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Multicenter Clinical Evaluation of Vitek 2 Meropenem-Vaborbactam for Susceptibility Testing of *Enterobacterales* and *Pseudomonas aeruginosa*

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ABSTRACT The carbapenem/beta-lactamase inhibitor meropenem-vaborbactam (MEV) used to treat complicated urinary tract infections and pyelonephritis in adults was approved in 2017 by the U.S. Food and Drug Administration (FDA). Here, we evaluated Vitek 2 MEV (bioMérieux, Durham, NC) compared to the reference broth microdilution (BMD) method. Of 449 *Enterobacterales* isolates analyzed per FDA/CLSI breakpoints, the overall performance was 98.2% essential agreement (EA), 98.7% category agreement (CA), and 0% very major errors (VME) or major errors (ME). For 438 FDA intended-for-use *Enterobacterales* isolates, performance was 98.2% EA, 98.6% CA, and 0% VME or ME. Evaluable EA was 81.0%, but with only 42 on-scale evaluable results. Individual species demonstrated EA and CA rates of ≥90% without any VME or ME. When evaluated using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, overall Vitek 2 MEV performance for *Enterobacterales* and *Pseudomonas aeruginosa* demonstrated 97.3% EA, 99.2% CA, 2.3% VME, and 0.6% ME (after error resolution: 97.3% EA, 99.4% CA, 2.2% VME, and 0.4% ME) compared to the reference BMD method. Performance for *P. aeruginosa* included 92.2% EA, 97.4% CA, 0% VME, and 3.0% ME (after error resolution: 92.2% EA, 98.7% CA, 0% VME, and 1.5% ME). Performance for *Enterobacterales* included 98.2% EA, 99.6% CA, 3.0% VME, and 0.2% ME. Evaluable EA was 80.6% but was based on only 67 evaluable results. These findings support Vitek 2 MEV as an accurate automated system for MEV susceptibility testing of *Enterobacterales* and *P. aeruginosa* and could be an alternate solution to the manual-labor-intensive reference BMD method.

KEYWORDS automated susceptibility testing, Vitek 2, meropenem-vaborbactam, AST

A new antimicrobial combination of the carbapenem and boronic acid beta-lactamase inhibitor (meropenem-vaborbactam; MEV) was reported effective in the treatment of complicated urinary tract infections and pyelonephritis caused by Gram-negative bacteria, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* species complex (1, 2). The combination also demonstrated superiority to treat carbapenem-resistant *Enterobacterales* (CRE) infection associated with bacteremia, hospital-acquired/ventilator-associated bacterial pneumonia, complicated intra-abdominal infection, and complicated urinary tract infection/acute pyelonephritis compared to the best available mono or combination therapies (3, 4). For example, MEV proved to be a potent inhibitor of serine carbapenemases, and *Klebsiella pneumoniae* carbapenemase (KPC) producing *Enterobacterales* compared to meropenem. Due to vaborbactam, MEV has potent activity against KPC as well as against Ambler Class A and Class C enzymes. MEV, however, lacks activity against metallo-β-lactamases or OXA-type

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β-lactamase enzymes (5). MEV showed better *in vitro* activity against multidrug-resistant and extensively drug-resistant isolates of *Pseudomonas aeruginosa* compared to that of meropenem alone (6). Susceptibility testing is critical to appropriately prescribe MEV since MEV resistance has recently been reported among KPC-producing *K. pneumoniae* strains resistant to ceftazidime-avibactam (7). The Vitek 2 (bioMérieux, Inc., Durham, NC) antimicrobial susceptibility test (AST) Gram-negative meropenem-vaborbactam (MEV) is an automated *in vitro* quantitative testing system for determining the antimicrobial susceptibility of *Enterobacterales* and *P. aeruginosa* against MEV as applicable per breakpoints developed by the U.S. Food and Drug Administration (FDA)/Clinical and Laboratory Standards Institute (CLSI) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST). This multicenter clinical evaluation of Vitek 2 MEV performance was conducted compared to the reference CLSI broth microdilution (BMD) method.

MATERIALS AND METHODS

Ethics. As applicable, each study site performing testing on clinical strains acquired local institutional review board approval or a waiver prior to study initiation.

Trial product. The Vitek 2 AST-GN cards had concentrations of MEV that included 0.5/8, 2/8, 8/8, and 32/8 μg/mL (equivalent standard method concentration by efficacy in μg/mL). The meropenem-vaborbactam *Enterobacterales* MIC result range for the Vitek 2 was $\leq$0.5/8 to $\geq$64/8 μg/mL. The Vitek 2 AST cards were used in conjunction with Vitek 2 systems (8). The BMD test was performed in accordance with CLSI standards (9, 10). BMD panels were prepared using meropenem concentrations of 0.003 to 256 μg/mL with vaborbactam at a fixed concentration of 8 μg/mL.

Setting. Testing was performed at Cleveland Clinic (CC; Cleveland, OH), the Clinical Microbiology Institute (CMI; Wilsonville, OR), the University of California–Los Angeles Medical Center (UCLA; Los Angeles, CA), and bioMérieux, Inc. (BMX; Hazelwood, MO).

Culture isolates. Challenge, reproducibility, and quality control (QC) organisms were provided by bioMérieux. Isolates were shipped frozen in Trypticase soy broth (Remel, San Diego, CA) with 15% glycerol and immediately upon receipt were stored at −70°C.

Clinical isolates were provided by the test sites as instructed according to Table 1 in the FDA guidance document (11), which requires a minimum of three sites and 100 isolates per site with isolates of species indicated for use (12), and no more than 50% of isolates tested can be from frozen stocks; in addition, for the EUCAST application, isolates of *P. aeruginosa* were included at approximately 25 isolates per site, as follows: CC, 100 isolates comprising 61 fresh ($\leq$7 days old) and 39 frozen (>7 days old); CMI, 103 isolates comprising 86 fresh ($\leq$7 days old) and 17 frozen (>7 days old); UCLA, 100 isolates comprising 70 fresh ($\leq$7 days old) and 30 frozen (>7 days old); and BMX, 28 frozen isolates (>7 days old).

Fresh isolates were subcultured once from primary plates to Trypticase soy agar with 5% sheep blood (Remel) and incubated at 35°C for 18 to 24 h prior to testing.

