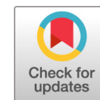
AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

SUSCEPTIBILITY



In Vitro Activity of Meropenem-Vaborbactam against Clinical Isolates of KPC-Positive *Enterobacteriaceae*

Meredith A. Hackel,[a] Olga Lomovskaya,[b] Michael N. Dudley,[b] James A. Karlowsky,[c] Daniel F. Sahm[a]

[a]International Health Management Associates, Inc., Schaumburg, Illinois, USA

[b]The Medicines Company, San Diego, California, USA

[c]Department of Medical Microbiology and Infectious Diseases, College of Medicine, University of Manitoba, Winnipeg, Manitoba, Canada

ABSTRACT Vaborbactam (formerly RPX7009) is a novel inhibitor of serine β-lactamases, including Ambler class A carbapenemases, such as KPCs. The current study evaluated the *in vitro* activity of the combination agent meropenem-vaborbactam against a global collection of 991 isolates of KPC-positive *Enterobacteriaceae* collected in 2014 and 2015 using the Clinical and Laboratory Standards Institute (CLSI) standard broth microdilution method. The MIC_{90} of meropenem (when tested with a fixed concentration of 8 μg/ml of vaborbactam) for isolates of KPC-positive *Enterobacteriaceae* was 1 μg/ml, and MIC values ranged from ≤0.03 to >32 μg/ml; 99.0% (981/991) of isolates had meropenem-vaborbactam MICs of ≤4 μg/ml, the U.S. FDA-approved MIC breakpoint for susceptibility to meropenem-vaborbactam (Vabomere). Vaborbactam lowered the meropenem MIC_{50} from 32 to 0.06 μg/ml and the MIC_{90} from >32 to 1 μg/ml. There were no differences in the activity of meropenem-vaborbactam when the isolates were stratified by KPC variant type. We conclude that meropenem-vaborbactam demonstrates potent *in vitro* activity against a worldwide collection of clinical isolates of KPC-positive *Enterobacteriaceae* collected in 2014 and 2015.

KEYWORDS meropenem, vaborbactam, KPC, carbapenemase, *Enterobacteriaceae*, Vabomere

Carbapenem resistance has emerged worldwide in clinical isolates of *Enterobacteriaceae* (1–3). The spread of carbapenem-resistant *Enterobacteriaceae*, facilitated by either the horizontal spread of carbapenemase genes or the clonal expansion of carbapenem-resistant isolates (e.g., *Klebsiella pneumoniae* sequence type 258), has been identified to be a global public health threat and is of particular concern for patients afflicted with health care-associated infections (4, 5). Factors associated with escalating carbapenem resistance rates include the increased reliance on (and selective pressure from) carbapenems as treatment for the burgeoning number of infections caused by extended-spectrum β-lactamase (ESBL)-positive *Enterobacteriaceae* that have occurred worldwide over the last 2 decades as well as substandard infection control practices and the absence of antimicrobial stewardship programs in many hospitals (6, 7). The spread of carbapenemase genes both to colonizing flora and to potential pathogens is of particular concern because carbapenemases frequently confer resistance to all β-lactams, the most widely prescribed class of antimicrobial agents (8). The majority of carbapenemase-producing *Enterobacteriaceae* are also multidrug resistant (1, 2), limiting the treatment options available for empirical and directed therapy (9). The list of antimicrobial agents currently available to treat patients infected with carbapenem-resistant Gram-negative bacilli is short (aminoglycosides, tigecycline, colistin) and includes agents commonly associated with significant toxicities and increasing resistance or agents to which some species of *Enterobacteriaceae* show intrinsic resistance

Received 12 September 2017 **Returned for modification** 13 October 2017 **Accepted** 20 October 2017

Accepted manuscript posted online 30 October 2017

Citation Hackel MA, Lomovskaya O, Dudley MN, Karlowsky JA, Sahm DF. 2018. *In vitro* activity of meropenem-vaborbactam against clinical isolates of KPC-positive *Enterobacteriaceae*. Antimicrob Agents Chemother 62:e01904-17. https://doi.org/10.1128/AAC.01904-17.

Copyright © 2017 Hackel et al. This is an open-access article distributed under the terms of the Creative Commons Attribution 4.0 International license.

Address correspondence to Meredith A. Hackel, mhackel@ihmainc.com.

(6, 7, 10). New antimicrobial agents are urgently needed to address the increasing prevalence of carbapenem-resistant *Enterobacteriaceae* (4, 5).

Carbapenemases are classified into three of the four Ambler (molecular) classes of β-lactamase enzymes: class A (e.g., KPC), class B (e.g., NDM, VIM, IMP), and class D (e.g., OXA-48) enzymes. Class A, class C (AmpC), and class D β-lactamase have serine-based active sites. Class B enzymes have zinc-based active sites and are known as metallo-β-lactamases (MBLs). AmpC β-lactamases and ESBLs (a subset of class A β-lactamases) may also confer carbapenem resistance to isolates of Gram-negative bacilli when combined with porin mutations/loss, expression of efflux pumps, and/or alterations in penicillin-binding proteins (1–3, 6, 7, 9). KPCs have the greatest global distribution of all carbapenemases associated with *Enterobacteriaceae* (1–3, 7, 9). KPC-positive isolates are the most common carbapenemase-producing *Enterobacteriaceae* in the United States and have also been reported to be widespread in South and Central America, the Middle East, and China (7). In Europe, the highest incidences of KPC-positive *Enterobacteriaceae* are found in Italy and Greece (1–3, 7, 9).

