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Multicenter Evaluation of Ceftazidime/Avibactam MIC Results for *Enterobacteriaceae* and *Pseudomonas aeruginosa* Using MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Background: A multicenter study was performed to evaluate the accuracy of ceftazidime/avibactam 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 618 *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates were tested using the turbidity and Prompt™* methods of inoculation. For Challenge, a set of 116 organisms were tested on MSDGN panels at one site. For reproducibility, a subset of 16 organisms was tested on MSDGN panels at each site. MSDGN panels were incubated at 35 ± 2ºC and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. at 16-20 hours. Frozen reference panels, prepared according to CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 2ºC and read visually at 16-18 hours. FDA breakpoints (µg/ml) used for interpretation of MIC results were: *Enterobacteriaceae* and *Pseudomonas aeruginosa* ≤ 8/4 S and ≥ 16/4 R.

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

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors* %		Major Errors* %	
	T	P	T	P	T	P	T	P
Visually	98.8 (725/734)	97.7 (717/734)	99.2 (728/734)	99.0 (727/734)	6.5 (2/31)	3.2 (1/31)	0.1 (1/703)	0.3 (2/703)
WalkAway	98.9 (726/734)	95.2 (699/734)	98.8 (725/734)	98.4 (722/734)	3.2 (1/31)	3.2 (1/31)	0.3 (2/703)	1.0 (7/703)
autoSCAN-4	98.8 (725/734)	97.3 (714/734)	99.2 (728/734)	98.9 (726/734)	6.5 (2/31)	6.5 (2/31)	0.0 (0/703)	0.0 (0/703)

T = Turbidity inoculation method, P = Prompt inoculation method
* = calculated without 1 well dilution errors

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

Conclusions: This multicenter study showed that ceftazidime/avibactam MIC results for *Enterobacteriaceae* and *Pseudomonas aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels.

INTRODUCTION

A multicenter study was performed to evaluate the performance of a MicroScan Dried Gram Negative MIC panel with ceftazidime/avibactam using *Enterobacteriaceae* and *Pseudomonas aeruginosa* 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 734 *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates were tested among the three sites.

Quality Control Expected Results

Escherichia coli ATCC 25922: 0.06/4 - 0.5/4 µg/ml
Pseudomonas aeruginosa ATCC 27853: 0.5/4 - 4/4 µg/ml
Klebsiella pneumoniae ATCC 700603: 0.25/4 - 2/4 µg/ml
Escherichia coli ATCC 35218: ≤ 0.03/4 – 0.12/4 µg/ml

METHODS (Continued)

Panels

•Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of ceftazidime/avibactam 0.25/4-64/4 µg/ml in cation-adjusted Mueller-Hinton broth.
•Reference panels were prepared and frozen following CLSI recommendations.

Reproducibility

•Reproducibility organisms with known results on-scale for ceftazidime/avibactam 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.

Quality Control

•Quality control (QC) testing was performed daily using ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa*, ATCC 700603 *K. pneumoniae*, ATCC 35218 *E. coli* using FDA and CLSI QC ranges.

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, R) agree using FDA breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa*. (Table 1).

Table 1. Ceftazidime/Avibactam FDA Interpretive Breakpoints(µg/ml)

Organism Group	S	R
Enterobacteriaceae	≤ 8/4	≥ 16/4
Pseudomonas aeruginosa	≤ 8/4	≥ 16/4

•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 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 is S or R when MSGDN panel MIC is I or MSDGP panel MIC is S or R when frozen reference 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 (Tables 2 and 3)

•A total of 734 *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates were tested among three sites. MSDGN panels were inoculated using the turbidity inoculation method.
•Essential Agreement for *Enterobacteriaceae* and *Pseudomonas aeruginosa* between MSDGN panel and frozen reference panel was 98.8% (725/734) for manual read method, 98.9% (726/734) for WalkAway System, 98.8% (725/734) for autoSCAN-4 instrument using the turbidity inoculation method.
•Categorical Agreement for *Enterobacteriaceae* and *Pseudomonas aeruginosa* between MSDGN panel and frozen reference panel was 99.2% (728/734) for manual read method, 98.8% (725/734) for WalkAway System, 99.2% (728/734) for autoSCAN-4 instrument using the turbidity inoculation method.