Frozen stock isolates were subcultured twice onto Trypticase soy agar with 5% sheep blood before being tested in this study. Primary subcultures (day 1 plates) were incubated until sufficient growth appeared, usually within 18 to 24 h at 35°C. Secondary subcultures (day 2 plates) were transferred from day 1 plates and incubated at 35°C for 18 to 24 h prior to testing.

All isolates were evaluated against EUCAST criteria (13), while only *Enterobacterales* isolates were evaluated against FDA/CLSI criteria (14).

Reproducibility study. The reproducibility set consisted of 10 Gram-negative organisms, including *E. coli* (n = 1), *Klebsiella aerogenes* (n = 1), and *Klebsiella pneumoniae* (n = 8). Each reproducibility isolate was tested in triplicate for 3 days by Vitek 2 automatic and manual dilution and Vitek 2 Compact manual dilution methods at three participating sites. At the end of the study, each reproducibility isolate had 27 card results for each method. Reproducibility was calculated assuming that all off-scale results were greater than one well from the mode.

Quality control study. Quality control (QC) was performed for the Vitek 2 and BMD each day of comparative testing at each site using the FDA/CLSI-recommended QC set, which includes *E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, *K. pneumoniae* ATCC BAA-1705, *K. pneumoniae* ATCC BAA-2814, and *K. pneumoniae* ATCC 700603. *Staphylococcus aureus* ATCC 29213 was tested as ancillary QC. QC testing was performed a minimum of 20 times at each site.

Comparative (clinical and challenge) study. A total of 449 *Enterobacterales* isolates (413 susceptible [S], 3 intermediate [I], and 33 resistant [R], per reference BMD results and FDA/CLSI breakpoint interpretations) were included in the comparative analysis. This included 331 clinical isolates comprising 217 (65.6%) fresh isolates (never frozen and tested within 7 days from isolation in culture) and 114 (34.4%) stock isolates (frozen for <3 years before testing). The clinical isolates were tested once each at the test site of origin using Vitek 2 automatic dilution at all three sites. The 118 challenge isolates (87 susceptible, 3 intermediate, and 28 resistant) were tested at one external site using the Vitek 2 automatic and manual dilutions and the Vitek 2 Compact manual dilution. A separate performance analysis on was also performed on 438 FDA intended for use (IFU) isolates out of 449 (97.6%). The FDA IFU species are shown in the results tables discussed below. For the EUCAST breakpoints, the comparative study included a total of 526 *Enterobacterales* and *P. aeruginosa* isolates, including 408 clinical isolates (392 susceptible and 16 resistant) and 118 challenge isolates (90 susceptible and 28 resistant), i.e., the

TABLE 1 Reproducibility performance

Test isolate	Method	No. of dilution differences between card result and card result mode							Card result mode
		Off scale (negative)	−2	−1	0	1	2	Off scale (positive)	
E. coli	Vitek 2 autodilution	5			22				32
	Vitek 2 manual dilution	3		1	23				32
	Compact manual dilution	4			23				32
K. aerogenes	Vitek 2 autodilution				23	4			4
	Vitek 2 manual dilution	1	2		22	2			4
	Compact manual dilution	1	1		23	2			4
K. pneumoniae	Vitek 2 autodilution			3	23	1			16
	Vitek 2 manual dilution				27				16
	Compact manual dilution			2	24	1			16
K. pneumoniae	Vitek 2 autodilution			3	17	6		1	2
	Vitek 2 manual dilution			3	15	9			2
	Compact manual dilution			2	20	3	1	1	2
K. pneumoniae	Vitek 2 autodilution			5	22				16
	Vitek 2 manual dilution			4	22	1			16
	Compact manual dilution			2	25				16
K. pneumoniae	Vitek 2 autodilution			2	24	1			16
	Vitek 2 manual dilution			1	25	1			16
	Compact manual dilution			1	26				16
K. pneumoniae	Vitek 2 autodilution				27				16
	Vitek 2 manual dilution				27				16
	Compact manual dilution				27				16
K. pneumoniae	Vitek 2 autodilution				16	11			16
	Vitek 2 manual dilution				26			1	16
	Compact manual dilution				22	4		1	16
K. pneumoniae	Vitek 2 autodilution			1	25	1			16
	Vitek 2 manual dilution			1	26				16
	Compact manual dilution			1	25	1			16
K. pneumoniae	Vitek 2 autodilution				27				16
	Vitek 2 manual dilution				26	1			16
	Compact manual dilution				27				16
All species[a]	Vitek 2 autodilution	5		14	226	24		1	
	Vitek 2 manual dilution	4	2	10	239	14		1	
	Compact manual dilution	5	1	8	242	11	1	2	

[a]Combined reproducibility: Vitek 2 autodilution, 264/270 = 97.8%; Vitek 2 manual dilution, 263/270 = 97.4%; Compact manual dilution, 261/270 = 96.7%.

same set of 449 *Enterobacterales* isolates (IFU and non-IFU) plus 77 (66 susceptible and 11 resistant) isolates of *P. aeruginosa* evaluated against the EUCAST breakpoints described below in "Data analysis."

Susceptibility testing. Each isolate was first subcultured on Trypticase soy agar with 5% sheep blood. After an 18- to 24-h culture at 35°C ± 2°C, a suspension in the 0.5 to 0.63 McFarland range was prepared in 0.45% aqueous NaCl (bioMérieux) using the DensiChek Plus instrument (bioMérieux). Clinical, challenge, reproducibility, and QC isolates were all tested using the Vitek 2 automatic dilution method. In addition, the Vitek 2 and Vitek 2 Compact manual dilution methods were evaluated for QC, reproducibility, and challenge testing to demonstrate equivalence with the automatic dilution method. Either dilution method can be selected on Vitek 2, while Vitek 2 Compact only offers a manual dilution method. For each isolate, a single initial McFarland suspension was prepared for inoculation of Vitek 2 cards and the BMD method for comparative testing. Each method was performed once unless otherwise mentioned.