A proven strategy to overcome β-lactamase-driven resistance is to restore the activity of an inactivated β-lactam agent by combining it with an inhibitor of the β-lactamase(s) responsible for the degradation of that β-lactam. Vaborbactam (formerly RPX7009) was developed specifically to inactivate KPC β-lactamases (11). It is a novel (first-in-class), non-β-lactam, cyclic boronic acid pharmacophore that inhibits serine β-lactamases of class A and class C, including KPC, IMI, SME, NMC-A, BKC-1, and FR-1 carbapenemases (8, 11–13), with no inhibition of mammalian serine proteases (11). Vaborbactam was optimized to be a potent inhibitor of serine β-lactamases using *in silico* modeling of the active sites of key serine β-lactamases, principally, KPCs (11). Vaborbactam possesses no antibacterial activity alone (MIC, >64 μg/ml) (12, 14). Mechanistically, the affinity of boronates, such as vaborbactam, for serine-based active sites of β-lactamases is due to the formation of a covalent complex between the catalytic serine side chain and the boronate moiety, which mimics the tetrahedral transition state of the acylation or deacylation reaction complex (11). Vaborbactam is structurally distinct from other new β-lactamase inhibitors, such as avibactam and relebactam, which are diazabicyclooctane inhibitors and also inhibit KPCs (1, 2). Avibactam is approved for use in combination with ceftazidime and is in clinical development in combination with other β-lactams, and relebactam is part of a combination with imipenem. KPCs are poorly inhibited by clavulanate, tazobactam, and sulbactam; and β-lactam–β-lactamase inhibitor combinations including these three older β-lactamase inhibitors have no utility in the treatment of infections due to carbapenem-resistant *Enterobacteriaceae*.

Meropenem-vaborbactam (Vabomere) in a fixed-dose combination of meropenem and vaborbactam has recently been approved by the U.S. FDA for the treatment of complicated urinary tract infections and acute pyelonephritis. The New Drug Application (NDA) included a phase 3 clinical trial to evaluate its efficacy in the treatment of complicated urinary tract infection, including acute pyelonephritis, in comparison to that of piperacillin-tazobactam (TANGO I; ClinicalTrials.gov identifier NCT02166476). A second phase 3 clinical trial in which meropenem-vaborbactam is being assessed for its efficacy for the treatment of serious infections due to carbapenem-resistant *Enterobacteriaceae*, including hospital-acquired and ventilator-associated pneumonia, in comparison to that of the best available antimicrobial therapy (TANGO II; ClinicalTrials.gov identifier NCT02168946) was ongoing at the time of NDA submission and review.

The current study evaluated the *in vitro* activities of meropenem-vaborbactam and seven comparator agents against a recent global collection of 991 clinical isolates of KPC-positive (OXA-48-negative and MBL-negative) *Enterobacteriaceae* collected in 2014 and 2015.

RESULTS

Table 1 depicts the *in vitro* activities of meropenem-vaborbactam and its comparators against 991 clinical isolates of *Enterobacteriaceae* known to be KPC positive (and

TABLE 1 *In vitro* activities of meropenem-vaborbactam and comparator agents against 991 clinical isolates of KPC-positive *Enterobacteriaceae*

Family, genus, or species[a] (no. of isolates)	Antimicrobial agent(s)	MIC[b] (μg/ml)			% of isolates with the following MIC interpretation[c]:		
		Range	50%	90%	Susceptible	Intermediate	Resistant
All *Enterobacteriaceae*[d] (991)	Meropenem-vaborbactam	≤0.03 to >32	0.06	1	99.0	0.6	0.4
	Meropenem	2 to >32	32	>32	0	4.1	95.9
	Ceftazidime-avibactam	≤0.06 to >64	1	4	98.2		1.8
	Ceftazidime	1 to >64	>64	>64	3.0	2.5	94.5
	Tigecycline	≤0.06 to 8	1	2	95.8	3.6	0.6
	Minocycline	0.5 to >64	8	32	44.5	30.4	25.1
	Gentamicin	≤0.06 to >64	1	>64	63.4	6.3	30.4
	Polymyxin B	0.25 to >16	0.5	16	NA	NA	NA
K. pneumoniae (878)	Meropenem-vaborbactam	≤0.03 to >32	0.12	1	98.9	0.7	0.5
	Meropenem	2 to >32	>32	>32	0	1.9	98.1
	Ceftazidime-avibactam	≤0.06 to >64	1	4	98.2		1.8
	Ceftazidime	1 to >64	>64	>64	1.6	2.0	96.4
	Tigecycline	0.12 to 8	1	2	95.9	3.4	0.7
	Minocycline	1 to >64	8	32	44.2	32.0	23.8
	Gentamicin	≤0.06 to >64	1	>64	64.7	6.1	29.2
	Polymyxin B	0.25 to >16	0.5	16	NA	NA	NA
E. coli (35)	Meropenem-vaborbactam	≤0.03 to 0.12	≤0.03	≤0.03	100	0	0
	Meropenem	2 to 32	4	16	0	25.7	74.3
	Ceftazidime-avibactam	≤0.06 to 1	0.5	1	100		0
	Ceftazidime	2 to >64	64	>64	8.6	5.7	85.7
	Tigecycline	≤0.06 to 1	0.25	0.5	100	0	0
	Minocycline	0.5 to >64	4	32	51.4	11.4	37.1
	Gentamicin	0.25 to >64	1	>64	57.1	5.7	37.1
	Polymyxin B	0.25 to 1	0.5	0.5	NA	NA	NA
Enterobacter spp.[e] (29)	Meropenem-vaborbactam	≤0.03 to 0.12	≤0.03	0.12	100	0	0
	Meropenem	2 to >32	8	>32	0	27.6	72.4
	Ceftazidime-avibactam	0.25 to 2	1	2	100		0
	Ceftazidime	4 to >64	32	>64	3.5	6.9	89.7
	Tigecycline	0.25 to 4	1	2	93.1	6.9	0
	Minocycline	1 to >64	16	64	41.4	3.5	55.2
	Gentamicin	0.25 to >64	4	>64	51.7	13.8	34.5
	Polymyxin B	0.25 to 16	0.5	1	NA	NA	NA
K. oxytoca (19)	Meropenem-vaborbactam	≤0.03 to 0.25	≤0.03	0.25	100	0	0
	Meropenem	2 to >32	4	32	0	21.1	78.9
	Ceftazidime-avibactam	≤0.06 to 16	0.5	4	94.7		5.3
	Ceftazidime	4 to >64	64	>64	15.8	5.3	78.9
	Tigecycline	0.12 to 2	0.5	2	100	0	0
	Minocycline	1 to >64	4	>64	52.6	36.9	10.5
	Gentamicin	0.25 to >64	8	>64	42.1	10.5	47.4
	Polymyxin B	0.5 to 1	0.5	0.5	NA	NA	NA
S. marcescens (16)	Meropenem-vaborbactam	≤0.03 to 2	0.06	1	100	0	0
	Meropenem	2 to >32	16	>32	0	6.2	93.8
	Ceftazidime-avibactam	≤0.06 to 32	0.5	2	93.8		6.2
	Ceftazidime	2 to >64	8	>64	37.5	12.5	50.0
	Tigecycline	0.5 to 4	1	4	81.3	18.7	0
	Minocycline	2 to 32	4	16	50.0	31.2	18.8
	Gentamicin	0.5 to >64	1	>64	68.8	0	31.2
	Polymyxin B	4 to >16	>16	>16	NA	NA	NA
Citrobacter spp.[f] (13)	Meropenem-vaborbactam	≤0.03 to 0.12	≤0.03	0.06	100	0	0
	Meropenem	2 to 32	4	8	0	15.4	84.6
	Ceftazidime-avibactam	0.12 to 2	0.5	2	100		0
	Ceftazidime	4 to >64	64	>64	23.1	0	76.9
	Tigecycline	0.25 to 4	0.5	2	92.3	7.7	0
	Minocycline	1 to >64	8	>64	30.8	23.1	46.1
	Gentamicin	0.25 to >64	16	>64	46.2	0	53.8
	Polymyxin B	0.25 to 1	0.5	0.5	NA	NA	NA

