Multicenter Evaluation of Meropenem/Vaborbactam MIC Results for *Enterobacteriaceae* Using MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Background: A multicenter study was performed to evaluate the accuracy of meropenem/vaborbactam on a MicroScan Dried Gram Negative (MSDGN) MIC Panel when compared to a frozen CLSI broth microdilution reference panel.

Materials/Methods: An evaluation was conducted at three US sites by comparing MIC values obtained using the MSDGN to MICs using a CLSI broth microdilution reference panel. A total of 560 *Enterobacteriaceae* clinical isolates were tested using the turbidity and MicroScan PROMPT inoculation® (Prompt) methods of inoculation in the efficacy phase. For challenge, 95 *Enterobacteriaceae* isolates were tested on MSDGN panels at one site. For reproducibility, a subset of 14 organisms was tested on MSDGN panels at each site. MSDGN panels were incubated at 35 ± 2℃ and read on the MicroScan WalkAway *plus* (WalkAway), the MicroScan autoSCAN-4 instrument (autoSCAN-4), and read visually. Read times for the MSDGN panels were 16–20 hours. Frozen reference panels, prepared according to CLSI/ISO methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 2℃ and read visually. Frozen reference panels were read at 16–20 hours. CLSI/FDA breakpoints (µg/ml) used for interpretation of MIC results were: *Enterobacteriaceae* ≤ 4/8 S, 8/8 I, and ≥ 16/8 R.

Results: When compared to frozen reference panel results, essential and categorical agreements for isolates tested in the Efficacy and Challenge are as follows:

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %		Minor Errors%	
	T	P	T	P	T	P	T	P	T	P
Visually	97.9 (641/655)	96.5 (632/655)	98.5 (645/655)	99.1 (649/655)	3.2 (1/31)	0.0 (0/31)	0.0 (0/619)	0.0 (0/619)	1.4 (9/655)	0.9 (6/655)
WalkAway	98.2 (643/655)	98.3 (644/655)	98.5 (645/655)	99.1 (649/655)	3.2 (1/31)	0.0 (0/31)	0.0 (0/619)	0.0 (0/619)	1.4 (9/655)	0.9 (6/655)
autoSCAN-4	97.9 (641/655)	97.3 (637/655)	98.5 (645/655)	99.1 (649/655)	3.2 (1/31)	0.0 (0/31)	0.0 (0/619)	0.0 (0/619)	1.4 (9/655)	0.9 (6/655)

T = Turbidity inoculation method, P = Prompt inoculation method

Reproducibility among the three sites were greater than 95% for all read methods for both the turbidity and Prompt inoculation methods.

Conclusion: This multicenter study showed that meropenem/vaborbactam MIC results for *Enterobacteriaceae* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI/FDA interpretive criteria.

INTRODUCTION

A multicenter study was performed to evaluate the performance of a MicroScan Dried Gram Negative MIC panel with meropenem/vaborbacatam using *Enterobacteriaceae* isolates with CLSI/FDA interpretive breakpoints.

METHODS

Study Design: MicroScan Dried Gram Negative MIC panels were tested concurrently with a CLSI frozen broth microdilution reference panel at three sites using both the turbidity and Prompt Inoculation methods.
A total of 655 *Enterobacteriaceae* clinical isolates were tested among the three sites.

Quality Control Expected Results, CLSI M100-ED29*
Escherichia coli ATCC 25922: ≤0.03/8–0.06/8 µg/ml
Pseudomonas aeruginosa ATCC 27853: 0.12/8–1/8 µg/ml
Escherichia coli ATCC 35218 : ≤0.03/8–0.06/8 µg/ml
Klebsiella pneumoniae ATCC 700603: ≤0.03/8–0.06/8 µg/ml
Klebsiella pneumoniae ATCC BAA-1705: ≤0.03/8–0.06/8 µg/ml
*Dilutions are extrapolated to validation panel.

METHODS (Continued)

Panels
Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of meropenem/vaborbactam 0.03/8–64/8 µg/ml in cation-adjusted Mueller-Hinton broth.
Reference panels were prepared and frozen following CLSI/ISO recommendations.