Data analysis. Comparative data analysis was performed using the FDA Guidance for Industry and FDA Class II Special Controls Guidance Document "Antimicrobial Susceptibility Test [AST] Systems" (11) and ISO 20776-2 (15) susceptibility performance criteria and the FDA-recognized CLSI (*Enterobacterales*, ≤4 [S], 8 [I], ≥16 [R]) (14) and EUCAST (13) breakpoints (*Enterobacterales* and *P. aeruginosa*, ≤8 [S], ≥16 [R]). Comparative performance analysis of clinical and challenge testing was conducted using essential agreement (EA), categorical agreement (CA), very major error (VME), major error (ME), and minor error (mE) rates. EA was calculated as the percentage of total isolates with the test and reference method results within one doubling-dilution of each other and including all (on-scale and off-scale) results. Evaluable EA was calculated as the percentage of test results that were on-scale and in EA with the reference method, defined as follows: "Evaluable (on-scale) organisms are those that fall within the test range of the reference and have the opportunity for a result on

TABLE 2 Quality control performance for meropenem/vaborbactam

Species (ATCC no.)	CLSI QC range	Vitek 2 MEV range	No. (%) of results within range			No. (%) of reference results within range
			Auto	Manual	Compact	
QC						
E. coli (25922)	0.008–0.06	≤0.5	116 (100)	90 (100)	90 (100)	116 (100)
E. coli (35218)	0.008–0.06	≤0.5	116 (100)	91 (100)	91 (100)	116 (100)
K. pneumoniae (BAA-1705)	0.008–0.06	≤0.5	114 (100)	89 (100)	89 (100)	114 (100)
K. pneumoniae (BAA-2814)	0.12–0.5	≤0.5	115 (100)	89 (98.9)	89 (100)	111 (96.5)
K. pneumoniae (700603)	0.016–0.06	≤0.5	115 (100)	89 (100)	89 (100)	114 (99.1)
P. aeruginosa (27853)	0.12–1	≤0.5–1	117 (100)	92 (100)	92 (100)	117 (100)
Ancillary QC						
Staphylococcus aureus (29213)	0.03–0.12					119 (100)

the test method that could also be on-scale. Any reference result that falls in the < or > category is considered not evaluable" (11). CA was calculated as the percentage of total test results in agreement of interpretive results (susceptible, intermediate, and resistant) of the reference method using FDA/CLSI or EUCAST interpretive criteria as indicated above. The VME rate was defined as the percentage of isolates interpreted as resistant by the reference method and as susceptible by the Vitek 2 method. The ME rate was defined as the percentage of isolates interpreted as susceptible by the reference method but resistant by the Vitek 2 method. The mE rate was defined as the percentage of isolates interpreted as intermediate by the reference method but susceptible or resistant by the Vitek 2 method or intermediate by the Vitek 2 method but susceptible or resistant by the reference method. Isolates that by definition had VME or ME but were within EA of the reference method were noted as errors, but were considered acceptable when taking the EA into consideration. Regarding ISO criteria, error resolution was performed per ISO standard 20776-2, section 5.2.7 (15), and overall performance was recorded after error resolution. Any discrepancy was resolved by triplicate testing of the reference method using separate bacterial inocula. The mode of the triplicate results of the reference method replaced the original result for determining the error rate.

RESULTS

Reproducibility of Vitek 2 MEV. Ten on-scale isolates (*K. aerogenes*, *n* = 1; *E. coli*, *n* = 1; and *K. pneumoniae*, *n* = 8) were tested at CC, CMI, and UCLA study sites as described in Materials and Methods. A total of 270 tests (90 per site) were performed. Mode values of MEV MICs for each isolate tested for Vitek 2 auto and manual and Vitek Compact manual dilution methods are shown in Table 1. The reproducibility set performance, including the mode MIC value, for Vitek 2 automatic and manual and Compact manual dilution methods is included. The Vitek 2 reproducibility for autodilution was 97.8% and for manual dilution was 97.4%. The Vitek 2 Compact reproducibility was 96.7%. All reproducibility results met the ≥ 95% reproducibility requirement for FDA and ISO (11, 15) (Table 1).

Quality control of Vitek 2 MEV. QC testing was performed for Vitek 2 autodilution, Vitek 2 manual dilution, Vitek 2 Compact manual dilution, and the reference method at each site. QC results were within acceptable limits (11, 15) of ≥95% of QC tests performed on each quality control organism for all Vitek 2 methods (Table 2).

Vitek 2 MEV challenge study. To examine the test accuracy, MICs of challenge organisms derived by Vitek 2 autodilution and BMD methods were compared. All 118 challenge *Enterobacterales* isolates grew in the Vitek 2 cards. FDA/CLSI breakpoints have not been established for MEV and *P. aeruginosa*, so *P. aeruginosa* is not included in the U.S. FDA indication of Vitek 2 MEV. The overall challenge set performance for Vitek 2 (FDA/CLSI breakpoint, *Enterobacterales*, ≤4 [S], 8 [I], ≥16 [R]) included 95.8% (113/118) EA, 96.6% (114/118) CA, and no VME or ME (Table 3). There were 36 evaluable results of which 31 (86.1%) were within evaluable EA. Of the six species that included evaluable results, only *K. aerogenes*, *Klebsiella oxytoca*, and *K. pneumoniae* showed an evaluable EA of >90%. The challenge performance for Vitek 2 manual dilution included 94.9% (112/118) EA, 96.6% (114/118) CA, 1.1% ME (1/87), and no VME. Vitek 2 Compact challenge performance included 94.1% (111/118) EA, 97.5% (115/118) CA, and no VME or ME (data not shown). For FDA/CLSI breakpoints, all individual challenge set species had ≥90% EA and CA, with the exceptions of *E. cloacae* with 88.9% (16/18) EA, 94.4% (17/18) CA, and no VME or ME and *M. morganii* with 0.0% (0/1) EA and 0.0% (0/1) CA with no VME or ME (Table 3).