(Continued on next page)

negative for genes for both OXA-48 and MBLs). The MIC_{50}s and MIC_{90}s of meropenem-vaborbactam for all isolates of *Enterobacteriaceae* tested were 0.06 and 1 μg/ml, respectively. These concentrations were 512-fold and >64-fold lower, respectively, than the MIC_{50} (32 μg/ml) and MIC_{90} (>32 μg/ml) of meropenem alone. The modal MIC of meropenem for all 991 isolates of *Enterobacteriaceae* also fell in the range of from >32 to ≤0.03 μg/ml in the presence of vaborbactam (Table 2). The MIC_{50} and MIC_{90} values of meropenem-vaborbactam for *K. pneumoniae* (MIC_{50}, 0.12 μg/ml; MIC_{90}, 1 μg/ml) were >4-fold higher and 4 to >32-fold higher, respectively, than the MIC_{50} and MIC_{90} values for *Escherichia coli*, *Enterobacter* spp., *Klebsiella oxytoca*, and *Citrobacter* spp. (Table 2). The MIC_{90} value of meropenem-vaborbactam for *Serratia marcescens* (1 μg/ml) was identical to that of *K. pneumoniae*. Reductions in MIC_{50} and MIC_{90} values similar to those demonstrated for meropenem-vaborbactam and meropenem alone were also observed when the values for ceftazidime-avibactam were compared with those for ceftazidime alone (Table 1). The MIC distributions of meropenem-vaborbactam for *K. pneumoniae* and *S. marcescens* were demonstrated to be much broader than those for the other species of *Enterobacteriaceae* tested (Table 2). Meropenem-vaborbactam (MIC_{90}, 1 μg/ml) was more potent than all other agents tested, including tigecycline (MIC_{90}, 2 μg/ml), ceftazidime-avibactam (MIC_{90}, 4 μg/ml), and polymyxin B (MIC_{90}, 16 μg/ml) (Table 1).

The percent susceptibility for all isolates of *Enterobacteriaceae* was greater for meropenem-vaborbactam (99.0% susceptible) than for ceftazidime-avibactam (98.2%) and tigecycline (95.8%). Of the 991 isolates of KPC-positive *Enterobacteriaceae* tested, 6 isolates (0.6%) tested as meropenem-vaborbactam intermediate, 4 isolates (0.4%) tested as meropenem-vaborbactam resistant, and 18 isolates (1.8%) tested as ceftazidime-avibactam resistant (Table 1). Of the 991 isolates tested, 5.3% (53/991) had a ceftazidime-avibactam MIC of 8 μg/ml (data not shown). The observed difference in percent nonsusceptibility between meropenem-vaborbactam (1.0% of isolates) and ceftazidime-avibactam (1.8% of isolates) was due to 14 of 18 ceftazidime-avibactam-resistant isolates (MIC, ≥16 μg/ml) testing as susceptible to meropenem-vaborbactam (MIC, ≤4 μg/ml) and 6 of 10 meropenem-vaborbactam-nonsusceptible isolates (MIC, ≥8 μg/ml) testing as susceptible to ceftazidime-avibactam (MIC, ≤8 μg/ml) (data not shown). In general, cross-resistance between meropenem-vaborbactam and ceftazidime-avibactam was uncommon, occurring in only 20.8% (5/24 isolates) of isolates resistant to either agent.

The MIC range for meropenem-vaborbactam for 991 isolates of KPC-positive *Enterobacteriaceae* was ≤0.03 to >32 μg/ml, with the MICs for only 2 isolates exceeding 16 μg/ml (both were *K. pneumoniae* isolates with MIC values of >32 μg/ml; 1 isolate was from Greece [and produced KPC-2], and the other isolate was from Italy [and produced KPC-3]). Of the two isolates with meropenem-vaborbactam MIC values of >32 μg/ml, one was resistant to ceftazidime-avibactam (MIC, 32 μg/ml) and had a polymyxin MIC of 0.5 μg/ml, whereas the other isolate was susceptible to ceftazidime-avibactam (MIC, 2 μg/ml) and had a polymyxin B MIC of >16 μg/ml.

The cumulative MIC distributions of meropenem-vaborbactam stratified by KPC variant are shown in Table 3. There were no appreciable differences in the activity of meropenem-vaborbactam against different KPC variants (KPC-2, KPC-3), suggesting

TABLE 1 (Continued)