Quality Control
Quality control (QC) testing was performed daily using ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa*, ATCC 35218 *E. coli*, ATCC 700603 *K. pneumoniae*, ATCC BAA-1705 *K. pneumoniae* using extrapolated CLSI M100-ED29 QC ranges based on the panel dilutions.

Panel Inoculation, Incubation, and Reading
All isolates were subcultured onto trypticase soy agar (TSA) with 5% sheep blood and incubated for 18–24 hours at 34–37ºC prior to testing. Isolates from frozen stocks were subcultured twice before testing.
Inoculum suspensions for each strain were prepared with the direct standardization (turbidity standard) method for MSDGN MIC and frozen reference panels. MSDGN MIC panels were also inoculated using the Prompt Inoculation method.
Following inoculation, MSDGN MIC panels were incubated at 35±2ºC in the WalkAway system for 18±2 hours. All panels were read by the WalkAway, autoSCAN-4, and visually.

Reproducibility
Reproducibility organisms with known results on-scale for meropenem/vaborbactam were tested in triplicate (for each inoculation method) on the MicroScan Dried Gram Negative MIC panels and singly on the frozen reference panel on three different days at each site.
MicroScan Dried Gram Negative MIC panels were tested using both the turbidity and Prompt inoculation methods and read on the WalkAway system, autoSCAN-4 instrument and manually.

Data Analysis
Essential Agreement (EA) = MSDGN panel MIC within +/- 1 dilution of the frozen reference MIC result.
Categorical Agreement (CA) = MSDGN panel and reference categorical results (S, I, R) agree using CLSI/FDA breakpoints for *Enterobacteriaceae* (Table 1).

Table 1. Meropenem/Vaborbactam (CLSI M100-ED29) Interpretive Breakpoints (µg/ml)

Organism Group	Susceptible	Intermediate	Resistant
Enterobacteriaceae	≤ 4/8	8/8	≥ 16/8

Major Errors = Frozen reference MIC is S and MSDGN panel MIC is R; calculated for susceptible strains only.

$$\% \text{ Major Errors} = \frac{\text{No. Major Errors}}{\text{Total No. S Isolates tested}} \times 100$$

Very Major Errors = Frozen reference MIC is R and MSDGN panel MIC is S; calculated for resistant strains only.

$$\% \text{ Very Major Errors} = \frac{\text{No. Very Major Errors}}{\text{Total No. R Isolates tested}} \times 100$$

Minor Errors = Frozen reference MIC is S or R when MSGDN panel MIC is I or MSDGN panel MIC is S or R when frozen reference MIC is I; calculated for all isolates tested.

$$\% \text{ Minor Errors} = \frac{\text{No. Minor Errors}}{\text{Total No. Isolates tested}} \times 100$$

RESULTS

Efficacy & Challenge Combined (Tables 2 and 3)
A total of 560 *Enterobacteriaceae* clinical isolates were tested among 3 sites on the MSDGN during efficacy. For challenge, 95 *Enterobacteriaceae* isolates were tested at one site. The tables below are the results from efficacy and challenge combined with the indicated inoculation method. (See https://www.fda.gov/STIC for indicated species).

Turbidity (Table 2)
Essential Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 97.9% (641/655) for manual read method, 98.2% (643/655) for WalkAway System, 97.9% (641/655) for autoSCAN-4 instrument using the turbidity inoculation method.
Categorical Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 98.5% (645/655) for manual read method, 98.5% (645/655) for WalkAway System, 98.5% (645/655) for autoSCAN-4 instrument using the turbidity inoculation method.