TABLE 3 Performance by FDA criteria using the autodilution mode

Organism(s)	Source	No. tested	CLSI/FDA interpretive criteria (IFU and non-IFU)[a]								
			No. (%) of results by broth microdilution			Performance [no. [%])					
			S	I	R	EA	No. evaluable (% evaluable EA)	CA	VME	ME	mE
Citrobacter braakii[b]	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
C. freundii	Clinical	7	7 (100)	0 (0)	0 (0)	7 (100)	0 (NA)	7 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	8	8 (100)	0 (0)	0 (0)	8 (100)	0 (NA)	8 (100)	0 (0)	0 (0)	0 (0)
C. koseri	Clinical	5	5 (100)	0 (0)	0 (0)	5 (100)	0 (NA)	5 (100)	0 (0)	0 (0)	0 (0)
Enterobacter cloacae	Clinical	10	10 (100)	0 (0)	0 (0)	10 (100)	0 (NA)	10 (100)	0 (0)	0 (0)	0 (0)
	Challenge	18	16 (88.9)	1 (5.6)	1 (5.6)	16 (88.9)	2 (0.0)	17 (94.4)	0 (0)	0 (0)	1 (5.6)
	Both	28	26 (92.9)	1 (3.6)	1 (3.6)	26 (92.9)	2 (0.0)	27 (96.4)	0 (0)	0 (0)	1 (3.6)
E. cloacae complex	Clinical	22	22 (100)	0 (0)	0 (0)	22 (100)	0 (NA)	22 (100)	0 (0)	0 (0)	0 (0)
Escherichia coli	Clinical	97	95 (97.9)	0 (0)	2 (2.1)	96 (99)	2 (50)	97 (100)	0 (0)	0 (0)	0 (0)
	Challenge	10	3 (30)	0 (0)	7 (70)	9 (90)	7 (85.7)	10 (100)	0 (0)	0 (0)	0 (0)
	Both	107	98 (91.6)	0 (0)	9 (8.4)	105 (98.1)	9 (77.8)	107 (100)	0 (0)	0 (0)	0 (0)
E. vulneris[b]	Clinical	2	2 (100)	0 (0)	0 (0)	2 (100)	0 (NA)	2 (100)	0 (0)	0 (0)	0 (0)
Klebsiella (*Enterobacter*) *aerogenes*	Clinical	39	39 (100)	0 (0)	0 (0)	38 (97.4)	1 (0)	38 (97.4)	0 (0)	0 (0)	1 (2.6)
	Challenge	1	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	40	40 (100)	0 (0)	0 (0)	39 (97.5)	2 (50)	39 (97.5)	0 (0)	0 (0)	1 (2.5)
K. oxytoca	Clinical	23	22 (95.7)	0 (0)	1 (4.3)	23 (100)	1 (100)	23 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	24	23 (95.8)	0 (0)	1 (4.2)	24 (100)	2 (100)	24 (100)	0 (0)	0 (0)	0 (0)
K. pneumoniae subsp. *ozaenae*	Challenge	1	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
K. pneumoniae subsp. *pneumoniae*	Clinical	4	3 (75)	0 (0)	1 (25)	4 (100)	1 (100)	3 (75)	0 (0)	0 (0)	1 (25)
	Challenge	7	7 (100)	0 (0)	0 (0)	7 (100)	2 (100)	7 (100)	0 (0)	0 (0)	0 (0)
	Both	11	10 (90.9)	0 (0)	1 (9.1)	11 (100)	3 (100)	10 (90.9)	0 (0)	0 (0)	1 (9.1)
K. pneumoniae	Clinical	52	51 (98.1)	0 (0)	1 (1.9)	52 (100)	0 (NA)	52 (100)	0 (0)	0 (0)	0 (0)
	Challenge	77	57 (74)	1 (1.3)	19 (24.7)	76 (98.7)	21 (95.2)	75 (97.4)	0 (0)	0 (0)	2 (2.6)
	Both	129	108 (83.7)	1 (0.8)	20 (15.5)	128 (99.2)	21 (95.2)	127 (98.4)	0 (0)	0 (0)	2 (1.6)
Lelliotia amnigena[b]	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
Morganella morganii	Clinical	9	9 (100)	0 (0)	0 (0)	9 (100)	0 (NA)	9 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	0 (0)	1 (100)	0 (0)	0 (0)	1 (0)	0 (0)	0 (0)	0 (0)	1 (100)
	Both	10	9 (90)	1 (10)	0 (0)	9 (90)	1 (0)	9 (90)	0 (0)	0 (0)	1 (10)
Pantoea dispersa[b]	Clinical	2	2 (100)	0 (0)	0 (0)	2 (100)	0 (NA)	2 (100)	0 (0)	0 (0)	0 (0)

(Continued on next page)

TABLE 3 (Continued)

Organism(s)	Source	No. tested	CLSI/FDA interpretive criteria (IFU and non-IFU)[a]								
			No. (%) of results by broth microdilution			Performance [no. [%])					
			S	I	R	EA	No. evaluable (% evaluable EA)	CA	VME	ME	mE
Proteus mirabilis	Clinical	25	25 (100)	0 (0)	0 (0)	24 (96)	1 (0)	25 (100)	0 (0)	0 (0)	0 (0)
P. penneri[b]	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
P. vulgaris[b]	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
Providencia rettgeri	Clinical	3	3 (100)	0 (0)	0 (0)	3 (100)	0 (NA)	3 (100)	0 (0)	0 (0)	0 (0)
P. stuartii	Clinical	4	4 (100)	0 (0)	0 (0)	4 (100)	0 (NA)	4 (100)	0 (0)	0 (0)	0 (0)
Raoultella ornithinolytica[b]	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
Serratia fonticola[b]	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
S. marcescens	Clinical	20	20 (100)	0 (0)	0 (0)	20 (100)	0 (NA)	20 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	0 (0)	0 (0)	1 (100)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	21	20 (95.2)	0 (0)	1 (4.8)	21 (100)	0 (NA)	21 (100)	0 (0)	0 (0)	0 (0)
S. rubidaea[b]	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
All species (IFU and non-IFU)	Clinical	331	326 (98.5)	0 (0)	5 (1.5)	328 (99.1)	6 (50)	329 (99.4)	0 (0)	0 (0)	2 (0.6)
	Challenge	118	87 (73.7)	3 (2.5)	28 (23.7)	113 (95.8)	36 (86.1)	114 (96.6)	0 (0)	0 (0)	4 (3.4)
	Both	449	413 (92)	3 (0.7)	33 (7.3)	441 (98.2)	42 (81)	443 (98.7)	0 (0)	0 (0)	6 (1.3)
All species (IFU only)	Clinical	320	315 (98.4)	0 (0)	5 (1.6)	317 (99.1)	6 (50)	318 (99.4)	0 (0)	0 (0)	2 (0.6)
	Challenge	118	87 (73.7)	3 (2.5)	28 (23.7)	113 (95.8)	36 (86.1)	114 (96.6)	0 (0)	0 (0)	4 (3.4)
	Both	438	402 (91.8)	3 (0.7)	33 (7.5)	430 (98.2)	42 (81)	432 (98.6)	0 (0)	0 (0)	6 (1.4)

[a]S, susceptible; I, intermediate; R, resistant; EA, essential agreement; CA, category agreement; VME, very major error; ME, major error; mE, minor error; NA, not applicable.
[b]Non-IFU species.

