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Multicenter Evaluation of Ceftolozane/Tazobactam 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 ceftolozane/tazobactam on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to frozen CLSI broth microdilution reference panels.
Material/Methods: For efficacy and challenge, an evaluation was conducted at three sites by comparing MICs obtained using the MSDGN to MICs using a CLSI broth microdilution reference panel. A total of 823 *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates were tested using the turbidity and Prompt®* methods of inoculation. For reproducibility, a set of 17 organisms was tested on MSDGN panels at all three sites. 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 ISO/CLSI 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 16-18 hours. EUCAST breakpoints (mg/L) used for interpretation of MIC results were: *Enterobacteriaceae* ≤ 1/4 S and > 1/4 R and *Pseudomonas aeruginosa* ≤ 4/4 S and > 4/4 R.
Results: When compared to frozen reference panel results, essential and categorical agreements for all isolates tested in Efficacy are as follows:

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %	
	T	P	T	P	T	P	T	P
Visually	95.4 (785/823)	93.6 (770/823)	98.2 (808/823)	97.7 (804/823)	1.1 (1/93)	1.1 (1/93)	1.2 (9/730)	1.5 (11/730)
WalkAway	94.0 (774/823)	90.9 (748/823)	98.4 (810/823)	95.9 (789/823)	4.3 (4/93)	5.4 (5/93)	0.8 (6/730)	3.7 (27/730)
autoSCAN-4	93.6 (770/823)	93.2 (767/823)	98.4 (810/823)	98.2 (808/823)	4.3 (4/93)	4.3 (4/93)	0.7 (5/730)	1.4 (10/730)

T = Turbidity inoculation method, P = Prompt inoculation method

Reproducibility among the three sites was greater than 95% for all read methods for both the turbidity and Prompt inoculation methods.
Conclusions: This multicenter study showed that ceftolozane/tazobactam MIC results for *Enterobacteriaceae* and *Pseudomonas aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using EUCAST interpretative criteria.

INTRODUCTION

A multicenter study was performed to evaluate the performance of a MicroScan Dried Gram Negative MIC panel with ceftolozane/tazobactam using *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolates with EUCAST 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 823 *Enterobacteriaceae* and *Pseudomonas aeruginosa* clinical isolates were tested among the three sites.
Quality Control Expected Results, EUCAST v9.0
Escherichia coli ATCC 25922: 0.12/4 - 0.5/4 mg/L
Pseudomonas aeruginosa ATCC 27853: 0.25/4 - 1/4 mg/L
Klebsiella pneumoniae ATCC 700603: 0.5/4 - 2/4 mg/L
Escherichia coli ATCC 35218: 0.06/4 - 0.25-4 mg/L

METHODS (Continued)

Panels
•Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of ceftolozane/tazobactam 0.03/4-64/4 mg/L in cation-adjusted Mueller-Hinton broth.
•Reference panels were prepared and frozen following CLSI/ISO recommendations.
Reproducibility
•Reproducibility organisms with known results on-scale for ceftolozane/tazobactam 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*, and ATCC 35218 *E. coli* using EUCAST 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 also 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 EUCAST breakpoints for *Enterobacteriaceae* and *Pseudomonas aeruginosa*. (Table 1).

Table 1. Ceftolozane/Tazobactam EUCAST, v9.0 Interpretive Breakpoints (mg/L)

Organism Group	Susceptible	Resistant
Enterobacteriaceae	≤1/4	>1/4
Pseudomonas aeruginosa	≤4/4	>4/4

•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.

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

RESULTS

Efficacy (Tables 2 and 3)
•A total of 823 *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 95.4% (785/823) for manual read method, 94.0% (774/823) for WalkAway System, and 93.6% (770/823) for autoSCAN-4 instrument using the turbidity inoculation method.
•Categorical Agreement for *Enterobacteriaceae* and *Pseudomonas aeruginosa* between MSDGN panel and frozen reference panel was 98.2% (808/823) for manual read method, 98.4% (810/823) for WalkAway System, and 98.4% (810/823) for autoSCAN-4 instrument using the turbidity inoculation method.

Table 2. Efficacy – Turbidity Inoculation Method

	Essential Agreement		Categorical Agreement		Major Errors		Very Major Errors	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	785/823	95.4	808/823	98.2	9/730	1.2	1/93	1.1
WalkAway	774/823	94.0	810/823	98.4	6/730	0.8	4/93	4.3
autoSCAN-4	770/823	93.6	810/823	98.4	5/730	0.7	4/93	4.3

•A total of 823 *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 93.6% (770/823) for manual read method, 90.9% (748/823) for WalkAway System, and 93.2% (767/823) for autoSCAN-4 instrument using the Prompt inoculation method.
•Categorical Agreement for *Enterobacteriaceae* and *Pseudomonas aeruginosa* between MSDGN panel and frozen reference panel was 97.7% (804/823) for manual read method, 95.9% (789/823) for WalkAway System, and 98.2% (808/823) for autoScan-4 instrument using the Prompt inoculation method.

Table 3. Efficacy – Prompt Inoculation Method

	Essential Agreement		Categorical Agreement		Major Errors		Very Major Errors	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	770/823	93.6	804/823	97.7	11/730	1.5	1/93	1.1
WalkAway	748/823	90.9	789/823	95.9	27/730	3.7	5/93	5.4
autoSCAN-4	767/823	93.2	808/823	98.2	10/730	1.4	4/93	4.3

Efficacy (continued)
•Very major errors were repeated in triplicate. One very major error resolved upon repeat testing for all inoculation and read method comparisons. In addition, the following limitation of procedure has been implemented: If a ceftolozane/tazobactam instrument result of resistant occurs with *Serratia liquefaciens*, *Morganella morganii*, or *Providencia rettgeri*, manually verify results.

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

Table 4. Reproducibility Testing with C/T Best Case – 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 All Sites Combined
Manual	Turbidity	447/459 (97.4)
WalkAway		455/459 (99.1)
autoSCAN-4		451/459 (98.3)
Manual	Prompt	449/459 (97.8)
WalkAway		453/459 (98.7)
autoSCAN-4		438/459 (95.4)

Reproducibility testing for ceftolozane/tazobactam with worst case comparisons yielded identical results to the best case comparisons.

Quality Control (Table 5)
•Overall QC results for the frozen reference panel were 100% in range for *E. coli, K. pneumoniae, P. aeruginosa,* and *E. coli*.

Table 5. Quality Control Results

Organism (Range)	Percent (%) in Range					
	Manual		WalkAway		autoSCAN-4	
	Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt
E. coli **ATCC 25922 (0.12/4-0.5/4)**	163/164 99.3%	164/164 100%	163/164 99.3%	162/162 100%	163/164 99.3%	163/163 100%
P. aeruginosa **ATCC 27853 (0.25/4 – 1/4)**	164/164 100%	164/164 100%	164/164 100%	161/161 100%	163/163 100%	164/164 100%
K. pneumoniae **ATCC 700603 (0.5/4-2/4)**	162/164 98.7%	161/164 98.1%	164/164 100%	160/161 99.3%	162/164 98.7%	161/164 98.1%
E. coli **ATCC 35218 (0.06/4-0.25/4)**	164/164 100%	159/164 96.9%	164/164 100%	159/162 98.1%	164/164 100%	159/162 98.1%

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 ceftolozane/tazobactam and *Enterobacteriaceae* and *Pseudomonas aeruginosa* in a multicenter study using EUCAST interpretive criteria.

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