Table 2. Clinical Isolates - Turbidity Inoculation Method

	Essential Agreement		Categorical Agreement		Major Errors		Very Major Errors*	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	725/734	98.8	728/734	99.2	1/703	0.1	2/31	6.5
WalkAway	726/734	98.9	725/734	98.8	2/703	0.3	1/31	3.2
autoSCAN-4	725/734	98.8	728/734	99.2	0/703	0.0	2/31	6.5

•A total of 734 *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates were tested among three sites. MSDGN panels were inoculated using the Prompt inoculation method.
•Essential Agreement for *Enterobacteriaceae* and *Pseudomonas aeruginosa* between MSDGN panel and frozen reference panel was 97.7% (717/734) for manual read method, 95.2% (699/734) for WalkAway System, 97.3% (714/734) for autoSCAN-4 instrument using the Prompt inoculation method.
•Categorical Agreement for *Enterobacteriaceae* and *Pseudomonas aeruginosa* between MSDGN panel and frozen reference panel was 99.0% (727/734) for manual read method, 98.4% (722/734) for WalkAway System, 98.9% (726/734) for autoScan-4 instrument using the Prompt inoculation method.

Table 3. Clinical Isolates – Prompt Inoculation Method

	Essential Agreement		Categorical Agreement		Major Errors		Very Major Errors*	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	717/734	97.7	727/734	99.0	2/703	0.3	1/31	3.2
WalkAway	699/734	95.2	722/734	98.4	7/703	1.0	1/31	3.2
autoSCAN-4	714/734	97.3	726/734	98.9	0/703	0.0	2/31	6.5

*Errors that were within essential agreement were excluded from analysis due to variability acceptance of the test. As a result of the very major error rate, the following limitations have been implemented:
•Results obtained with *S. marcescens* when using the Prompt Inoculation system and WalkAway read were outside of essential agreement compared to the reference method and should be confirmed using manual read.

Efficacy & Challenge (continued)

•Results obtained with *P. rettgeri* and ceftazidime/avibactam with the Prompt Inoculation system and WalkAway read were outside of essential agreement compared to the reference method; results should be confirmed using manual read. In addition, isolates of *P. rettgeri* providing MIC values ≥16 µg/mL with ceftazidime/avibactam should be retested using an alternate method to avoid major errors.
•Due to the occurrence of very major errors with ceftazidime/avibactam and with all inoculation and read methods, isolates of *Providencia stuartii* that provide MICs of 4 and 8 µg/mL and isolates of *P. aeruginosa* that provide MICs of 8 µg/mL should be retested using an alternative/ reference method.

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 with CZA – All Sites Combined with Manual, WalkAway, and autoScan-4 Instrument Reads of MicroScan Dried Gram-Negative Panel

Read Method	Inoculation Method	No. (%) Agreement Best Case & Worst Case All Sites Combined
Manual	Turbidity	431/432 (100)
WalkAway		431/432 (100)
autoSCAN-4		420/432 (97)
Manual	Prompt	429/432 (99)
WalkAway		425/432 (98)
autoSCAN-4		426/432 (99)

Quality Control (Table 6)

•Overall QC results for the frozen reference panel were 100% (164/164) in range for ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa*, ATCC 700603 *K. pneumoniae*, ATCC 35218 *E. coli*.

Organism	QC Range (µg/mL)	Percent (%) in Range					
		Manual		WalkAway		autoSCAN-4	
		Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt
***E. coli* ATCC 25922**	**0.06/4-0.5/4**	164/164 100%	164/164 100%	164/164 100%	162/162 100%	163/164 99%	163/163 100%
***P. aeruginosa* ATCC 27853**	**0.5/4 – 4/4**	164/164 100%	164/164 100%	164/164 100%	161/161 100%	163/163 100%	164/164 100%
K. pneumoniae ATCC 700603	**0.25/4-2/4**	163/164 99%	163/164 99%	164/164 100%	161/161 100%	164/164 100%	164/164 100%
***E. coli* ATCC 35218**	**0.03/4-0.12/4**	164/164 100%	160/164 98%	164/164 100%	159/162 98%	164/164 100%	159/162 98%

CONCLUSION

There is a correlation between the MIC results obtained using MicroScan Dried Gram-Negative panel and MICs obtained using a CLSI broth microdilution frozen reference panel for susceptibility testing of the new ceftazidime/avibactam formulation for *Enterobacteriaceae* and *Pseudomonas aeruginosa* in a multicenter study using FDA interpretive criteria.

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