TABLE 4 Performance by EUCAST/ISO criteria using the autodilution mode (before ISO error resolution)

Organism(s)	Source	No. tested	EUCAST/ISO interpretive criteria[a]								
			No. (%) of results by broth microdilution			Performance (no. [%])					
			S	I	R	EA	Evaluable EA	CA	VME	ME	mE
Citrobacter braakii	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
C. freundii	Clinical	7	7 (100)	0 (0)	0 (0)	7 (100)	0 (NA)	7 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	8	8 (100)	0 (0)	0 (0)	8 (100)	0 (NA)	8 (100)	0 (0)	0 (0)	0 (0)
C. koseri	Clinical	5	5 (100)	0 (0)	0 (0)	5 (100)	0 (NA)	5 (100)	0 (0)	0 (0)	0 (0)
Enterobacter cloacae	Clinical	10	10 (100)	0 (0)	0 (0)	10 (100)	0 (NA)	10 (100)	0 (0)	0 (0)	0 (0)
	Challenge	18	17 (94.4)	0 (0)	1 (5.6)	16 (88.9)	2 (0.0)	18 (100)	0 (0)	0 (0)	0 (0)
	Both	28	27 (96.4)	0 (0)	1 (3.6)	26 (92.9)	2 (0.0)	28 (100)	0 (0)	0 (0)	0 (0)
E. cloacae complex	Clinical	22	22 (100)	0 (0)	0 (0)	22 (100)	0 (NA)	22 (100)	0 (0)	0 (0)	0 (0)
Escherichia coli	Clinical	97	95 (97.9)	0 (0)	2 (2.1)	96 (99)	2 (50)	97 (100)	0 (0)	0 (0)	0 (0)
	Challenge	10	3 (30)	0 (0)	7 (70)	9 (90)	7 (85.7)	10 (100)	0 (0)	0 (0)	0 (0)
	Both	107	98 (91.6)	0 (0)	9 (8.4)	105 (98.1)	9 (77.8)	107 (100)	0 (0)	0 (0)	0 (0)
E. vulneris	Clinical	2	2 (100)	0 (0)	0 (0)	2 (100)	0 (NA)	2 (100)	0 (0)	0 (0)	0 (0)
Klebsiella (*Enterobacter*) *aerogenes*	Clinical	39	39 (100)	0 (0)	0 (0)	38 (97.4)	1 (0)	39 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	40	40 (100)	0 (0)	0 (0)	39 (97.5)	2 (50)	40 (100)	0 (0)	0 (0)	0 (0)
K. oxytoca	Clinical	23	22 (95.7)	0 (0)	1 (4.3)	23 (100)	1 (100)	23 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	24	23 (95.8)	0 (0)	1 (4.2)	24 (100)	2 (100)	24 (100)	0 (0)	0 (0)	0 (0)
K. pneumoniae subsp. *ozaenae*	Challenge	1	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	0 (0)	0 (0)	0 (0)
K. pneumoniae subsp. *pneumoniae*	Clinical	4	4 (100)	0 (0)	1 (25)	4 (100)	1 (100)	3 (75)	1 (100)	0 (0)	0 (0)
	Challenge	7	7 (100)	0 (0)	0 (0)	7 (100)	2 (100)	7 (100)	0 (0)	0 (0)	0 (0)
	Both	11	11 (100)	0 (0)	1 (9.1)	11 (100)	3 (100)	10 (90.9)	1 (100)	0 (0)	0 (0)
K. pneumoniae	Clinical	52	51 (98.1)	0 (0)	1 (1.9)	52 (100)	0 (NA)	52 (100)	0 (0)	0 (0)	0 (0)
	Challenge	77	58 (75.3)	0 (0)	19 (24.7)	76 (98.7)	21 (95.2)	76 (98.7)	0 (0)	1 (1.7)	0 (0)
	Both	129	109 (84.5)	0 (0)	20 (15.5)	128 (99.2)	21 (95.2)	128 (99.2)	0 (0)	1 (0.9)	0 (0)
Lelliotia amnigena	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
Morganella morganii	Clinical	9	9 (100)	0 (0)	0 (0)	9 (100)	0 (NA)	9 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	1 (100)	0 (0)	0 (0)	0 (0)	1 (0)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	10	10 (100)	0 (0)	0 (0)	9 (90)	1 (0)	10 (100)	0 (0)	0 (0)	0 (0)
Pantoea dispersa	Clinical	2	2 (100)	0 (0)	0 (0)	2 (100)	0 (NA)	2 (100)	0 (0)	0 (0)	0 (0)

(Continued on next page)

TABLE 4 (Continued)

