Performance of ETEST® Meropenem/Vaborbactam for Antimicrobial Susceptibility Testing of *Enterobacteriaceae*

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ABSTRACT (REVISED)

Background:
Meropenem/vaborbactam (MEV) is a combination of meropenem, a penem antibacterial, and vaborbactam, a beta-lactamase inhibitor, indicated for the treatment of patients 18 years and older with complicated urinary tract infections (cUTI), including pyelonephritis, caused by designated susceptible bacteria. This study evaluated the performance of ETEST® MEV (bioMérieux), a new gradient diffusion strip FDA cleared for determining antimicrobial susceptibility of *Enterobacteriaceae* as compared to the Clinical and Laboratory Standards Institute (CLSI) broth microdilution reference method (BMD).

Method:
A total of 629 *Enterobacteriaceae* isolates (550 clinical and 79 challenge isolates), including 12 species, were tested at 4 clinical trial sites, (including one internal laboratory) using ETEST® MEV and BMD. Isolates were subcultured on tryptic soy or Columbia agar plates supplemented with 5% sheep blood. After overnight incubation bacterial suspensions were prepared in saline and used to inoculate ETEST® MEV and BMD. Reading was performed after 16 – 20 hours incubation at 35±2°C. Results were analyzed for essential agreement (EA), category agreement (CA), minor (mE), major (ME) and very major (VME) error rates using the FDA approved breakpoints for meropenem/vaborbactam (Susceptible ≤ 4/8 µg/mL, Intermediate 8/8 µg/mL, Resistant ≥ 16/8 µg/mL). Performance was evaluated using the FDA performance acceptance criteria, EA and CA (≥ 90%), major error rate (≤ 3.0%) and very major error rate (≤ 2.0%)*. Minor error rate is included for informational purposes.

*per FDA/STMA presentation dated on 05 Dec 2017

Results:
Results are summarized in Table 1. ETEST® MEV performance met all the FDA performance acceptance criteria when testing *Enterobacteriaceae.*

Table 1

Organism	EA	CA	CA VME Rate	CA ME Rate	CA mE Rate
*Enterobacteriaceae***	95.8% (569/594)	99.3% (590/594)	0.0% (0/13)	0.0% (0/580)	0.7% (4/594)

**excluding *P. mirabilis* species from the intended use of the device label due to the low MIC essential agreement compared to the reference method.

Conclusion:
When compared to the BMD reference method, results of this multicenter trial support the accuracy of ETEST® MEV for determining the MIC of *Enterobacteriaceae* in a clinical setting.

INTRODUCTION

ETEST MEV strip is an *in vitro* quantitative device for determining the antimicrobial susceptibility of *Enterobacteriaceae* against meropenem/vaborbactam (MEV). The device consists of a thin, inert and non-porous plastic strip that has an exponential gradient of MEV one side and a MIC reading scale on the other. Application of the strip to an inoculated agar surface produces an elliptical zone of bacterial growth on the agar surface following overnight incubation. The point at which the edge of the elliptical zone of inhibition meets the strip is interpreted as the Minimum Inhibitory Concentration (MIC) endpoint, measured in µg/mL.

OBJECTIVE

Evaluate ETEST MEV performance in a clinical setting as compared to the CLSI BMD reference for FDA submission.

MATERIALS AND METHODS

Clinical isolates were evaluated at four clinical trial sites. Twelve species of *Enterobacteriaceae* were tested for a total of 629 isolates. Each isolate was first subcultured on TSA with sheep blood agar. From an 18-24 hour culture, a 0.5 McFarland suspension (1 McFarland for mucoid strains) was prepared in 0.85% saline using visual comparison against a McFarland standard. This suspension was used to inoculate a Mueller Hinton agar plate for the ETEST MEV and also for CLSI broth microdilution (BMD). Both ETEST MEV plates and the BMD panels were incubated at 35±2°C in ambient air. BMD panels and ETEST MEV were read after 16-20 hour incubation. As meropenem/vaborbactam is a bactericidal agent, the MIC results were determined at the point of complete inhibition of bacterial growth.

Figure 1: Characteristic ellipses observed with QC strains.