[a]Species of *Enterobacteriaceae* with <10 isolates were grouped together with other species in their genus, and data are presented as data for the genus only.
[b]MIC_{50} and MIC_{90} values for individual genus or species were calculated when 10 or more isolates were tested.
[c]NA, not available. There are no CLSI, EUCAST, or U.S. FDA MIC breakpoints published for polymyxin B. Current CLSI MIC interpretative breakpoints were used for meropenem (susceptible, ≤1 μg/ml; intermediate, 2 μg/ml; resistant, ≥4 μg/ml), ceftazidime (susceptible, ≤4 μg/ml; intermediate, 8 μg/ml; resistant, ≥16 μg/ml), minocycline, and gentamicin. Current U.S. FDA MIC interpretative breakpoints were used for meropenem-vaborbactam (susceptible, ≤4 μg/ml; intermediate, 8 μg/ml; resistant, ≥16 μg/ml), ceftazidime-avibactam (susceptible, ≤8 μg/ml; resistant, ≥16 μg/ml), and tigecycline (susceptible, ≤2 μg/ml; intermediate, 4 μg/ml; resistant, ≥8 μg/ml). Vaborbactam was tested at a final concentration of 8 μg/ml.
[d]There was one isolate of *Raoultella ornithinolytica* in the study. It was included in the data set for all *Enterobacteriaceae* but not in a genus-specific subset of isolates. The MICs of meropenem and meropenem-vaborbactam for this isolate were >32 and 0.25 μg/ml, respectively.
[e]The 29 isolates of *Enterobacter* spp. comprised 17 *Enterobacter cloacae* isolates, 8 *Enterobacter aerogenes* isolates, 3 *Enterobacter asburiae* isolates, and 1 *Enterobacter hormaechi* isolate.
[f]The 13 isolates of *Citrobacter* spp. comprised 11 *Citrobacter freundii* isolates and 2 *Citrobacter koseri* isolates.

TABLE 2 Cumulative MIC distributions for meropenem-vaborbactam and meropenem against 991 clinical isolates of KPC-positive *Enterobacteriaceae*

Family, genus, species (no. of isolates)	Antimicrobial agent(s)	No. of isolates (cumulative % of isolates) inhibited at MIC (μg/ml)[a] of:											
		≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
All *Enterobacteriaceae*[b] (991)	Meropenem-vaborbactam	460 (46.4)	55 (52.0)	58 (57.8)	135 (71.4)	139 (85.5)	**80 (93.5)**	39 (97.5)	15 (99.0)	6 (99.6)	2 (99.8)	0 (99.8)	2 (100)
	Meropenem							41 (4.1)	78 (12.0)	125 (24.6)	148 (39.6)	140 (53.7)	**459 (100)**
K. pneumoniae (878)	Meropenem-vaborbactam	372 (42.4)	47 (47.7)	49 (53.3)	131 (68.2)	138 (83.9)	**78 (92.8)**	38 (97.2)	15 (98.9)	6 (99.5)	2 (99.8)	0 (99.8)	2 (100)
	Meropenem							17 (1.9)	46 (7.2)	104 (19.0)	130 (33.8)	130 (48.6)	451 (100)
E. coli (35)	Meropenem-vaborbactam	**34 (97.1)**	0 (97.1)	1 (100)									
	Meropenem							9 (25.7)	14 (65.7)	7 (85.7)	**3 (94.3)**	2 (100)	
Enterobacter spp. (29)	Meropenem-vaborbactam	22 (75.9)	3 (86.2)	**4 (100)**									
	Meropenem							8 (27.6)	5 (44.8)	3 (55.2)	9 (86.2)	1 (89.7)	**3 (100)**
K. oxytoca (19)	Meropenem-vaborbactam	17 (89.5)	0 (89.5)	0 (89.5)	**2 (100)**								
	Meropenem							4 (21.1)	7 (57.9)	5 (84.2)	1 (89.5)	**1 (94.7)**	1 (100)
S. marcescens (16)	Meropenem-vaborbactam	4 (25.0)	4 (50.0)	3 (68.8)	1 (75.0)	1 (81.3)	**2 (93.8)**	1 (100)					
	Meropenem							1 (6.3)	0 (6.3)	2 (18.8)	5 (0.50)	5 (81.3)	**3 (100)**
Citrobacter spp. (13)	Meropenem-vaborbactam	11 (84.6)	**1 (92.3)**	1 (100)									
	Meropenem							2 (15.4)	6 (61.5)	**4 (92.3)**	0 (92.3)	1 (100)	

[a] MIC_{90}s are in boldface.
[b] There was one isolate of *Raoultella ornithinolytica* in the study. It was included in the data set for all *Enterobacteriaceae* but not in a genus-specific subset of isolates. The MICs of meropenem and meropenem-vaborbactam for this isolate were >32 and 0.25 μg/ml, respectively.

TABLE 3 Cumulative MIC distributions for meropenem-vaborbactam stratified by KPC variant

KPC variant (no. of isolates)	No. of isolates (cumulative % of isolates) inhibited at MIC (μg/ml)[a] of:											
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	>32
KPC-2 (610)	294 (48.2)	42 (55.1)	39 (61.4)	67 (72.5)	79 (85.4)	**46 (93.0)**	27 (97.4)	11 (99.2)	4 (99.8)	0 (99.8)	0 (99.8)	1 (100)
KPC-3 (373)	161 (43.2)	13 (46.6)	18 (51.5)	68 (69.7)	58 (85.3)	**34 (94.4)**	12 (97.6)	4 (98.7)	2 (99.2)	2 (99.7)	0 (99.7)	1 (100)
KPC-5 (2)	1 (50.0)	0 (50.0)	1 (100)									
KPC-6 (1)	1 (100)											
KPC-9 (2)	0 (0)	0 (0)	0 (0)	0 (0)	2 (100)							
KPC-18 (3)	3 (100)											
All isolates (991)	460 (46.4)	55 (52.0)	58 (57.8)	135 (71.4)	139 (85.5)	**80 (93.5)**	39 (97.5)	15 (99.0)	6 (99.6)	2 (99.8)	0 (99.8)	2 (100)

[a]MIC_{90}s are in boldface when 10 or more isolates of a KPC variant were present.

that the differences in the activity of meropenem-vaborbactam observed between *K. pneumoniae* and *S. marcescens* and the other species of *Enterobacteriaceae* were due to factors other than the KPC variant present. Of the 18 ceftazidime-avibactam-resistant isolates (MIC, ≥16 μg/ml), 77.8% (14/18) produced KPC-3 (data not shown). A difference was observed between KPC variants with meropenem-vaborbactam MICs of ≥8 μg/ml (0.8% [5/610] of isolates producing KPC-2 and 1.3% [5/373] of isolates producing KPC-3) (Table 3) and those with ceftazidime-avibactam MICs of ≥16 μg/ml (0.7% [4/610] of isolates producing KPC-2 and 3.8% [14/373] of isolates producing KPC-3) (data not shown).

