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Multicenter Clinical Evaluation of Etest Meropenem-Vaborbactam (bioMérieux) for Susceptibility Testing of *Enterobacterales* (*Enterobacteriaceae*) and *Pseudomonas aeruginosa*

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ABSTRACT Meropenem-vaborbactam (MEV) is a novel carbapenem–beta-lactamase inhibitor combination antibiotic approved by the U.S. Food and Drug Administration (FDA) for treatment of complicated urinary tract infections, including pyelonephritis, in adults. In this study, we evaluated the performance of Etest MEV (bioMérieux, Marcy l'Etoile, France) compared to that of broth microdilution for 629 *Enterobacterales* and 163 *Pseudomonas aeruginosa* isolates. According to CLSI/FDA breakpoints, 13 *Enterobacterales* isolates (12 clinical and 1 challenge) were resistant to MEV. Overall, Etest MEV demonstrated 92.4% essential agreement (EA), 99.2% category agreement (CA), 0% very major errors (VME), 0% major errors (ME), and 0.8% minor errors (mE) with clinical and challenge isolates of *Enterobacterales*. Individual species demonstrated EA rates of ≥80%, with the exception of *Proteus mirabilis*, for which clinical and challenge isolates demonstrated 34.3% EA, 97.1% CA, 0% ME, and 2.9% mE, precluding the use of Etest MEV with this species. Excluding *P. mirabilis*, MEV Etest MEV demonstrated 95.8% EA, 99.3% CA, 0% VME, 0% ME, and 0.7% mE with *Enterobacterales* isolates. When evaluated using European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints, Etest MEV performance with clinical (16 MEV resistant) and challenge (12 MEV resistant) isolates of *Enterobacterales* (excluding *P. mirabilis*) and *P. aeruginosa* demonstrated an unacceptably high VME rate of 7.1% despite 95.2% EA, 99.2% CA, and 0.5% ME compared to the reference method. In conclusion, we report that Etest MEV is accurate and reproducible for MEV susceptibility testing for *P. aeruginosa* and *Enterobacterales*, with the exception of *P. mirabilis*, using CLSI/FDA breakpoints. Etest MEV should not be used with *P. mirabilis* due to unacceptable analytical performance.

KEYWORDS susceptibility testing, gradient diffusion, meropenem-vaborbactam

Meropenem-vaborbactam (MEV) is a novel beta-lactamase combination agent composed of a carbapenem, meropenem, and a cyclic boronic acid compound and novel β-lactamase inhibitor, vaborbactam. MEV was approved by the U.S. Food and Drug Administration (FDA) for treatment of complicated urinary tract infections (cUTI), including pyelonephritis, in adults after it was demonstrated to be noninferior to piperacillin-tazobactam for treatment of cUTI (1, 2). Recently, therapy with MEV was also shown to result in reduced all-cause mortality and a statistically significant increase in

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cure rates for serious infections due to carbapenem-resistant *Enterobacterales* (CRE), including cUTI/pyelonephritis, complicated intraabdominal infection (cIAI), health care/ventilator-associated bacterial pneumonia (HCABP/VABP), and/or bloodstream infection, compared to the best alternative therapy (3).

Vaborbactam was developed to have potent activity against *Klebsiella pneumoniae* carbapenemases (KPC) but also has activity against strains producing Ambler class A enzymes such as imipenemase (IMI) and Guiana extended-spectrum (GES) enzymes and class C carbapenemases. However, MEV lacks activity against metallo-β-lactamases (Ambler class B) or Ambler class D/OXA-type enzymes and thus is not clinically useful in the treatment of organisms that produce these enzymes (4). Additionally, MEV does not have improved *in vitro* activity against carbapenem-resistant *Pseudomonas aeruginosa*, *Acinetobacter* spp., and other nonfermenting Gram-negative bacilli compared to meropenem alone, as a result of the predominance of noncarbapenemase mechanisms of resistance in these species and the presence of Ambler class B (*P. aeruginosa*) or class D (*Acinetobacter* spp.) enzymes most commonly when carbapenemases are present.