Organism(s)	Source	No. tested	EUCAST/ISO interpretive criteria[a]								
			No. (%) of results by broth microdilution			Performance (no. [%])					
			S	I	R	EA	Evaluable EA	CA	VME	ME	mE
Proteus mirabilis	Clinical	25	25 (100)	0 (0)	0 (0)	24 (96)	1 (0)	25 (100)	0 (0)	0 (0)	0 (0)
P. penneri	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
P. vulgaris	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
Providencia rettgeri	Clinical	3	3 (100)	0 (0)	0 (0)	3 (100)	0 (NA)	3 (100)	0 (0)	0 (0)	0 (0)
P. stuartii	Clinical	4	4 (100)	0 (0)	0 (0)	4 (100)	0 (NA)	4 (100)	0 (0)	0 (0)	0 (0)
Pseudomonas aeruginosa	Clinical	77	66 (85.7)	0 (0)	11 (14.3)	71 (92.2)	20 (80)	75 (97.4)	0 (0)	2 (3)	0 (0)
Raoultella ornithinolytica	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
Serratia fonticola	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
S. marcescens	Clinical	20	20 (100)	0 (0)	0 (0)	20 (100)	0 (NA)	20 (100)	0 (0)	0 (0)	0 (0)
	Challenge	1	0 (0)	0 (0)	1 (100)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
	Both	21	20 (95.2)	0 (0)	1 (4.8)	21 (100)	0 (NA)	21 (100)	0 (0)	0 (0)	0 (0)
S. rubidaea	Clinical	1	1 (100)	0 (0)	0 (0)	1 (100)	0 (NA)	1 (100)	0 (0)	0 (0)	0 (0)
All species	Clinical	408	392 (96.1)	0 (0)	16 (3.9)	399 (97.8)	31 (74.2)	405 (99.3)	1 (6.3)	2 (0.5)	0 (0)
	Challenge	118	90 (76.3)	0 (0)	28 (23.7)	113 (95.8)	36 (86.1)	117 (99.2)	0 (0)	1 (1.1)	0 (0)
	Both[b]	526	482 (91.6)	0 (0)	44 (8.4)	512 (97.3)	67 (80.6)	522 (99.2)	1 (2.3)	3 (0.6)	0 (0)

[a]S, susceptible; I, intermediate; R, resistant; EA, essential agreement; CA, category agreement; VME, very major error; ME, major error; mE, minor error; NA, not applicable.
[b]After error resolution: 97.3% EA, 99.4% CA, 2.2% VME, and 0.4% ME.

The challenge set for EUCAST included 118 *Enterobacterales* isolates with 95.8% (113/118) EA and 99.2% (117/118) CA, no VME, and 1.1% (1/90) ME for Vitek 2 autodilution (Table 4). For Vitek 2 manual dilution, 94.9% EA (112/118) and 98.3% (116/118) CA were reported, with no VME and 2.2% (2/90) ME. There were 67 evaluable results, of which 54 (80.6%) were within evaluable EA. Of the six species that included evaluable results, only *K. aerogenes*, *K. oxytoca*, and *K. pneumoniae* showed an evaluable EA of >90%. For Vitek 2 Compact dilution, 94.1% EA and 99.2% CA were reported, with no VME and 1.1% (1/90) ME (data not shown). All individual species tested had ≥90% EA and CA, with the exceptions of *E. cloacae* with 88.9% (16/18) EA and 100% (18/18) CA and *M. morganii* with 0.0% EA (0/1) and 100% CA (1/1). As noted earlier, no VME was reported among any species, and the only ME observed was with *K. pneumoniae* (1/58) (Table 4).

Clinical performance of Vitek 2 MEV. The clinical performance using FDA/CLSI criteria was evaluated using a total of 331 *Enterobacterales* isolates, including 217 (65.6%) fresh and 114 (34.4%) stock isolates. EA was 99.1% (328/331) and CA was 99.4% (329/331) with 0% (0/5) VME and 0% (0/326) ME. There were 6 evaluable results, of which 3 (50.0%) were within evaluable EA. Of the six species that included evaluable results, only *K. oxytoca* and *K. pneumoniae* showed an evaluable EA of >90%. All individual species tested demonstrated an EA of >90%. CA for all species was >90%, except for *K. pneumoniae* subsp. *pneumoniae*, which was 75% (3/4), for which one isolate (1/4) had a minor error. Out of these 331 clinical isolates, performance analysis for FDA indicated for use (IFU) isolates only was for 320 isolates, excluding *Citrobacter braakii* (*n* = 1), *Escherichia vulneris* (*n* = 2), *Lelliottia amnigena* (*n* = 1), *Pantoea dispersa* (*n* = 2), *Proteus penneri* (*n* = 1), *Proteus vulgaris* (*n* = 1), *Raoultella ornithinolytica* (*n* = 1), *Serratia fonticola* (*n* = 1), and *Serratia rubidaea* (*n* = 1). For FDA IFU organisms only, performance was 99.1% (317/320) EA, 99.4% CA (318/320), and no VME or ME (Table 3).

EUCAST MEV breakpoints for *Enterobacterales* (≤8 [S] and ≥16 [R]) and *P. aeruginosa* (≤8 [S] and ≥16 [R]) do not include an "intermediate" category; rather, isolates with MICs of ≤8 μg/mL are susceptible, and those with MICs of >8 μg/mL are resistant. For EUCAST breakpoint analysis, a total of 408 clinical *Enterobacterales* and *Pseudomonas* isolates were analyzed; performance included 97.8% (399/408) EA, 99.3% (405/408) CA, 6.3% (1/16) VME, and 0.5% (2/392) ME (after error resolution, 97.8% [399/408] EA, 99.5% [406/408] CA, 5.9% [1/17] VME, and 0.3% [1/391] ME) (Table 4). There were 31 evaluable results, of which 23 (74.2%) were within evaluable EA. Of the six species that included evaluable results, only *K. oxytoca* and *K. pneumoniae* showed an evaluable EA of >90%.

Overall analysis. For FDA/CLSI breakpoints, overall performance combining clinical and challenge *Enterobacterales* isolates was 98.2% (441/449) EA, 98.7% (443/449) CA, 0% (0/33) VME, and 0% (0/413) ME. The MIC distribution of all *Enterobacterales* isolates using FDA/CLSI breakpoints is shown in Table S1 in the supplemental material. For FDA IFU organisms only, overall performance included 98.2% (430/438) EA, 98.6% (432/438) CA, 0% (0/33) VME, and 0% (0/402) ME (Table 3). The overall MIC distribution of FDA IFU organisms only is shown in Table S2.