The cumulative MIC distributions for meropenem-vaborbactam stratified by KPC, AmpC, and ESBL genotypes are shown in Table 4. There were no appreciable differences in the activity of meropenem-vaborbactam against isolates which co-produced AmpC enzymes or ESBLs and KPC (MIC_{90} values of 0.06 μg/ml and 1 μg/ml, respectively, compared to an MIC_{90} of 1 μg/ml for isolates which produced only KPC) (Table 4).

DISCUSSION

In the current study, we observed that meropenem-vaborbactam inhibited 99.0% of KPC-positive isolates of *Enterobacteriaceae* at ≤4 μg/ml, the U.S. FDA MIC breakpoint for susceptibility (15). In the current study, the *in vitro* activity of meropenem-vaborbactam was equivalent to that of ceftazidime-avibactam (to which 98.2% of isolates were susceptible) and tigecycline (to which 95.8% of isolates were susceptible) (Table 1). Meropenem-vaborbactam was demonstrated to be a more potent antimicrobial agent *in vitro* than ceftazidime-avibactam, tigecycline, and all other antimicrobial agents tested against the recent worldwide collection of clinical isolates of KPC-positive *Enterobacteriaceae* tested (Table 1). On the basis of the MIC_{90}s, meropenem-vaborbactam (MIC_{90}, 1 μg/ml) was four times more potent than ceftazidime-avibactam and >64 times more potent than meropenem alone.

Four previous studies that determined the *in vitro* activity of meropenem-vaborbactam against clinical isolates of Gram-negative bacilli and in which subsets of isolates were phenotypically or molecularly characterized for ESBLs, carbapenemases,

TABLE 4 Cumulative MIC distributions for meropenem-vaborbactam stratified by genotype

Genotype (no. of isolates)	No. of isolates (cumulative % of isolates) inhibited at MIC (μg/ml)[a] of:										
	≤0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	>32
KPC AmpC[b] (34)	29 (78.4)	**2 (94.1)**	3 (100)								
KPC AmpC ESBL[c] (8)	6 (75.0)	1 (87.5)	1 (100)								
KPC ESBL[d] (346)	171 (49.4)	23 (56.1)	16 (60.7)	42 (72.8)	47 (86.4)	**27 (94.2)**	16 (98.8)	1 (99.1)	2 (99.7)	0 (0)	1 (100)
KPC only (603)	254 (42.1)	29 (46.9)	38 (53.2)	93 (68.7)	92 (83.9)	**53 (92.7)**	23 (96.5)	14 (98.8)	4 (99.5)	2 (99.8)	1 (100)

[a]MIC_{90}s are is boldface when 10 or more isolates of a genotype were present.
[b]AmpC enzymes comprised ACT/MIR (*n* = 13 isolates) and CMY II (*n* = 21 isolates).
[c]AmpC enzymes comprised ACT/MIR (*n* = 2 isolates) and CMY II (*n* = 6 isolates); extended spectrum β-lactamase (ESBL) enzymes comprised CTX-M (*n* = 7 isolates) and SHV (*n* = 1 isolates).
[d]ESBL enzymes comprised CTX-M (*n* = 167 isolates), CTX-M and SHV (*n* = 9 isolates), and SHV (*n* = 170 isolates).

or other mechanisms of carbapenem resistance have been published (11, 14, 16, 17). Initially, Hecker et al. demonstrated that vaborbactam, at a fixed concentration of 4 μg/ml, reduced the MICs of biapenem, meropenem, ertapenem, and imipenem by ≥64-, ≥32-, ≥16-, and ≥32-fold, respectively, when tested against KPC-positive *E. coli*, *Enterobacter cloacae*, and *Klebsiella* isolates (11). These investigators also reported that vaborbactam potentiated the activity of cefepime against isolates producing class A and D β-lactamases with extended-spectrum activity against cephalosporins (CTX-M, SHV, TEM, OXA-2, OXA-1/OXA-30) and isolates with chromosomally encoded or transferable AmpC β-lactamases (11).

Lapuebla et al. tested meropenem in combination with vaborbactam (8 μg/ml) against a panel of 121 carbapenem-resistant KPC-positive isolates of *K. pneumoniae* and reported that the MIC_{50}, MIC_{90}, and MIC range were 0.03, 0.5, and ≤0.004 to >64 μg/ml, respectively, and that 98.5% (131/133) of KPC-positive *Enterobacteriaceae* (including 5 isolates of *E. coli* and 7 isolates of *Enterobacter* spp.) were inhibited by meropenem-vaborbactam at a concentration of 1 μg/ml (16). Lapuebla et al. also observed that vaborbactam had little to no effect on meropenem MICs for meropenem-nonsusceptible *Acinetobacter baumannii* isolates containing OXA-type carbapenemases or for *Pseudomonas aeruginosa* isolates (16). In addition, Lapuebla et al. reported that meropenem-vaborbactam MICs were 8- to 16-fold higher for isolates with diminished *ompK35* and *ompK36* expression than for isolates producing the same β-lactamases without permeability changes (16).

Castanheira et al. evaluated the activity of meropenem-vaborbactam against 315 serine carbapenemase-producing *Enterobacteriaceae* isolates, including 308 KPC-positive isolates, using checkerboard-designed panels and reported a maximum potentiation for vaborbactam activity at a concentration of 8 μg/ml (14). Castanheira et al. also reported that 93.7% of the 315 serine carbapenemase-producing isolates of *Enterobacteriaceae* were inhibited at a meropenem MIC of ≤1 μg/ml (vaborbactam concentration, 8 μg/ml) and that 96.5% of isolates were inhibited at a meropenem concentration of ≤2 μg/ml (14). The MIC_{50} and MIC_{90} for the 315 isolates were ≤0.06 and 1 μg/ml, respectively (14). These investigators identified seven isolates with meropenem-vaborbactam MICs of ≥16 μg/ml. All seven isolates were *K. pneumoniae*, four of which coproduced an MBL (MIC, 16 to >64 μg/ml); the other three isolates demonstrated decreased expression of *ompK37* and/or elevated expression of the AcrAB-TolC efflux system (MIC, 16 μg/ml) (14). Earlier, Livermore and Mushtaq also reported that an outer membrane porin deficiency combined with the presence of β-lactamases can diminish the effect of vaborbactam combined with biapenem, an observation that suggested that the utility of a carbapenem–β-lactamase inhibitor combination against certain isolates may be limited (12). Livermore and Mushtaq tested vaborbactam in combination with biapenem against 300 *Enterobacteriaceae* isolates, including isolates carrying KPC-type enzymes or another class A serine β-lactamase alone or in combination with an ESBL, derepressed AmpC, or an intrinsic resistance mechanism (12). These investigators determined that vaborbactam potentiated the activity of biapenem against KPC-positive isolates; however, the activity of biapenem-vaborbactam against isolates producing class B or D (OXA-48) β-lactamases was limited (12).

