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Multicenter Evaluation of Eravacycline 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 eravacycline 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 sites by comparing MIC values obtained using the MSDGN to MICs using a CLSI broth microdilution reference panel. A total of 414 *Enterobacteriaceae* clinical isolates were tested using the turbidity and MicroScan PROMPT inoculation® (Prompt) methods of inoculation during the efficacy phase. For challenge, 79 *Enterobacteriaceae* isolates were tested on MSDGN panels at one site. For reproducibility, a subset of 11 organisms was tested on MSDGN panels at each site. MSDGN panels were incubated at 35 ± 1°C 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 at 18 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°C and read visually. Frozen reference panels were read at 16–20 hours. FDA breakpoints (µg/ml) used for interpretation of MIC results were: *Enterobacteriaceae* ≤ 0.5 S. Potential major and very major errors were calculated using the non-susceptible (NS) result in place of resistant (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 (EA) %		Categorical Agreement (CA) %		Potential Very Major Errors (VMJ)* %		Potential Major Errors (MAJ) %	
	T	P	T	P	T	P	T	P
Visually	99.0 (488/493)	97.0 (478/493)	98.8 (487/493)	98.2 (484/493)	0.0 (0/44)	0.0 (0/44)	0.4 (2/449)	1.3 (6/449)
WalkAway	98.0 (483/493)	96.8 (477/493)	98.2 (484/493)	98.4 (485/493)	0.0 (0/44)	0.0 (0/44)	1.3 (6/449)	1.6 (7/449)
autoSCAN-4	96.1 (474/493)	92.3 (455/493)	98.4 (485/493)	98.0 (483/493)	0.0 (0/44)	0.0 (0/44)	0.4 (2/449)	1.1 (5/449)

T = Turbidity inoculation method, P = Prompt inoculation method.
* = Calculation of Potential VMJ excluding 1 well errors.

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 eravacycline MIC results for *Enterobacteriaceae* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using FDA interpretive criteria.

INTRODUCTION

A multicenter study was performed to evaluate the performance of a MicroScan Dried Gram Negative MIC panel with eravacycline using *Enterobacteriaceae* isolates with 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 493 *Enterobacteriaceae* clinical isolates were tested among the three sites.
Quality Control Expected Results, https://www.fda.gov/STIC
Escherichia coli ATCC 25922: 0.03 – 0.12 µg/ml
Pseudomonas aeruginosa ATCC 27853: 2 – 16 µg/ml

METHODS (Continued)

Panels
Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of eravacycline 0.016–32 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* and ATCC 27853 *P. aeruginosa* (see https://www.fda.gov/STIC).
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 eravacycline 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, NS) agree using FDA breakpoints for *Enterobacteriaceae*. (Table 1).

Table 1. Eravacycline FDA Interpretive Breakpoints (µg/ml) (https://www.fda.gov/STIC)

Organism Group	Susceptible	Resistant
Enterobacteriaceae	≤ 0.5	--

Eravacycline does not have an intermediate or resistant category, therefore potential major and very major errors were calculated using the Non-Susceptible (NS) result in place of resistant (R) as well as categorical errors. Calculation of very major errors excluded one well errors as those errors are within essential agreement.

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

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

Potential Very Major Errors = Frozen reference MIC is NS and MSDGN panel MIC is S; calculated for non-susceptible strains only.

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

RESULTS

Efficacy & Challenge Combined (Tables 2 and 3)
A total of 414 *Enterobacteriaceae* clinical isolates were tested among 3 sites on the MSDGN during efficacy. For challenge, 79 *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 99.0% (488/493) for manual read method, 98.0% (483/493) for WalkAway System, 96.1% (474/493) for autoSCAN-4 instrument using the turbidity inoculation method.
Categorical Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 98.8% (487/493) for manual read method, 98.2% (484/493) for WalkAway System, 98.4% (485/493) for autoSCAN-4 instrument using the turbidity inoculation method.