A total of 526 *Enterobacterales* and *P. aeruginosa* isolates were analyzed using EUCAST breakpoints, which demonstrated overall 97.3% (512/526) EA, 99.2% (522/526) CA, 2.3% (1/44) VME, and 0.6% (3/482) ME (after error resolution, 97.3% [512/526] EA, 99.4% [523/526] CA, 2.2% [1/45] VME, and 0.4% [2/481] ME). Overall performance of *Enterobacterales* was 98.2% (441/449) EA, 98.7% (443/449) CA, 0% (0/33) VME, and 0% (0/413) ME. Overall performance for *P. aeruginosa* remained the same as clinical performance, since no challenge performance was evaluated (Table 4). The overall MIC distribution of organisms using EUCAST breakpoints is shown in Table S3.

Molecular characterization of *Enterobacterales* isolates tested with Vitek 2 MEV. *Enterobacterales* isolates characterized for resistance markers harboring mostly β-lactamases, including AmpC (ACT/MIR), extended-spectrum β-lactamases (ESBLs; CTX-M, TEM, and SHV), and carbapenemases (KPC and OXA), were included in the Vitek 2 MEV performance analysis and were consistent with BMD results (Table 5). Consistent with MEV drug labeling (12), the isolates with metallo-β-lactamase (NDM and/or VIM) carbapenemases were resistant to MEV (data not shown). The performance of Vitek 2 could not be established for porin mutations combined with overexpression of efflux pumps, nor for SME or CMY, as these enzyme

TABLE 5 Molecular characterization profile of a subset of the *Enterobacterales* isolates[b]

Isolate name	Source	BMD MIC (μg/mL)	Vitek 2 MIC (μg/mL)	KPC				OXA				CTX-M-15	TEM				SHV					ACT	
				NOS[a]	2	3	6	1	9	48	181		NOS[a]	1A	1B	1D	NOS[a]	1	11	12	26	7	16
K. pneumoniae	CDC	0.5	≤0.5																	x			
K. pneumoniae	CDC	1	1					x	x			x		x						x			
K. pneumoniae subsp. *ozaenae*	CDC	4	4								x	x									x		
E. coli	CDC	0.015	≤0.5			x			x					x									
E. aerogenes	CDC	2	2							x													
K. pneumoniae	CDC	0.015	≤0.5			x																	
E. coli	CDC	0.03	≤0.5			x									x								
K. pneumoniae	CDC	0.03	≤0.5			x								x									
C. freundii	CDC	0.03	≤0.5		x																		
K. pneumoniae	CDC	0.125	≤0.5			x			x					x									
K. pneumoniae	CDC	0.03	≤0.5		x											x							
K. pneumoniae	CDC	0.03	≤0.5			x			x						x								
K. pneumoniae	CDC	0.03	≤0.5			x								x									
K. pneumoniae	CDC	2	1								x	x									x		
K. pneumoniae	CDC	2	4								x	x									x		
E. cloacae	CDC	0.03	≤0.5		x			x				x			x							x	
E. cloacae	CDC	0.06	≤0.5				x	x							x								x
K. pneumoniae subsp. *ozaenae*	CDC	0.125	≤0.5			x			x					x				x						
K. pneumoniae	CDC	0.25	≤0.5			x			x					x					x					
K. pneumoniae	CDC	0.015	≤0.5		x				x					x									
K. pneumoniae	CDC	0.03	≤0.5		x														x				
K. pneumoniae	CDC	0.015	≤0.5		x														x				
E. cloacae	Rempex	0.25	≤0.5			x																	
E. cloacae	Rempex	0.06	≤0.5	x									x										
K. pneumoniae	Rempex	0.25	≤0.5	x									x				x						
K. pneumoniae	Rempex	0.03	≤0.5	x									x				x						
K. pneumoniae	Rempex	0.015	≤0.5		x								x						x				
K. pneumoniae	Rempex	0.03	≤0.5		x																		
K. pneumoniae	Rempex	0.5	≤0.5		x								x						x				
K. pneumoniae	Rempex	0.015	≤0.5		x								x						x				
K. pneumoniae	Rempex	0.25	≤0.5			x							x						x				
K. pneumoniae	Rempex	0.03	≤0.5			x							x					x					
K. pneumoniae	Rempex	0.25	≤0.5			x							x						x				
K. pneumoniae	Rempex	0.25	≤0.5		x							x	x						x				
K. pneumoniae	Rempex	0.25	≤0.5			x							x						x				
K. pneumoniae	Rempex	0.25	≤0.5		x																		
K. pneumoniae	Rempex	0.03	≤0.5	x									x				x						
K. pneumoniae	Rempex	0.5	≤0.5	x								x	x				x						
K. pneumoniae	Rempex	4	2	x													x						
K. pneumoniae	Rempex	4	8	x									x				x						
K. pneumoniae	Rempex	1	≤0.5	x									x				x						
K. pneumoniae	Rempex	0.125	≤0.5	x									x				x						
K. pneumoniae	Rempex	0.06	≤0.5		x								x							x			
K. pneumoniae	Rempex	0.06	≤0.5			x							x						x	x			
K. pneumoniae	Rempex	0.25	≤0.5			x							x						x	x			
K. pneumoniae	Rempex	0.5	≤0.5	x													x						
K. pneumoniae	Rempex	0.5	≤0.5	x									x				x						
K. pneumoniae	Rempex	4	2		x												x						
K. pneumoniae	Rempex	0.06	≤0.5			x							x				x						
K. pneumoniae	Rempex	0.03	≤0.5			x							x				x						
K. pneumoniae	Rempex	0.25	≤0.5	x								x					x						
K. pneumoniae	Rempex	0.25	≤0.5	x								x					x						
K. pneumoniae	Rempex	0.5	≤0.5	x								x					x						
K. pneumoniae	Rempex	0.5	≤0.5	x								x					x						

[a]NOS, not otherwise specified.

[b]KPC, *K. pneumoniae* carbapenemase; OXA, oxacillinase; CTX-M, cefotaximase–Munich-type β-lactamase; TEM, Temoneira β-lactamase; SHV, sulfhydryl reagent variable β-lactamase; ACT, AmpC-type β-lactamase (21); x, marker present.

group characterizations for *Enterobacterales* were not available at the time of study and could not be analyzed.

DISCUSSION

MEV is a combination of meropenem and a novel β-lactamase inhibitor, vaborbactam, with a broad spectrum of enzyme inhibition that includes β-lactamases and ESBLs of the KPC, SME, TEM, SHV, CTX-M, CMY, and ACT groups but that does not demonstrate activity against metallo-β-lactamase-producing strains (4, 16).

