meropenem (MIC_{50} and MIC_{90}, 32 and >64 μg/ml, respectively) inhibited 0.0 and 1.3% of these isolates at the CLSI and EUCAST breakpoints, respectively. Meropenem combined with vaborbactam at a fixed concentration of 8 μg/ml (MIC_{50} and MIC_{90}, ≤0.06 and 1 μg/ml, respectively) inhibited 96.0 and 98.7% of the isolates at the CLSI and EUCAST breakpoints, respectively, including 68 *K. pneumoniae* isolates and 7 additional isolates representing four other bacterial species (Table 2).

Evaluation of intrinsic mechanisms of resistance to β-lactams. All seven isolates displaying meropenem-vaborbactam (fixed vaborbactam concentration, 8 μg/ml) MICs of ≥16 μg/ml were *K. pneumoniae*. Four of these isolates coproduced a metallo-β-lactamase along with KPC enzymes. Three genetically identical isolates were recovered in Italy; these isolates carried bla_{KPC-3} and bla_{VIM-1} and displayed meropenem-vaborbactam MICs of 16 μg/ml when tested using 8 μg/ml of vaborbactam. One strain producing KPC-2 and VIM-4, cultured from a clinical specimen in a U.S. hospital, had a meropenem-vaborbactam (fixed vaborbactam concentration, 8 μg/ml) MIC of >64 μg/ml. The remaining three isolates were collected from hospitals in the United States and Israel and exhibited MICs for meropenem-vaborbactam of 16 μg/ml. These three KPC-producing isolates were evaluated for expression of intrinsic genes encoding OMP and an efflux pump and showed reduced expression of *ompK37* and modest-to-high expression rates of the AcrAB-TolC resistance nodulation efflux system. In one isolate, the expression of *acrA* was 22.93-fold higher than in the control strain, and in the other two isolates, modest increases in *acrA* expression were observed (7.80- and 6.62-fold compared to the level for the control) (see Table S2 in the supplemental material).

DISCUSSION

Previous studies testing vaborbactam demonstrated favorable precovalent and covalent binding with β-lactamases of Ambler classes A and C, and specifically high inhibitory potency was exhibited with KPC enzymes. This inhibitor has pharmacokinetics similar to those of β-lactam agents and has displayed high efficacy in animal model studies using KPC-producing isolates (7).

When tested in combination with various carbapenems, vaborbactam reduced the MICs of those agents against KPC-producing *E. coli*, *E. cloacae*, and *Klebsiella* spp. ≥64-, ≥32-, ≥16-, and ≥32-fold for biapenem, meropenem, ertapenem, and imipenem, respectively (7). Additionally, this serine β-lactamase inhibitor potentiates the activity of cefepime against isolates producing commonly detected Ambler class A and D enzymes with extended-spectrum activity for cephalosporins, such as CTX-M, SHV, TEM, OXA-2, OXA-1/-30, and class C chromosomally encoded or transferable cephalosporinases (7).

Vaborbactam tested in combination with biapenem against 300 *Enterobacteriaceae* isolates, including strains harboring the prevalent carbapenemase groups and isolates with a combination of ESBL or derepressed AmpC and intrinsic resistance mechanisms, demonstrated that this inhibitor potentiated the activity of biapenem for KPC-producing isolates; however, the activity of this combination against isolates producing Ambler class B or D enzymes was limited (14). In a recent study, for KPC-producing *K. pneumoniae*, *E. cloacae*, *P. aeruginosa*, and *Acinetobacter* spp. collected in a hospital located in New York City, meropenem-vaborbactam increased the activity of carbapenems; however, decreased

TABLE 2 MIC distribution for meropenem alone and combined with vaborbactam at a fixed concentration of 8 μg/ml against most common *Enterobacteriaceae* species producing serine carbapenemases