Resistance to MEV among *Enterobacterales* has been reported even among KPC-producing CRE; thus, susceptibility testing is appropriate in settings where the prescription of this agent is anticipated. Additionally, in the absence of molecular diagnostics in many clinical laboratories, the availability of practical susceptibility testing is critical for detection of MEV-resistant organisms when considering MEV for treatment of meropenem-resistant *Enterobacterales*. Our objective was to conduct a multicenter evaluation of the analytical performance characteristics of Etest MEV (bioMérieux, Marcy l'Etoile, France) compared to the Clinical and Laboratory Standards Institute (CLSI) reference broth microdilution (BMD). Etest MEV was recently cleared for *in vitro* diagnostic (IVD) use by the U.S. Food and Drug Administration (FDA) (5).

MATERIALS AND METHODS

Ethics. Each study site performing testing on clinical strains acquired local institutional review board approval or waiver prior to study initiation.

Setting. Testing was performed at the University of California—Los Angeles Medical Center (UCLA) (Los Angeles, CA), Washington University School of Medicine (WU) (St. Louis, MO), University Hospital of Wales (Cardiff) (Wales, UK), and bioMérieux SA (Marcy) (Marcy l'Etoile, France). Challenge and quality control (QC) studies took place at Marcy, while clinical, reproducibility, and quality control studies took place at UCLA, WU, and Cardiff.

Susceptibility testing methodology. For Etest MEV, a visual calibrator was used to prepare a 0.5 McFarland suspension (for nonmucoid isolates) in 0.85% sterile saline from 18 to 24 h of colony growth on blood agar. All mucoid isolates were tested using a 1.0 McFarland standard inoculum. Within 15 min of preparation, a sterile cotton swab moistened with the standardized bacterial suspension was inoculated to BBL Mueller-Hinton II agar plates (BD, Sparks, MD), and Etest strips were applied to plates manually or automatically using a Retro C80 rota-plater and/or a Nema C88 vacuum pen (bioMérieux, Durham, NC). Plates were incubated in ambient air at 35 $\pm$ 2°C and read after 16 to 20 h of incubation. Using reflected light, the MIC was read where the elliptical zone of inhibition intersected the Etest strip or rounded up to the nearest doubling dilution as described in the Etest MEV instructions for use (6). For the broth microdilution (BMD) method, 96-well microtiter plates with 0.1 ml diluted antimicrobial suspension per well, consisting of 2-fold dilutions of meropenem ranging from 0.004 to 64 μg/ml and vaborbactam fixed at 8 μg/ml in cation-adjusted Mueller-Hinton broth, were prepared by bioMérieux and provided to each site; panels were stored frozen at $\leq$−60°C. Prior to use, BMD panels were thawed completely at room temperature for 30 min to 1 h. Using a repeater pipette, BMD panels were inoculated with 0.05 ml of a 100-fold dilution in cation-adjusted BBL Mueller-Hinton II broth (BD, Sparks, MD) of the same 0.5 McFarland suspension used for Etest MEV and incubated at 35 $\pm$ 2°C in ambient air for 16 to 20 h. The MIC was read as the lowest concentration of MEV showing complete inhibition of growth as described in CLSI document M7-A10 (7). Growth from BMD was inoculated to blood agar and assessed for purity after 20 to 24 and 44 to 48 h of incubation. Inoculum density checks were performed with quality control strains, reproducibility strains, and 10% of fresh clinical isolates. Briefly, a 100-μl aliquot of a 1:1,000 dilution of the growth control from broth microdilution panels was plated to blood agar and incubated at 35 $\pm$ 2°C in ambient air for 18 to 48 h. Colony counts after incubation were recorded and used to calculate the final inoculum density.

Reproducibility study. Twelve on-scale stock strains provided by bioMérieux (*Enterobacterales*, $n = 10$; *Pseudomonas aeruginosa*, $n = 2$) were each tested three times from a separate inoculum for 3 days at the UCLA, WU, and Cardiff study sites. Isolates were subcultured twice on blood agar before testing. A 0.5 McFarland suspension prepared in 0.85% sterile saline of each strain was used for the Etest, and an inoculum density check was performed. Tests from all three sites were used to establish a modal value for each strain. Test results within $\pm$1 doubling dilution were deemed acceptable and were used to calculate the reproducibility rate as the percentage of total tests performed with essential agreement

TABLE 1 CLSI/FDA and EUCAST interpretive criteria for meropenem-vaborbactam[a]

Organism(s)	MIC (μg/ml)[b]					
	CLSI/FDA criteria			EUCAST criteria		
	S	I	R	S	I	R
Enterobacterales	≤4/8	8/8	≥16/8	≤8/8		>8/8
Pseudomonas aeruginosa				≤8/8		>8/8

[a]CLSI MEV breakpoints are as reported in CLSI supplement M100, 29th edition (11). FDA breakpoints are available at www.fda.gov/STIC. EUCAST MEV breakpoints are as reported in reference 12.
[b]Abbreviations: S, susceptible; I, intermediate; R, resistant.