Most recently, Castanheira and coworkers studied >10,000 clinical isolates of *Enterobacteriaceae* collected worldwide in 2014 and reported MIC_{90}s of meropenem-vaborbactam of 0.06, 32, 0.5, >32, and 1 μg/ml (vaborbactam was tested at a fixed concentration of 8 μg/ml) for all isolates, carbapenem-resistant *Enterobacteriaceae*, KPC producers, non-KPC-producing carbapenem-resistant *Enterobacteriaceae* isolates, and multidrug-resistant isolates, respectively (17). Overall, meropenem-vaborbactam inhibited 99.1% of *Enterobacteriaceae* at a meropenem MIC of ≤1 μg/ml (17). All but 5 of 135 (3.7%) KPC-producing isolates were inhibited by meropenem-vaborbactam at 1 μg/ml, a rate slightly lower than that observed in the current study (6.5%) (Table 3). All KPC-producing isolates in the study by Castanheira et al. were inhibited by meropenem-vaborbactam at an MIC of 8 μg/ml (17).

Previously, vaborbactam was also demonstrated to possess pharmacokinetics similar to those of β-lactam agents, including carbapenems, and displayed efficacy as a treatment for infections caused by KPC-positive isolates of *Escherichia coli*, *Enterobacter cloacae*, and *K. pneumoniae* in a neutropenic mouse thigh infection model (11, 18, 19). Meropenem-vaborbactam has also shown activity in an *in vitro* hollow-fiber model that simulated human exposure, where the data generated support for the use of meropenem-vaborbactam (vaborbactam concentration, 8 μg/ml) for the treatment of infections caused by KPC-positive carbapenem-resistant *Enterobacteriaceae* isolates with meropenem MICs as high as 8 μg/ml (20).

In the current study, the difference between the KPC variants associated with meropenem-vaborbactam MICs of ≥2 μg/ml (7.0% [43/610 isolates] for isolates producing KPC-2 and 5.6% [21/373 isolates] for isolates producing KPC-3) and meropenem-vaborbactam MICs of ≥4 μg/ml (2.6% [16/610 isolates] for isolates producing KPC-2 and 2.4% [9/373 isolates] for isolates producing KPC-3) (Table 3) and those associated with ceftazidime-avibactam MICs of ≥16 μg/ml (0.7% [4/610 isolates] for isolates producing KPC-2 and 3.8% [14/373 isolates] for isolates producing KPC-3) is noteworthy. Other authors have reported higher MICs of ceftazidime-avibactam for isolates carrying KPC-3 than for those carrying KPC-2 (21) and the emergence of resistance to ceftazidime-avibactam due to plasmid-borne KPC-3 mutations during treatment of carbapenem-resistant *K. pneumoniae* infections (22). The differences observed between the subsets of isolates resistant to meropenem-vaborbactam and ceftazidime-avibactam may also be related to the observation that the inhibition of KPC-2 by vaborbactam does not involve S130, a residue important for inhibition by avibactam (R. Tsivkovski, M. Totrov, and O. Lomovskaya, presented at Microbe 2016, Boston, MA).

The intent of the current study was to add to the limited amount of available *in vitro* data on the meropenem-vaborbactam MICs for KPC-positive *Enterobacteriaceae* isolates (14, 16, 17). Our data align with the findings of these previous studies and show that vaborbactam restores the *in vitro* activity of meropenem against the majority of isolates of *Enterobacteriaceae* carrying KPCs that would otherwise be nonsusceptible to carbapenems. On the basis of the results of our current study, meropenem-vaborbactam appears to be a promising, novel carbapenem–β-lactamase inhibitor combination. Its continued clinical development may provide a valuable therapeutic option for treating infections caused by antimicrobial-resistant Gram-negative bacilli in the future.

MATERIALS AND METHODS

Bacterial isolates. The isolates of KPC-positive *Enterobacteriaceae* tested in this study ($n = 991$) were randomly selected from isolates in the frozen stock culture collection of International Health Management Associates, Inc. (IHMA; Schaumburg, IL, USA), collected in 2014 and 2015 for a global clinical isolate surveillance study on the basis of their β-lactamase content and year of isolation. In total, 580 isolates were selected in 2014 and 411 isolates were selected in 2015. All 991 KPC-positive isolates were previously determined to be OXA-48 negative and MBL negative by a protocol that included screening of the isolates for the presence of genes encoding the following β-lactamases using published multiplex PCR assays as described previously (24): ESBLs (TEM, SHV, CTX-M, VEB, PER, GES), AmpC enzymes (ACC, ACT, CMY, DHA, FOX, MIR, MOX), and carbapenemases (KPC, OXA-48, GES, IMP, VIM, NDM, SPM, GIM). The detected β-lactamase genes were amplified using flanking primers and sequenced. Enzyme subtypes were determined by comparison against the subtypes in the database maintained by the National Center for Biotechnology Information (www.ncbi.nlm.nih.gov). Isolates positive for OXA-48 or an MBL were excluded from the current study because previous publications have documented that vaborbactam does not inhibit these enzymes (13).

The 991 isolates comprised 878 *K. pneumoniae* (88.6% of isolates), 35 *E. coli* (3.5%), 19 *Klebsiella oxytoca* (1.9%), 17 *Enterobacter cloacae* (1.7%), 16 *Serratia marcescens* (1.6%), 11 *Citrobacter freundii* (1.1%), 8 *Enterobacter aerogenes* (0.8%), 3 *Enterobacter asburiae* (0.3%), and 2 *Citrobacter koseri* (0.2%) isolates and one isolate each of *Enterobacter hormaechi* (0.1%) and *Raoultella ornithinolytica* (0.1%). Of the 991 isolates, 496 isolates were from Europe (12 countries; 242 isolates producing KPC-2, 252 isolates producing KPC-3, 2 isolates producing KPC-9), 371 were from Latin America (9 countries; 326 isolates producing KPC-2, 42 isolates producing KPC-3, 2 isolates producing KPC-5, 1 isolate producing KPC-6), 96 from North America (2 countries; 19 isolates producing KPC-2, 74 isolates producing KPC-3, 3 isolates producing KPC-18), 16 from the Asia-Pacific region (3 countries; all 16 isolates produced KPC-2), and 12 from the Middle East (1 country; 7 isolates producing KPC-2, 5 isolates producing KPC-3). All isolates were originally grown in clinical microbiology laboratories from specimens from patients with documented infection, with a limit of one isolate per patient. The identities of all isolates were determined by IHMA

using matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics, Billerica, MA, USA).