Table 2. Clinical Isolates—Turbidity Inoculation Method

Read Method	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
Manual	641/655	97.9	645/655	98.5	9/655	1.4	0/619	0.0	1/31	3.2
WalkAway	643/655	98.2	645/655	98.5	9/655	1.4	0/619	0.0	1/31	3.2
autoSCAN-4	641/655	97.9	645/655	98.5	9/655	1.4	0/619	0.0	1/31	3.2

Prompt (Table 3)
Essential Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 96.5% (632/655) for manual read method, 98.3% (644/655) for WalkAway System, 97.3% (637/655) for autoSCAN-4 instrument using the Prompt inoculation method.
Categorical Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 99.1% (649/655) for manual read method, 99.1% (649/655) for WalkAway System, 99.1% (649/655) for autoScan-4 instrument using the Prompt inoculation method.

Table 3. Clinical Isolates—Prompt Inoculation Method

Read Method	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
Manual	632/655	96.5	649/655	99.1	6/655	0.9	0/619	0.0	0/31	0.0
WalkAway	644/655	98.3	649/655	99.1	6/655	0.9	0/619	0.0	0/31	0.0
autoSCAN-4	637/655	97.3	649/655	99.1	6/655	0.9	0/619	0.0	0/31	0.0

Performance of meropenem/vaborbactam when testing *Morganella morganii* using the Prompt Inoculation system with the autoSCAN-4 or manual read methods were outside of essential agreement compared to the reference method and should be tested using the turbidity inoculation method.
Meropenem/vaborbactam is not active against bacteria that produce metallo-beta lactamases or oxacillinases with carbapenemase activity.

Efficacy & Challenge Combined (continued)
The ability of the MicroScan Dried Gram Negative Panels to detect resistance to meropenem/vaborbactam is unknown with *C. koseri, E. aerogenes, K. oxytoca, M. morganii, P. mirabilis, Providencia* species and *S. marcescens* because resistant strains were not available at the time of comparative testing. If such isolates are observed, they should be tested on an alternate method and/or submitted to a reference lab.

Reproducibility (Table 4)
Overall agreement (within ± two-fold dilution) between all sites for the reproducibility phase was ≥ 95% for all combinations.

Table 4. Reproducibility Testing—All Sites Combined with Manual, WalkAway, and autoScan-4 Instrument Reads

Read Method	Inoculation Method	No. (%) Agreement All Sites Combined	
		Best Case	Worst Case
Manual	Turbidity	367/378 (97.1)	367/378 (97.1)
WalkAway		371/378 (98.1)	371/378 (98.1)
autoSCAN-4		371/378 (98.1)	367/378 (97.1)
Manual	Prompt	366/378 (96.8)	366/378 (96.8)
WalkAway		363/378 (96.0)	363/378 (96.0)
autoSCAN-4		362/378 (95.8)	359/378 (95.0)

Quality Control (Table 5)
Overall QC results for the MSDGN panel were 94.9-100% in range for organisms tested (Table 5). Overall QC results for frozen reference panel were 99.2–100% in range for organism tested.

Table 5. Quality Control

Organism	QC Range (µg/mL)	Percent (%) in Range					
		Manual		WalkAway		autoSCAN-4	
		Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt
E. coli ATCC 25922	≤0.03/8–0.06/8	120/121 (99.2)	121/121 (100)	119/120 (99.2)	121/121 (100)	120/121 (99.2)	121/121 (100)
P. aeruginosa ATCC 27853	0.12/8–1/8	121/121 (100)	118/121 (97.5)	121/121 (100)	117/120 (97.5)	121/121 (100)	118/121 (97.5)
K. pneumoniae ATCC 700603	≤0.03/8–0.06/8	112/117 (95.7)	112/118 (94.9)	112/117 (95.7)	112/118 (94.9)	112/117 (95.7)	112/118 (94.9)
E. coli ATCC 35218	≤0.03/8–0.06/8	118/118 (100)	117/118 (99.2)	117/117 (100)	116/117 (99.1)	118/118 (100)	117/118 (99.2)
K. pneumoniae ATCC BAA-1705	≤0.03/8–0.06/8	111/117 (94.9)	116/116 (100)	111/117 (94.9)	117/117 (100)	111/117 (94.9)	116/117 (99.1)

CONCLUSION

This multicenter study showed that meropenem/vaborbactam MIC results for *Enterobacteriaceae* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI/FDA interpretive criteria.

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