(EA). Best-case calculations for reproducibility assumed off-scale values within one doubling dilution of the mode, while worst-case reproducibility assumed that off-scale calculations were not within one doubling dilution.

QC study. Quality control (QC) testing was performed at each site every day that testing was set up, and a minimum of 20 times at each study site, with the following organisms: *Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Klebsiella pneumoniae* ATCC 700603, *K. pneumoniae* ATCC BAA-1705, and *P. aeruginosa* ATCC 27853. The QC ranges described in CLSI supplement M100 (28th edition) (8) were applied on each day of clinical or challenge testing, and an inoculum density check was conducted with all QC tests according to CLSI document M7-A10 (7) and supplement M100 (28th edition) (8) guidelines. Results were considered invalid if QC results were unacceptable. Quality control performance was calculated as the percentage of total results within the expected range.

Clinical and challenge study. Seven hundred four clinical isolates of *Enterobacterales* and *P. aeruginosa* and 88 challenge isolates were evaluated for MEV susceptibility using Etest MEV and reference BMD simultaneously according to the methods described above. Clinical isolates were tested at the UCLA ($n = 242$), WU ($n = 240$), and Cardiff ($n = 222$), sites while challenge isolate testing was performed entirely at the Marcy site. All clinical isolates were recovered from clinical cultures submitted to the clinical laboratory as part of standard care and were identified to the genus and species levels by the matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Vitek MS), Vitek 2 (bioMérieux), or API identification (bioMérieux) system per the procedures at each clinical study site. Only one isolate per species per patient could be included, and study personnel were blinded to the susceptibility results for clinical isolates. A target of 10 to 50 isolates representing each of the species indicated for use was set for each clinical study site. Of the 704 clinical isolates, 406 (57.7%) were fresh isolates (never frozen and tested within 7 days from isolation in culture), 131 (18.6%) were recent isolates (>7 days from isolation in culture or frozen for less than 1 year before testing), and 167 (23.7%) were stock (frozen for 1 to 3 years before testing). All clinical isolates were subcultured on blood agar before testing; frozen isolates were subcultured twice before testing. A single 0.5 McFarland suspension was prepared for Etest and reference BMD testing. For mucoid isolates, a 1.0 McFarland suspension was prepared for Etest and the 0.5 McFarland was used for the reference BMD. An inoculum density check as described above was performed on a minimum of 10% of fresh clinical isolates at each study site.

Data analysis. Clinical and challenge isolates tested for MEV susceptibility by Etest MEV and reference BMD were included in the performance evaluation. Essential agreement (EA) was defined as the percentage of total isolates where the test and reference methods were within one doubling dilution of each other. Evaluable essential agreement was defined as the percentage of test results that were on scale and in essential agreement with the reference method. Category agreement (CA) was defined as the percentage of total test results in agreement with interpretive results (susceptible, intermediate, and resistant) of the reference method. The very major error (VME) rate was defined as the percentage of isolates interpreted as resistant by the reference method and susceptible by the Etest method. The major error (ME) rate was defined as the percentage of isolates interpreted as susceptible by the reference method but resistant by the Etest method. The minor error (mE) rate was defined as the percentage of total isolates for which the reference method interpretation was resistant or susceptible and the Etest method interpretation was intermediate or vice versa. The FDA currently recognizes the CLSI supplement M100 29th edition breakpoints for MEV and *Enterobacterales* (www.fda.gov/STIC). The performance of Etest MEV was evaluated using CLSI/FDA or EUCAST interpretative criteria (Table 1) as indicated.