E. coli ATCC® 25922™ MIC = 0.016 µg/ml

P. aeruginosa ATCC® 27853™ MIC = 0.5 µg/ml

K. pneumoniae ATCC® 700603™ MIC = 0.032 µg/ml

K. pneumoniae ATCC® BAA-1705™ MIC = 0.023 µg/ml

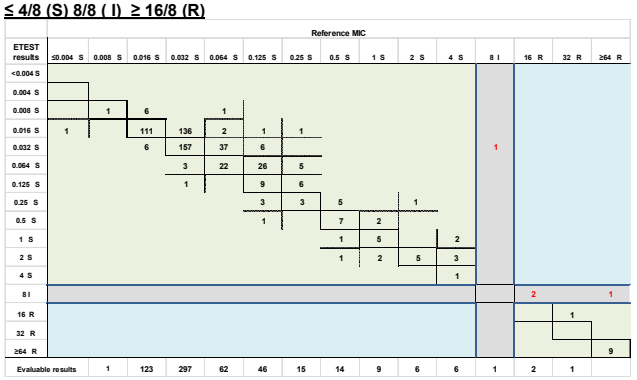
RESULTS

Overall performance with the FDA approved breakpoints (≤4/8 S, 8/8 I, and ≥16/8 R) for all *Enterobacteriaceae* species combined (excluding *P. mirabilis*), was 95.8% essential agreement (EA), 99.3% category agreement (CA), 0.0% very major errors (VME), 0.0% major errors (ME) and 0.7% minor errors (mE). Overall performance by species is detailed in Table 2. The performance of *Enterobacteriaceae* is summarized in the Frequency Table (Table 3) and the molecular characterizations for the resistant strains tested are shown in Table 4.

Table 2 : Summary of Overall Performance by Species

Organism	Total	EA	%EA	CA	%CA	#R	#VME	#ME	#mE
Citrobacter freundii	32	31	96.9%	32	100.0%	0	--	0	0
Citrobacter koseri	32	31	96.9%	32	100.0%	0	--	0	0
Enterobacter aerogenes	33	31	93.9%	33	100.0%	0	--	0	0
Enterobacter cloacae	21	21	100.0%	21	100.0%	0	--	0	0
E. cloacae complex	77	77	100.0%	77	100.0%	0	--	0	0
Escherichia coli	136	132	97.1%	135	99.3%	2	0	0	1
Klebsiella oxytoca	31	31	100.0%	31	100.0%	0	--	0	0
Klebsiella pneumoniae	128	123	96.1%	126	98.4%	11	0	0	2
Morganella morganii	31	26	83.9%	31	100.0%	0	--	0	0
Providencia rettgeri	21	17	81.0%	21	100.0%	0	--	0	0
Providencia stuartii	21	19	90.5%	21	100.0%	0	--	0	0
Serratia marcescens	31	30	96.8%	30	96.8%	0	--	0	1

Table 3: Frequency Table of Overall *Enterobacteriaceae FDA Breakpoint: ≤ 4/8 (S) 8/8 (I) ≥ 16/8 (R)**



*Excluding *P. mirabilis* species

Table 4: Molecular Characterizations of the Resistant Strains Tested with ETEST MEV

Organism	ETEST MIC	BMD MIC	Molecular Characterization
Escherichia coli	8	16	NDM-7, CMY-42
Escherichia coli	>=64	>64	NDM-5, TEM-1B, CMY-42
Klebsiella pneumoniae	8	64	NDM-1, OXA-9, TEM-1A, CTX-M15, SHV-11, OXA-1
Klebsiella pneumoniae	>=64	>64	NDM-1, CMY-4, CTX-M-15, SHV-11, OXA-10
Klebsiella pneumoniae	8	16	OXA-181, CTX-M-15, SHV-26
Klebsiella pneumoniae	>=64	>64	NDM-1, OXA-232, OXA-9, TEM-1A, CTX-M-15, SHV-11, OXA-1
Klebsiella pneumoniae	>=64	>64	OXA-232, CTX-M-15, SHV-1, OXA-1
Klebsiella pneumoniae	>=64	>64	VIM-1, SHV
Klebsiella pneumoniae	>=64	>64	VIM-1, SHV-11
Klebsiella pneumoniae	>=64	>64	TEM, SHV, CTX-M-15, OXA-48
Klebsiella pneumoniae	>=64	>64	TEM, SHV, NDM-1 CTX-M-15, OXA-232
Klebsiella pneumoniae	16	32	NDM-1, TEM, SHV, CTX-M-15, CMY
Klebsiella pneumoniae	>=64	>64	OXA-48

CONCLUSIONS

When compared to the broth microdilution reference method, ETEST MEV was found to be an accurate and reliable method for susceptibility testing of meropenem/vaborbactam providing MIC results for *Enterobacteriaceae,* excluding *P. mirabilis*. As a manual method it requires a minimal level of expertise and is easy to perform. Furthermore, ETEST requires no specific instrumentation thus adding flexibility to the clinical laboratories performing antimicrobial susceptibility testing.