Antimicrobial susceptibility testing. All aspects of antimicrobial susceptibility testing, including broth microdilution panel production, panel inoculation, incubation, MIC reading, and MIC interpretation, followed Clinical and Laboratory Standards Institute (CLSI) standard methods and were performed on-site at IHMA (10, 25). Broth microdilution panels included the following antimicrobial agents: meropenem-vaborbactam (doubling dilution range tested, 0.03/8 to 32/8 μg/ml), meropenem (0.03 to 32 μg/ml), ceftazidime-avibactam (0.06/4 to 64/4 μg/ml), ceftazidime (0.06 to 64 μg/ml), tigecycline (0.06 to 8 μg/ml), minocycline (0.03 to 64 μg/ml), gentamicin (0.06 to 64 μg/ml), and polymyxin B (0.12 to 16 μg/ml). Vaborbactam and avibactam were provided to IHMA by The Medicines Company (San Diego, CA). All other antimicrobial agents were purchased from the U.S. Pharmacopeia (Rockville, MD). The broth microdilution panels were incubated at 35°C for 16 to 20 h in ambient air before MIC endpoints were read. All compounds tested were dissolved according to CLSI specifications and then further diluted in cation-adjusted Mueller-Hinton broth (CAMHB) to generate the sequential dilutions required to produce the broth microdilution panels (25). Colonies were taken directly from a second-pass culture plate, and a suspension with a turbidity that was the equivalent to that of a 0.5 McFarland standard was prepared using normal saline. Inoculation of the MIC plates took place within 15 min of adjustment of the turbidity of the inoculum suspension. MICs were interpreted using current CLSI breakpoints (25), with the following exceptions: U.S. FDA guidelines were used to interpret the MICs of meropenem-vaborbactam (susceptible, ≤4 μg/ml; intermediate, 8 μg/ml; resistant, ≥16 μg/ml) (15), ceftazidime-avibactam (susceptible, ≤8 μg/ml; resistant, ≥16 μg/ml) (26), and tigecycline (susceptible, ≤2 μg/ml; intermediate, 4 μg/ml; resistant, ≥8 μg/ml) (27). Polymyxin B lacks CLSI, U.S. FDA, or European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints for *Enterobacteriaceae*. Quality control testing was performed on each day of testing using *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC 700603, *K. pneumoniae* 1074, and *K. pneumoniae* BAA1705. All quality control results were within specified CLSI ranges (10).

ACKNOWLEDGMENTS

This project has been funded in whole or in part with federal funds from the U.S. Department of Health and Human Services, Office of the Assistant Secretary for Preparedness and Response, Biomedical Advanced Research and Development Authority (BARDA), under contract no. HHSO100201400002 and HHSO100201600026C and The Medicines Company, San Diego, CA. Funding from The Medicines Company included compensation for preparation of the manuscript.

M.A.H. and D.F.S. are employees of International Health Management Associates, Inc. (IHMA). O.L. and M.N.D. are employees of The Medicines Company. J.A.K. is an employee of the University of Manitoba and Diagnostic Services Manitoba and a consultant to IHMA. The authors employed by IHMA and J.A.K. do not have personal financial interests in the sponsor of this study and preparation of the manuscript (The Medicines Company).

REFERENCES

1. Kazmierczak KM, Biedenbach DJ, Hackel M, Rabine S, de Jonge BLM, Bouchillon SK, Sahm DF, Bradford PA. 2016. Global dissemination of bla_{KPC} into bacterial species beyond *Klebsiella pneumoniae* and *in vitro* susceptibility to ceftazidime-avibactam and aztreonam-avibactam. Antimicrob Agents Chemother 60:4490–4500. https://doi.org/10.1128/AAC.00107-16.
2. de Jonge BLM, Karlowsky JA, Kazmierczak KM, Biedenbach DJ, Sahm DF, Nichols WW. 2016. *In vitro* susceptibility to ceftazidime-avibactam of carbapenem-nonsusceptible *Enterobacteriaceae* isolates collected during the INFORM global surveillance study (2012 to 2014). Antimicrob Agents Chemother 60:3163–3169. https://doi.org/10.1128/AAC.03042-15.
3. Biedenbach D, Bouchillon S, Hackel M, Hoban D, Kazmierczak K, Hawser S, Badal R. 2015. Dissemination of NDM metallo-β-lactamase genes among clinical isolates of *Enterobacteriaceae* collected during the SMART global surveillance study from 2008 to 2012. Antimicrob Agents Chemother 59:826–830. https://doi.org/10.1128/AAC.03938-14.
4. Centers for Disease Control and Prevention. 2013. Antibiotic resistance threats in the United States, p 1–114. Centers for Disease Control and Prevention, Atlanta, GA. https://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf.
5. World Health Organization. 2014. Antimicrobial resistance global report on surveillance. World Health Organization, Geneva, Switzerland. http://www.who.int/drugresistance/documents/surveillancereport/en/.
6. Logan LK, Weinstein RA. 2017. The epidemiology of carbapenem-resistant *Enterobacteriaceae*: the impact and evolution of a global menace. J Infect Dis 215(Suppl 1):S28–S36. https://doi.org/10.1093/infdis/jiw282.
7. van Duin D, Doi Y. 2017. The global epidemiology of carbapenemase-producing Enterobacteriaceae. Virulence 8:460–469. https://doi.org/10.1080/21505594.2016.1222343.
8. Bush K. 2015. A resurgence of β-lactamase inhibitor combinations effective against multidrug-resistant gram-negative pathogens. Int J Antimicrob Agents 46:483–493. https://doi.org/10.1016/j.ijantimicag.2015.08.011.
9. Walsh TR. 2010. Emerging carbapenemases: a global perspective. Int J Antimicrob Agents 36(Suppl 3):S8–S14. https://doi.org/10.1016/S0924-8579(10)70004-2.
10. Clinical and Laboratory Standards Institute. 2017. Performance standards for antimicrobial susceptibility testing. Twenty-seventh informational supplement. M100-S27. Clinical and Laboratory Standards Institute, Wayne, PA.
11. Hecker SJ, Reddy KR, Totrov M, Hirst GC, Lomovskaya O, Griffith DC, King P, Tsivkovski R, Sun D, Sabet M, Tazazi Z, Clifton MC, Atkins K, Raymond A, Potts KT, Abendroth J, Boyer SH, Loutit JS, Morgan EE, Durso S, Dudley MN. 2015. Discovery of a cyclic boronic acid β-lactamase inhibitor (RPX7009) with utility versus class A serine carbapenemases. J Med Chem 58:3682–3692. https://doi.org/10.1021/acs.jmedchem.5b00127.
12. Livermore DM, Mushtaq S. 2013. Activity of biapenem (RPX2003)