RESULTS

Reproducibility of Etest MEV. Twelve on-scale isolates (*Citrobacter freundii*, $n = 1$; *Klebsiella* [formerly *Enterobacter*] *aerogenes*, $n = 1$; *Enterobacter cloacae* complex, $n = 1$; *E. coli*, $n = 3$; *K. pneumoniae*, $n = 3$, *P. aeruginosa*, $n = 2$; and *Serratia marcescens*, $n = 1$) were tested at the UCLA, WU, and Cardiff study sites as described in Materials and Methods. A total of 324 tests (108/site) were performed. Mode values of MEV MICs for each isolate tested across all sites are shown in Table 2. The reproducibility rate for all strains, including *Pseudomonas aeruginosa*, between sites was 99.7%, with 323 tests within 1 doubling dilution of strain-specific modal values. Among only *Enterobacterales*,

TABLE 2 Reproducibility of Etest MEV

Organism	MIC mode for all tests (μg/ml)	No. of tests with doubling dilution from the mode:						
		Off scale	−2	−1	0	+1	+2	Off scale
Citrobacter freundii	0.016				21	6		
Klebsiella (*Enterobacter*) *aerogenes*	0.032			8	19			
Enterobacter cloacae	0.032			7	18	1	1	
Escherichia coli								
Strain A	0.016				16	11		
Strain B	0.016				21	6		
Strain C	0.016				27			
Klebsiella pneumoniae								
Strain A	0.5			1	24	2		
Strain B	0.125			3	24			
Strain C	0.032				26	1		
Pseudomonas aeruginosa								
Strain A	1.0				26	1		
Strain B	0.5			8	19			
Serratia marcescens	0.064			9	18			
Total		0	0	36	259	28	1	0

the reproducibility rate was 99.6%, with 269 of 270 tests within 1 doubling dilution of strain-specific modal values.

QC of Etest MEV. For quality control (QC) of Etest MEV, five (5) quality control organisms were tested a minimum of 20 times by the Etest and broth microdilution methods throughout the study at all study sites as described in Materials and Methods. One hundred percent of broth microdilution and Etest results for *E. coli* ATCC 25922, *E. coli* ATCC 35218, *P. aeruginosa* ATCC 27853, and *K. pneumoniae* ATCC BAA-1705 quality control results were within the expected range. Broth microdilution results for *K. pneumoniae* ATCC 700603 were out of range in two instances at 2 study sites, resulting in 97.7% (84/86) of quality control results being within range. All Etest quality control results for *K. pneumoniae* ATCC 700603 were within range (86/86; 100%).

Etest MEV challenge study. The accuracy of the Etest MEV was evaluated with 88 challenge isolates (79 *Enterobacterales* and 9 *P. aeruginosa*) tested at the Marcy study site. Twelve (15.2%) *Enterobacterales* isolates were resistant to MEV by broth microdilution according to CLSI/FDA breakpoints (Table 1). Among all *Enterobacterales* isolates, Etest MEV demonstrated 93.7% (74/79) essential agreement and 94.9% (75/79) category agreement. No very major or major errors were detected, but 5.1% (4/79) minor errors were observed (Table 3). All individual species tested had ≥90% essential agreement and category agreement, with the exception of *Proteus mirabilis*, for which 50% (2/4) essential agreement and 75% (3/4) category agreement were observed. Excluding *P. mirabilis*, the performance for Etest MEV among *Enterobacterales* was increased to 96.0% (72/75) essential agreement and category agreement with 0 very major or major errors and 4.0% (3/75) minor errors.

CLSI/FDA breakpoints have not been established for MEV and *P. aeruginosa*; accordingly, *P. aeruginosa* is not included in the U.S. FDA indication for Etest MEV. However, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) includes breakpoints for both *Enterobacterales* and *P. aeruginosa* (Table 1). When assessed with EUCAST breakpoints, *P. aeruginosa* isolates demonstrated 88.9% (8/9) essential agreement and 100% (9/9) category agreement. Only one *P. aeruginosa* challenge isolate was resistant to MEV according to EUCAST breakpoints, and neither very major nor major errors were detected among the challenge isolates tested. When EUCAST breakpoints were applied to *Enterobacterales*, 11 challenge isolates were resistant to MEV and the essential agreement and category agreement of Etest MEV among challenge *Enterobacterales* isolates were 93.7% (74/79) and 96.2% (76/79), respectively, with *P. mirabilis*

TABLE 3 Clinical and challenge performance of Etest MEV[a]