Organism (no. of strains) and antimicrobial agent(s)	No. (cumulative %) of strains with indicated MIC (μg/ml)[b]												MIC (μg/ml)	
	≤0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	>64	50%	90%
KPC-producing *K. pneumoniae* (208)														
Meropenem	—	—	—	—	1 (0.5)	4 (2.4)	16 (10.1)	28 (23.6)	60 (52.4)	30 (66.8)	20 (76.4)	49 (100.0)	16	>64
Meropenem-vaborbactam[a]	136 (65.4)	11 (70.7)	15 (77.9)	20 (87.5)	12 (93.3)	5 (95.7)	2 (96.6)	0 (96.6)	6 (99.5)	0 (99.5)	0 (99.5)	1 (100.0)	≤0.06	1
E. cloacae (39)														
Meropenem	—	—	—	—	1 (2.6)	3 (10.3)	9 (33.3)	11 (61.5)	10 (87.2)	2 (92.3)	1 (94.9)	2 (100.0)	8	32
Meropenem-vaborbactam[a]	28 (71.8)	6 (87.2)	2 (92.3)	0 (92.3)	0 (92.3)	2 (97.4)	1 (100.0)						≤0.06	0.25
KPC-producing *E. coli* (21)														
Meropenem	—	—	—	1 (4.8)	3 (19.0)	4 (38.1)	4 (57.1)	8 (95.2)	1 (100.0)				4	8
Meropenem-vaborbactam[a]	21 (100.0)												≤0.06	≤0.06
KPC-producing *K. oxytoca* (14)														
Meropenem	—	—	—	—	—	3 (21.4)	6 (64.3)	0 (64.3)	3 (85.7)	1 (92.9)	0 (92.9)	1 (100.0)	4	32
Meropenem-vaborbactam[a]	12 (85.7)	0 (85.7)	0 (85.7)	1 (92.9)	0 (92.9)	1 (100.0)							≤0.06	0.5
KPC-producing *C. freundii* (12)														
Meropenem	—	—	—	—	1 (8.3)	1 (16.7)	5 (58.3)	5 (100.0)					4	8
Meropenem-vaborbactam[a]	9 (75.0)	2 (91.7)	1 (100.0)										≤0.06	0.12
Isolates carrying multiple β-lactamases (75)														
Meropenem	—	—	—	—	—	1 (1.3)	6 (9.3)	9 (21.3)	21 (49.3)	10 (62.7)	9 (74.7)	19 (100.0)	32	>64
Meropenem-vaborbactam[a]	49 (65.3)	4 (70.7)	7 (80.0)	6 (88.0)	6 (96.0)	2 (98.7)	0 (98.7)	0 (98.7)	1 (100.0)				≤0.06	1

[a] Meropenem combined with vaborbactam at a fixed concentration of 8 μg/ml.
[b] —, dilution not tested or no isolates displaying MIC values at this concentration.

expression of *ompK36* also reduced the effect of the β-lactamase inhibitor 8- to 16-fold compared to the level for isolates producing the same β-lactamases (15).

In this study, we showed that vaborbactam restored the activity of meropenem against a diverse and representative large collection of isolates producing serine carbapenemases. Higher vaborbactam concentrations in the combination resulted in the inhibition of a greater number of isolates than meropenem alone (Table 1). Inhibitor concentrations of 4 μg/ml or greater consistently restored the activity of meropenem, but more-limited improvements in meropenem potency were observed when 16 or 32 μg/ml of vaborbactam was used; thus, a concentration of 8 μg/ml was considered optimal. These results are also supported by pharmacokinetics and pharmacodynamics (PK-PD) model results (16), and those concentrations are also compatible with exposures following a 2-g intravenous dose.

Further studies on KPC-producing isolates that did not coproduce metallo-β-lactamase enzymes but still had elevated meropenem-vaborbactam MICs (≥16 μg/ml) demonstrated that these isolates exhibited reduced expression of the gene encoding OmpK37 combined with elevated expression of the tripartite efflux system AcrAB-TolC.

In summary, meropenem-vaborbactam demonstrated potent activity against *Enterobacteriaceae* isolates producing serine carbapenemases that are often MDR where therapeutic options to treat infections caused by these pathogens are compromised. As with other β-lactamase inhibitors currently marketed or in late-stage clinical development, vaborbactam does not inhibit class B metallo-β-lactamases, and isolates producing these enzymes displayed elevated MICs for the meropenem-vaborbactam combination.

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