Table 2. Clinical Isolates—Turbidity Inoculation Method

	Essential Agreement		Categorical Agreement		Potential Major Errors		Potential Very Major Errors*	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	488/493	99.0	487/493	98.8	2/449	0.4	0/44	0
WalkAway	483/493	98.0	484/493	98.2	6/449	1.3	0/44	0
autoSCAN-4	474/493	96.1	485/493	98.4	2/449	0.4	0/44	0

*Calculation of Potential VMJ excluding 1 well errors.

Prompt: (Table 3)
Essential Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 97.0% (478/493) for manual read method, 96.8% (477/493) for WalkAway System, 92.3% (455/493) for autoSCAN-4 instrument using the Prompt inoculation method.
Categorical Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 98.2% (484/493) for manual read method, 98.4% (485/493) for WalkAway System, 98.0% (483/493) for autoScan-4 instrument using the Prompt inoculation method.

Table 3. Clinical Isolates—Prompt Inoculation Method

	Essential Agreement		Categorical Agreement		Potential Major Errors		Potential Very Major Errors*	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	478/493	97.0	484/493	98.2	6/449	1.3	0/44	0
WalkAway	477/493	96.8	485/493	98.4	7/449	1.6	0/44	0
autoSCAN-4	455/493	92.3	483/493	98.0	5/449	1.1	0/44	0

*Calculation of Potential VMJ excluding 1 well errors.

Results obtained with *C. freundii* and eravacycline with turbidity inoculation and the Prompt Inoculation System with the autoSCAN-4 read were outside of essential agreement compared to the reference method, results should be confirmed using a manual read.

Efficacy & Challenge Combined (continued)
Due to the lack of an intermediate interpretive category for eravacycline, results obtained with *C. freundii, E. cloacae, K. oxytoca* and *K. pneumoniae* with both the Prompt and turbidity inoculation methods and read using the autoSCAN-4 and manual read showed potential very major errors compared to the reference method. If critical to patient care, testing should be repeated using an alternative testing/reference method prior to reporting results when the eravacycline MIC is 0.5 µg/mL for *C. freundii, E. cloacae, K. oxytoca* and *K. pneumoniae*.
The ability of the MicroScan Dried Gram negative Panels to detect non-susceptible isolates to eravacycline is unknown for *E. coli, C. koseri, E. (K.) aerogenes* and *K. oxytoca* because an insufficient number of non-susceptible strains were available at the time of comparative testing.
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	292/297 (98.3)	292/297 (98.3)
WalkAway	Turbidity	294/297 (99.0)	294/297 (99.0)
autoSCAN-4	Turbidity	293/297 (98.7)	293/297 (98.7)
Manual	Prompt	294/297 (99.0)	294/297 (99.0)
WalkAway	Prompt	294/297 (99.0)	294/297 (99.0)
autoSCAN-4	Prompt	294/297 (99.0)	294/297 (99.0)

Quality Control (Table 5)
Overall QC results for the frozen reference and dried test results were 100% in range for ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa* when assessed with CLSI M100-ED30 QC ranges

Table 5: Quality Control

Organism	QC Range (µg/mL)	Ref	Percent (%) in Range					
			Manual		WalkAway		autoSCAN-4	
			Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt
E. coli ATCC 25922	0.03–0.12	100%	121/121 100%	121/121 100%	120/120 100%	121/121 100%	104/121 86.0%	103/121 85.1%
E. coli ATCC 25922	0.016–0.12†	100%	121/121 100%	121/121 100%	120/120 100%	121/121 100%	121/121 100%	121/121 100%
P. aeruginosa ATCC 27853	2–16	100%	121/121 100%	121/121 100%	121/121 100%	120/120 100%	121/121 100%	121/121 100%

†Recently approved CLSI range for M100-ED30.

CONCLUSION

This multicenter study showed that eravacycline MIC results for *Enterobacteriaceae* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using FDA interpretive criteria. FDA cleared April 18, 2019.

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