Organism(s)	No. of isolates	No. (%[b]) of isolates															
		CLSI/FDA interpretative criteria									EUCAST interpretative criteria[c]						
		Results by broth microdilution			Performance						Results by broth microdilution		Performance				
		S	I	R	EA	Evaluable EA[d]	CA	VME	ME	mE	S	R	EA	Evaluable EA	CA	VME	ME
Challenge																	
Enterobacterales[e]	79	66 (83)	1 (0)	12 (15)	74 (94)	66/70 (94)	75 (95)	0 (0)	0	4 (5)	68 (86)	11 (14)	74 (94)	66/70 (94)	76 (96)	2(18)	1(2)
Enterobacterales[f]	75	63 (84)	0 (0)	12 (16)	72 (96)	64/66 (97)	72 (96)	0 (0)	0	3 (4)	64 (85)	11 (14.7)	72 (96)	64/66 (97)	73 (97)	2 (18)	0
Citrobacter freundii	2	2 (100)	0 (0)	0 (0)	2 (100)		2 (100)	—[i]	0	0	2 (100)	0 (0)	2 (100)	2/2 (100)	2 (100)	—	0
C. koseri	2	2 (100)	0 (0)	0 (0)	2 (100)		2 (100)	—	0	0	2 (100)	0 (0)	2 (100)	2/2 (100)	2 (100)	—	0
Klebsiella (formerly *Enterobacter*) *aerogenes*	3	3 (100)	0 (0)	0 (0)	3 (100)		3 (100)	—	0	0	3 (100)	0 (0)	3 (100)	3/3 (100)	3 (100)	—	0
E. cloacae	1	1 (100)	0 (0)	0 (0)	1 (100)		1 (100)	—	0	0	1 (100)	0 (0)	1 (100)	1/1 (100)	1 (100)	—	0
E. cloacae complex	7	7 (100)	0 (0)	0 (0)	7 (100)		7 (100)	—	0	0	7 (100)	0 (0)	7 (100)	7/7 (100)	7 (100)	—	0
Escherichia coli	16	14 (88)	0 (0)	2 (12)	16 (100)	15/15 (100)	15 (94)	0 (0)	0	1 (6)	14 (878)	2 (12)	16 (100)	16/16 (100)	15 (94)	1 (50)	0
Klebsiella oxytoca	1	1 (100)	0 (0)	0 (0)	1 (100)		1 (100)	—	0	0	1 (100)	0 (0)	1 (100)	1/1 (100)	1 (100)	—	0
K. pneumoniae	40	30 (75)	0 (0)	10 (25)	37 (92)	30/32 (94)	38 (95)	0 (0)	0	2 (5)	31 (78)	9 (22)	37 (92)	30/32 (94)	39 (98)	1 (11)	0
Morganella morganii	1	1 (100)	0 (0)	0 (0)	1 (100)		1 (100)	—	0	0	1 (100)	0 (0)	1 (100)	1/1 (100)	1 (100)	—	0
Proteus mirabilis[g]	4	3 (75)	1 (25)	0 (0)	2 (50)	2/4 (50)	3 (75)	—	0	1 (25)	3 (100)	0 (0)	2 (50)	2/4 (50)	3 (75)	—	1 (25)
Providencia stuartii	1	1 (100)	0 (0)	0 (0)	1 (100)		1 (100)	—	0	0	1 (100)	0 (0)	1 (100)	1/1 (100)	1 (100)	—	0
Serratia marcescens	1	1 (100)	0 (0)	0 (0)	1 (100)		1 (100)	—	0	0	1 (100)	0 (0)	1 (100)	1/1 (100)	1 (100)	—	0
Pseudomonas aeruginosa[h]	9	NA	NA	NA	8 (89)	7/8 (88)	NA	NA	NA	NA	8 (89)	1 (11)	8 (89)	7/8 (88)	9 (100)	0 (0)	0
Clinical																	
Enterobacterales[e]	550	548 (100)	1 (0)	1 (0)	507 (92)	506/548 (92)	549 (100)	0 (0)	0	1 (0)	549 (100)	1 (0)	507 (92)	506/548 (92)	550 (100)	0 (0)	0 (0)
Enterobacterales[f]	519	517 (100)	1 (0)	1 (0)	497 (96)	496 (96)	518 (100)	0 (0)	0	1 (0)	518 (100)	1 (0)	497 (96)	496/517 (96)	519 (100)	0 (0)	0
C. freundii	30	30 (100)	0 (0)	0 (0)	29 (97)		30 (100)	—	0	0	30 (100)	0 (0)	29 (97)	29/30 (97)	30 (100)	—	0
C. koseri	30	30 (100)	0 (0)	0 (0)	29 (97)		30 (100)	—	0	0	30 (100)	0 (0)	29 (97)	29/30 (97)	30 (100)	—	0
K. aerogenes	30	30 (100)	0 (0)	0 (0)	28 (93)		30 (100)	—	0	0	30 (100)	0 (0)	28 (93)	28/30 (93)	30 (100)	—	0
E. cloacae	20	20 (100)	0 (0)	0 (0)	20 (100)		20 (100)	—	0	0	20 (100)	0 (0)	20 (100)	20/20 (100)	20 (100)	—	0
E. cloacae complex	70	70 (100)	0 (0)	0 (0)	70 (100)		70 (100)	—	0	0	70 (100)	0 (0)	70 (100)	70/70 (100)	70 (100)	—	0
Escherichia coli	120	120 (100)	0 (0)	0 (0)	116 (97)	116/119 (98)	120 (100)	—	0	0	120 (100)	0 (0)	116 (97)	116/119 (98)	120 (100)	—	0
K. oxytoca	30	30 (100)	0 (0)	0 (0)	30 (100)		30 (100)	—	0	0	30 (100)	0 (0)	30 (100)	30/30 (100)	30 (100)	—	0
K. pneumoniae	88	87 (99)	0 (0)	1 (1)	86 (98)	85/87 (98)	88 (100)	0 (0)	0	0	87 (99)	1 (1)	86 (98)	85/87 (98)	88 (100)	0 (0)	0
Morganella morganii	30	30 (100)	0 (0)	0 (0)	25 (83)	25/30 (83)	30 (100)	—	0	0	30 (100)	0 (0)	25 (83)	25/30 (83)	30 (100)	—	0
Proteus mirabilis[g]	31	31 (100)	0 (0)	0 (0)	10 (32)	10/31 (32)	31 (100)	—	0	0	31 (100)	0 (0)	10 (32)	10/31 (32)	31 (100)	—	0
Providencia rettgeri	21	21 (100)	0 (0)	0 (0)	17 (81)	17/21 (81)	21 (100)	—	0	0	21 (100)	0 (0)	17 (81)	17/21 (81)	21 (100)	—	0
Providencia stuartii	20	20 (100)	0 (0)	0 (0)	18 (90)	18/20 (90)	20 (100)	—	0	0	20 (100)	0 (0)	18 (90)	18/20 (90)	20 (100)	—	0
Serratia marcescens	30	29 (97)	1 (3)	0 (0)	29 (97)	29/30 (97)	29 (97)	—	0	1 (3)	30 (100)	0 (0)	29 (97)	29/30 (97)	30 (100)	0	0
Pseudomonas aeruginosa[h]	154	NA	NA	NA	144 (93)	137/147 (93)	NA	NA	NA	NA	139 (90)	15 (10)	144 (93)	137/147 (93)	150 (97)	0 (0)	4 (3)

