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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 MIC (MSDGN) Panel when compared to frozen CLSI broth microdilution reference panels.

Material/Methods: For efficacy, an evaluation was conducted at three sites by comparing MICs obtained using the MSDGN panel to MICs using a CLSI broth microdilution reference panel. A total of 414 *Enterobacteriaceae* clinical isolates were tested using the turbidity and Prompt®* methods of inoculation. MSDGN panels were incubated at 35 ± 2ºC and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGN panels were at 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 ± 2ºC 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.

Results: When compared to frozen reference panel results, essential and categorical agreements for all clinical isolates tested are as follows:

Read Method	Essential Agreement %		Categorical Agreement %		Categorical Errors %^	
	T	P	T	P	T	P
Visually	98.8 (409/414)	96.4 (399/414)	99.8 (413/414)	99.0 (410/414)	0.2 (1/414)	1.0 (4/414)
WalkAway	97.8 (405/414)	96.6 (400/414)	99.5 (412/414)	99.0 (410/414)	0.5 (2/414)	1.0 (4/414)
autoSCAN-4	95.7 (396/414)	92.5 (383/414)	99.8 (413/414)	98.8 (409/414)	0.2 (1/414)	1.2 (5/414)

T = Turbidity inoculation method, P = Prompt inoculation method

^Eravacycline has susceptible-only breakpoints, therefore minor, major and very major error designations as defined in the FDA document 'Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) System; Guidance for Industry and FDA', dated August 28, 2009 are not applicable. Categorical Errors occur when the CLSI Reference is susceptible (S) and the test is non-susceptible (NS), or the CLSI Reference is non-susceptible (NS) and the test is susceptible (S)

Conclusions: 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 414 *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.

Data Analysis
•Essential Agreement (EA) = MSDGN panel MIC within +/- 1 dilution of the frozen reference result MIC.
•Categorical Agreement (CA) = MSDGN panel and reference categorical results (S) 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 nor 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.

•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 is NS and MSDGN panel MIC is S; calculated for non-susceptible strains only.

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

•Categorical Errors = Frozen reference MIC is S and MSDGN panel MIC is NS or frozen reference MIC is NS and the MSDGN MIC is S; calculated for all isolates tested.

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

RESULTS

Efficacy (Tables 2 and 3)
Turbidity A total of 414 *Enterobacteriaceae* clinical isolates were tested among three sites. MSDGN panels were inoculated using the turbidity inoculation method (Table 2).
•Essential Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 98.8% (409/414) for manual read method, 97.8% (405/414) for WalkAway System, 95.7% (396/414) for autoSCAN-4 instrument using the turbidity inoculation method.
•Categorical Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 99.8% (413/414) for manual read method, 99.5% (412/414) for WalkAway System, 99.8% (413/414) 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	409/414	98.8	413/414	99.8	0/403	0	0/11	0
WalkAway	405/414	97.8	412/414	99.5	0/403	0	0/11	0
autoSCAN-4	396/414	95.7	413/414	99.8	0/403	0	0/11	0

**Calculation of Potential MAJ and VMJ excluding 1 well errors

Prompt: A total of 414 *Enterobacteriaceae* clinical isolates were tested among three sites MSDGN panels were inoculated using the Prompt inoculation method (Table 3)
•Essential Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 96.4% (399/414) for manual read method, 96.6% (400/414) for WalkAway System, 92.5% (383/414) for autoSCAN-4 instrument using the Prompt inoculation method.
•Categorical Agreement for *Enterobacteriaceae* between MSDGN panel and frozen reference panel was 99.0% (410/414) for manual read method, 99.0% (410/414) for WalkAway System, 98.8% (409/414) 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	399/414	96.4	410/414	99.0	2/403	0.5	0/11	0
WalkAway	400/414	96.6	410/414	99.0	2/403	0.5	0/11	0
autoSCAN-4	383/414	92.5	409/414	98.8	2/403	0.5	0/11	0

**Calculation of Potential MAJ and VMJ excluding 1 well errors

Efficacy (Table 4)
•Eravacycline has susceptible-only breakpoints, therefore minor, major and very major error designations as defined in the FDA document 'Class II Special Controls Guidance Document: Antimicrobial Susceptibility Test (AST) System; Guidance for Industry and FDA, dated August 28, 2009 are not applicable. Categorical Errors have been calculated as described in the Data Analysis section for the 414 *Enterobacteriaceae* isolates tested in efficacy.

Table 4. Clinical Isolates – Turbidity Inoculation Method

	Categorical Errors	
Read Method	**No.**	**%**
Manual	1/414	0.2
WalkAway	2/414	0.5
autoSCAN-4	1/414	0.2

Table 5. Clinical Isolates – Prompt Inoculation Method

	Categorical Errors	
Read Method	**No.**	**%**
Manual	4/414	1.0
WalkAway	4/414	1.0
autoSCAN-4	5/414	1.2

Quality Control (Table 6)
•Overall QC results for the frozen reference panel were 100% in range for ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa*

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%
***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%

•All results for ATCC 25922 *E. coli*, including those indicated as out of range for the AS4 (MIC ≤ 0.016 µg/mL), are 100% in range compared to the 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 18/APR/2019.

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