It is critical to take advantage of antimicrobial susceptibility testing for new drugs to facilitate the clinical use of these antimicrobial agents. Automated AST systems provide advantages over the reference BMD method, since they are less labor-intensive, automated determination standardizes results compared to manual interpretation, and they provide relatively rapid results that are better suited for routine testing in clinical microbiology laboratories. As MEV was recently approved for the treatment of complicated urinary tract infections, including pyelonephritis caused by susceptible isolates of *E. coli*, *K. pneumoniae*, and *E. cloacae* species complex, commercial AST methods have only recently become available. Systematic assessment of alternative methods to BMD has not been widely published for MEV susceptibility testing. Prior data on the accuracy of commercial AST methods for MEV are limited.

There is no report on the systematic assessment of an automated susceptibility testing system except a multicenter study using the MicroScan dried Gram-negative MIC panel (17). In that study, 98.2% EA, 98.5% CA, 3.2% VME, and no ME were reported when 560 *Enterobacterales* clinical isolates were analyzed using the WalkAway system with a turbidity inoculation preparation method.

In the current study, the performance of Vitek 2 MEV was also compared to that of BMD according to EUCAST (*Enterobacterales* and *P. aeruginosa*) breakpoints using ISO criteria. Overall, Vitek MEV performance, including that for *Enterobacterales* and *P. aeruginosa*, was 97.3% (512/526) EA, 99.2% (522/526) CA, 2.3% (1/44) VME, and 0.6% (3/482) ME (after error resolution, 97.3% EA, 99.4% CA, 2.2% VME, and 0.4% ME). In a previous study using the gradient method Etest, the performance of *Enterobacterales* (excluding *Proteus mirabilis*) demonstrated acceptable performance when evaluated using the FDA/CLSI breakpoints. Using the EUCAST breakpoints that also include *P. aeruginosa*, authors found an unacceptably high VME rate of 7.1%, reflecting the lack of an intermediate (I) category, despite 95.2% EA, 99.2% CA, and 0.5% ME compared to the reference method (18). Disk diffusion may also be used for susceptibility testing of MEV according to the CLSI standard (19), while EUCAST has not yet released clinical breakpoints for disk diffusion testing of the drug (13).

In this multicenter study, geographically diverse study locations in the United States were used to evaluate the performance of Vitek 2 as an alternate automated method for antimicrobial susceptibility testing of MEV. The MIC values derived by the Vitek 2 and reference BMD methods demonstrated a very high agreement for *Enterobacterales* that exceeded the $\geq$90% threshold of EA required by the FDA and for both *Enterobacterales* and *P. aeruginosa* as per the ISO criteria. Neither *Enterobacterales* in general nor any species demonstrated any significant trend for Vitek 2 MEV.

The CA for *Enterobacterales* using FDA criteria and for *Enterobacterales* and *P. aeruginosa* using ISO criteria exceeded the $\geq$90% required CA threshold. As no VME (false susceptibility) or ME (false resistance) were observed, Vitek 2 MEV met both the FDA and ISO criteria for CA and errors. Although the study included isolates across the MIC range, there was only a small number of isolates at the intermediate breakpoint.

MEV resistance is rare among *Enterobacterales* worldwide but is more prevalent in CRE, particularly in those that possess a metallo-β-lactamase. In this study, *Enterobacterales* isolates with β-lactamases, including AmpC (ACT/MIR), ESBL (CTX-M, TEM, and SHV), and carbapenemases (KPC, OXA, NDM, and VIM) were included for the performance assessment (20). MEV is known to be ineffective against isolates harboring metallo-β-lactamase (NDM and/or VIM) carbapenemases (12). Porin mutations combined with overexpression of efflux pumps, and enzyme groups SME and CMY were not available at the time of comparative testing.

Therefore, the performance of Vitek 2 MEV is unknown for these markers and it remains one of the limitations of this study.

Automated susceptibility testing methods have major advantages compared to reference BMD methods in that they are relatively faster and do not require manual reading of results. Here, the Vitek 2 automated dilution system reported a mean time to call of 8.76 ± 2.04 h for 331 clinical isolates and 10.57 ± 3.60 h for 118 challenge isolates when FDA/CLSI breakpoints were analyzed. While this rapid time to call is much faster than overnight results from BMD or disk diffusion methods, the advantage can only be realized in a laboratory that employs multiple shifts of technologists able to release these more rapid AST results.

Overall, this multicenter evaluation of Vitek 2 AST-GN meropenem-vaborbactam on a large number of clinical strains from geographically different regions was conducted using the CLSI reference BMD method. We conclude that bioMérieux Vitek 2 MEV showed substantial equivalence for *Enterobacterales* using FDA/CLSI breakpoints and for both *Enterobacterales* and *P. aeruginosa* using EUCAST breakpoints. The quality control, reproducibility, and challenge studies establish that Vitek 2 is a reproducible and accurate method for MEV susceptibility testing. MEV resistance is rare in *Enterobacterales*, but it does occur, emphasizing the need for susceptibility testing when this antibiotic is prescribed clinically. With the relative ease of use and strong performance characteristics, these data support the use of bioMérieux Vitek 2 MEV in routine clinical practice.

On-scale isolates are often limited with the introduction of new antimicrobials and this was a limitation of this study noted by the evaluable EA of <90%. This is to be expected when one considers that wild-type *Enterobacterales* isolates were highly susceptible to MEV. This lack of on-scale isolates is taken into account, and therefore all isolates were considered for the criterion of an EA of >90%. As the unavailability of on-scale isolates is a limitation of this study but not of the device, further studies are in progress to evaluate a larger population of on-scale global isolates for MEV.

While there is expectation that MEV is active in isolates that show elevated MICs to meropenem alone, a limitation of this study is that meropenem MICs were not available in parallel to the testing of MEV. The study was only intended to evaluate the performance of MEV as a validation of the newly developed AST assay, rather than as a clinical indication study for meropenem versus MEV. Further studies of MEV in comparison to meropenem in a clinical setting will be very informative for indications of the clinical use of MEV.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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Vitek MEV was cleared for *in vitro* diagnostic (IVD) use by the FDA (16).

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