[a]Abbreviations: 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]Percentages are rounded to nearest whole number.
[c]There is no intermediate category and thus no minor errors for EUCAST interpretive criteria.
[d]Evaluable EA is listed if different from essential agreement.
[e]Total *Enterobacterales*, including *P. mirabilis*.
[f]Total *Enterobacterales*, excluding *P. mirabilis*.
[g]*P. mirabilis* was not included in FDA indication for use.
[h]*P. aeruginosa* does not have CLSI/FDA breakpoints for MEV.
[i]—, no resistant isolates.

included, with 18.2% (2/11) very major errors and 1.5% (1/68) major errors. Excluding *P. mirabilis*, Etest MEV demonstrated 96% (72/75) essential agreement, 97.3% (73/75) category agreement with 18.2% (2/11) very major errors and 0 major errors.

Clinical performance of Etest MEV. To evaluate the performance of Etest MEV, a total of 704 clinical isolates (550 *Enterobacterales* and 154 *P. aeruginosa*) were tested at the UCLA ($n = 242$), WU ($n = 240$), and Cardiff ($n = 222$) study sites. Among *Enterobacterales*, 2 clinical isolates were not susceptible (1 was intermediate and 1 was resistant) to MEV by broth microdilution according to CLSI/FDA interpretive criteria. essential agreement was 92.2% (507/550), and category agreement was 99.8% (549/550), with 0% (0/1) very major error, 0% (0/548) major error, and 0.2% (1/550) minor error rates. The majority of individual species tested demonstrated essential agreement of >90%, with the exception of members of the *Morganellaceae* family, including *Morganella morganii* (83.3% EA), *P. mirabilis* (32.3%), *Providencia rettgeri* (81.0%), and *Providencia stuartii* (90.0%). Category agreement for all of the *Morganellaceae*, including *P. mirabilis*, was 100%. Given the poor essential agreement rate of Etest MEV for *P. mirabilis*, this species is specifically excluded from the list of FDA indicated-for-use species and should not be used for Etest MEV MIC determination. Excluding *P. mirabilis*, *Enterobacterales* clinical isolates had 95.8% (497/519) essential agreement and 99.8% (518/519) category agreement, with 0% very major and major errors and a minor error rate of 0.2% (1/519).