combined with the boronate β-lactamase inhibitor RPX7009 against carbapenem-resistant Enterobacteriaceae. J Antimicrob Chemother 68: 1825–1831. https://doi.org/10.1093/jac/dkt118.
13. Lomovskaya O, Tsivkovski R. 2017. Vaborbactam (RPX7009) plus meropenem is active against the newly discovered BKC-1 and FR-1 carbapenemases, abstr P1289. Abstr 27th Eur Congr Clin Microbiol Infect Dis. European Society of Clinical Microbiology and Infectious Diseases, Basel, Switzerland.
14. Castanheira M, Rhomberg PR, Flamm RK, Jones RN. 2016. Effect of the β-lactamase inhibitor vaborbactam combined with meropenem against serine carbapenemase-producing *Enterobacteriaceae*. Antimicrob Agents Chemother 60:5454–5458. https://doi.org/10.1128/AAC.00711-16.
15. The Medicines Company. 2017. Vabomere™ (meropenem and vaborbactam) for injection, for intravenous use, prescribing information. Revised August. The Medicines Company, Parsippany, NJ.
16. Lapuebla A, Abdallah M, Olafisoye O, Cortes C, Urban C, Quale J, Landman D. 2015. Activity of meropenem combined with RPX7009, a novel β-lactamase inhibitor, against Gram-negative clinical isolates in New York City. Antimicrob Agents Chemother 59:4856–4860. https://doi.org/10.1128/AAC.00843-15.
17. Castanheira M, Huband MD, Mendes RE, Flamm RK. 2017. Meropenem-vaborbactam tested against contemporary gram-negative isolates collected worldwide during 2014, including carbapenem-resistant, KPC-producing, multidrug-resistant, and extensively drug-resistant *Enterobacteriaceae*. Antimicrob Agents Chemother 61:e00567-17. https://doi.org/10.1128/AAC.00567-17.
18. Griffith DC, Loutit JS, Morgan EE, Durso S, Dudley MN. 2016. Phase 1 study of the safety, tolerability, and pharmacokinetics of the β-lactamase inhibitor vaborbactam (RPX7009) in healthy adult subjects. Antimicrob Agents Chemother 60:6326–6332. https://doi.org/10.1128/AAC.00568-16.
19. Sabet M, Tarazi Z, Nolan T, Parkinson J, Rubio-Aparicio D, Lomovskaya O, Dudley MN, Griffith DC. 2014. *In vivo* efficacy of Carbavance (meropenem/RPX7009) against KPC-producing *Enterobacteriaceae*, abstr F-958. Abstr 54th Intersci Conf Antimicrob Agents Chemother, Washington, DC. American Society of Microbiology, Washington, DC.
20. Tarazi Z, Sabet M, Rubio-Aparicio D, Nolan T, Parkinson J, Lomovskaya O, Dudley MN, Griffith DC. 2014. Efficacy of simulated human exposures of Carbavance (meropenem-RPX7009) against carbapenem-resistant *Enterobacteriaceae* in an *in vitro* hollow fiber model, abstr F-959. Abstr 54th Intersci Conf Antimicrob Agents Chemother, Washington, DC. American Society for Microbiology, Washington, DC.
21. Shields RK, Clancy CJ, Hao B, Chen L, Press EG, Iovine NM, Kreiswirth BN, Nguyen MH. 2015. Effects of *Klebsiella pneumoniae* carbapenemase subtypes, extended-spectrum β-lactamases, and porin mutations on the *in vitro* activity of ceftazidime-avibactam against carbapenem-resistant *K. pneumoniae*. Antimicrob Agents Chemother 59:5793–5797. https://doi.org/10.1128/AAC.00548-15.
22. Shields RK, Chen L, Cheng S, Chavada KD, Press EG, Synder A, Pandey R, Doi Y, Kreiswirth BN, Nguyen MH, Clancy CJ. 2017. Emergence of ceftazidime-avibactam resistance due to plasmid-borne bla_{KPC-3} mutations during treatment of carbapenem-resistant *Klebsiella pneumoniae* infections. Antimicrob Agents Chemother 61:e02097-16. https://doi.org/10.1128/AAC.02097-16.
23. Reference deleted.
24. Lob SH, Kazmierczak KM, Badal RE, Hackel MA, Bouchillon SK, Biedenbach DJ, Sahm DF. 2015. Trends in susceptibility of *Escherichia coli* from intra-abdominal infections to ertapenem and comparators in the United States according to data from the SMART Program, 2009 to 2013. Antimicrob Agents Chemother 59:3606–3610. https://doi.org/10.1128/AAC.05186-14.
25. Clinical and Laboratory Standards Institute. 2015. M07-A10. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard, 10th ed. Clinical and Laboratory Standards Institute, Wayne, PA.
26. Allergan USA, Inc. 2017. Avycaz® (ceftazidime and avibactam) for injection, for intravenous use, prescribing information. Revised January. Allergan USA, Inc, Irvine, CA.
27. Pfizer Inc. 2016. Tygacil® (tigecycline) injection, powder, lyophilized, for solution, prescribing information. Revised June. Pfizer Inc, Philadelphia, PA.