EUCAST MEV breakpoints for *Enterobacterales* and *P. aeruginosa* do not include an "intermediate" category; rather, isolates with MICs of ≤8 μg/ml are susceptible, while those with MICs of >8 μg/ml are resistant (Table 1). When interpreted using EUCAST breakpoints, 1 *Enterobacterales* and 15 *P. aeruginosa* clinical isolates were resistant to MEV. Etest MEV demonstrated 95.8% EA, 100% CA, 0% VME, and 0% ME for all *Enterobacterales* (excluding *P. mirabilis*). Of the 154 clinical *P. aeruginosa* isolates tested, 15 (9.7%) were resistant to MEV by broth microdilution. Etest MEV demonstrated 93.5% (144/154) essential agreement and 97.4% (150/154) category agreement, with 0% very major error and 2.9% (4/139) major error rates with *P. aeruginosa* isolates. The overall performance of Etest MEV, including all clinical and challenge isolates of all species (*Enterobacterales* and *P. aeruginosa*, but excluding *P. mirabilis*) was 95.2% EA, 99.2% CA, 7.1% VME, and 0.5% ME. Using EUCAST breakpoints, Etest MEV did not meet the acceptance criterion of the allowable very major error rate of 3% for either *Enterobacterales* alone (16.7%) or *Enterobacterales* combined with *P. aeruginosa* (7.1%). Given the high rate of very major errors for *Enterobacterales* isolates that are otherwise in essential agreement (95.8%) with the reference method, in the absence of an intermediate interpretive category, we suggest that testing should be repeated using an alternative testing/reference method prior to reporting results for *Enterobacterales* when the Etest MEV MIC is at or near (within 1 doubling dilution of) the EUCAST breakpoint, 8 μg/ml.

Molecular characterization of MEV-resistant *Enterobacterales* tested with Etest MEV. Among 629 clinical and challenge isolates of *Enterobacterales* tested, 12 challenge isolates and 1 clinical isolate were resistant to MEV by broth microdilution according to CLSI/FDA breakpoints. MEV-resistant isolates were identified as *E. coli* (2 isolates) and *K. pneumoniae* (11 isolates). The molecular characterization of these isolates is listed in Table 4. Nine of the 13 isolates harbored metallo-β-lactamases, (7, NDM; 2, VIM); it has been well established that MEV does not have activity against metallo-β-lactamases.

DISCUSSION

To facilitate the clinical use of new antimicrobial agents, it is crucial that clinical laboratories have the ability to perform susceptibility testing for these agents. The reference broth microdilution method is impractical for most clinical settings, and thus the availability of commercial methods such as gradient diffusion can facilitate susceptibility testing in clinical microbiology labs as part of routine practice. Here, we evaluated the performance of Etest MEV (bioMérieux, Marcy l'Etoile, France) compared to the broth microdilution reference method according to CLSI/FDA and EUCAST breakpoints for *Enterobacterales* and *P. aeruginosa* isolates. Overall, Etest MEV met FDA

TABLE 4 Molecular characterization of MEV-resistant *Enterobacterales* tested with Etest MEV

Isolate	Type	MIC by:		Molecular characterization
		Etest	BMD	
Escherichia coli	Challenge	8[a]	16	NDM-7, CMY-42
		≥64	>64	NDM-5, TEM-1B, CMY-42
Klebsiella pneumoniae	Challenge	8[a]	64	NDM-1, OXA-9, TEM-1A, CTX-M15, SHV-11, OXA-1
		≥64	>64	NDM-1, CMY-4, CTX-M15, SHV-11, OXA-10
		8	16	OXA-181, CTX-M15, SHV-26
		≥64	>64	NDM-1, OXA-232, OXA-9, TEM-1A, CTX-M15, SHV-11, OXA-1
		≥64	>64	OXA-232, CTX-M15, SHV-1, OXA-1
		≥64	>64	VIM-1, SHV
		≥64	>64	VIM-1, SHV-11
		≥64	>64	TEM, SHV, CTX-M15, OXA-48
		≥64	>64	TEM, SHV, NDM-1, CTX-M15, OXA-232
		16	32	NDM-1, TEM, SHV, CTX-M15, CMY
	Clinical	≥64	≥64	OXA-48

[a]The isolate resulted in very major errors when assessed with EUCAST breakpoints.

performance criteria, demonstrating 95.8% essential agreement and 99.3% category agreement with no very major or major errors observed for clinical and challenge *Enterobacterales*, excluding *P. mirabilis*. Etest MEV demonstrated unacceptable performance characteristics with clinical and challenge isolates of *P. mirabilis*, with 34.4% essential agreement, and is thus excluded from the FDA indication for use (9).

Prior data on the accuracy of gradient diffusion methods for MEV are limited. One previous study has reported on research-use-only (RUO) product versions of MEV gradient diffusion strips from bioMérieux and LioFilchem compared to broth microdilution for 120 isolates of carbapenem-resistant *Enterobacterales* (CRE), including *K. pneumoniae* (86 isolates), *Enterobacter cloacae* (17), *E. coli* (10) and *K. aerogenes* (7) (10). In that study, RUO Etest MEV displayed essential agreement of 82% and category agreement of ≥90%. However, the RUO Etest MEV had one very major error, two major errors, and three minor errors (10). It is important to note that these data were conducted with a prior iteration (RUO) of Etest MEV. Here, in a large, multicenter study, we illustrated the performance characteristics of the *in vitro* diagnostic (IVD) Etest MEV: 95.8% and 99.3%, essential agreement and category agreement, respectively; 0 major and very major errors, and 0.7% minor errors for 594 clinical and challenge isolates of *Enterobacterales* (excluding *P. mirabilis*). Given the expedited timeline of FDA clearance of the IVD Etest MEV and superior performance of the IVD product, the use of the RUO assay is discontinued.

To our knowledge, this is the first report of reduced essential agreement of MEV gradient diffusion strips compared to broth microdilution for *P. mirabilis* isolates. This is an interesting and unexpected finding. Members of the *Morganellaceae* family were not included in previous studies evaluating RUO gradient diffusion strips (10), and it is not clear whether this finding is specific to the IVD bioMérieux Etest gradient diffusion method. While *P. mirabilis* isolates were included in the clinical trials of other FDA-cleared gradient diffusion strips with no contraindication for use with *P. mirabilis* isolates indicated in the labeling, species-specific performance data were not shown (5). A future head-to-head study comparing the performance characteristics of the IVD versions of gradient diffusion strips for *P. mirabilis* from multiple manufacturers is needed to fully resolve this question.

MEV resistance is rare among *Enterobacterales* worldwide but is more prevalent in CRE, in particular those that possess a metallo-beta-lactamase (9). In keeping with this epidemiology, a limitation of this study is the lack of *Enterobacterales* isolates resistant to MEV, reflecting the relative rarity of resistant strains at the clinical study sites. Only 13 resistant *Enterobacterales* isolates were included, 12 of which were challenge isolates that were limited to *E. coli* and *K. pneumoniae*; the sole clinical isolate resistant to MEV was identified as *K. pneumoniae*. Evaluation of additional resistant isolates, such as

those included in the CDC's AR isolate bank, may have improved the assessment of Etest MEV with MEV-resistant isolates. This study also has many strengths. A large number of clinical isolates (704), including *Enterobacterales* (550) and *P. aeruginosa* (154), were tested across four independent, geographically diverse study sites. Additionally, Etest MEV performance was evaluated using the CLSI reference method, broth microdilution. Finally, this rigorous analysis also evaluated performance with CLSI/FDA and EUCAST MEV breakpoints.

Overall, we conclude that using CLSI/FDA breakpoints, the bioMérieux Etest MEV showed substantial equivalence for *Enterobacterales* compared to the reference method, broth microdilution, with the exception of *P. mirabilis*, where essential agreement was below acceptance criteria. Importantly, Etest MEV did not have acceptable performance when EUCAST breakpoints were applied, indicating that they should not be used with Etest MEV. Although resistance to MEV is rare in *Enterobacterales*, it does occur, highlighting the need for susceptibility testing when this antimicrobial agent is used clinically. Given the relative ease of use and the strong performance characteristics, these data support the use of the bioMérieux Etest MEV in routine clinical practice with only CLSI/FDA breakpoints.

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