Updated FDA/CLSI Ceftazidime Breakpoints from a Multicenter Assessment for Enterobacterales, *Acinetobacter* spp. and *Pseudomonas aeruginosa* Using MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Objectives: Updated US FDA/CLSI ceftazidime breakpoints were evaluated against data from a multicenter clinical study with Enterobacterales, *Acinetobacter* spp. and *P. aeruginosa* on a MicroScan Dried Gram-negative MIC (MSDGN) Panel. MIC results were compared to results obtained with frozen broth microdilution panels prepared according to CLSI methodology.
Materials/Methods: MSDGN panels were evaluated at five clinical sites by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI broth microdilution reference panel. Data from the combined phases of efficacy and challenge included 1,351 Enterobacterales, *Acinetobacter* spp. and *P. aeruginosa* clinical isolates tested using the turbidity and Prompt® methods of inoculation. To demonstrate reproducibility, a subset of 10 organisms were tested on MSDGN panels across four sites. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels were prepared and read according to CLSI methodology. FDA and CLSI breakpoints (µg/mL) used for interpretation of MIC results were: Enterobacterales ≤ 4 S, 8 I, ≥ 16 R; *Acinetobacter* spp. ≤ 8 S, 16 I, ≥ 32 R; *P. aeruginosa* ≤ 8 S, ≥ 16 R (FDA only).
Results: Essential and categorical agreement was calculated compared to frozen reference panel results. Results for isolates tested during efficacy and challenge with Prompt inoculation and manual read are as follows:

Organism Group	Essential Agreement (EA) %	Categorical Agreement (CA) %	Very Major Error (VMJ) %	Major Error (MAJ) %
Enterobacterales	93.5 (1010/1080)	96.8 (1045/1080)	1.8 (3/165)	1.1 (10/896)
Acinetobacter spp.	96.6 (84/87)	94.3 (82/87)	2.0 (1/50)	2.8 (1/36)
*P. aeruginosa**	93.5 (172/184)	97.3 (179/184)	0.0 (0/33)	2.0 (3/151)

*One well errors were excluded from categorical error calculations due to the lack of an intermediate breakpoint.

Conclusion: Ceftazidime MIC results for Enterobacterales, *Acinetobacter* spp., and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using updated FDA/CLSI interpretive criteria in this multicenter study.

INTRODUCTION

Data from a multicenter study was evaluated for the performance of a MicroScan Dried Gram Negative MIC panel with ceftazidime using Gram negative isolates with FDA/CLSI interpretive breakpoints.

METHODS

Study Design: MicroScan Dried Gram Negative MIC panels were tested concurrently with a CLSI frozen broth microdilution reference panel using both the turbidity and Prompt Inoculation methods. A total of 1,351 Gram negative clinical isolates were tested among five sites.

Quality Control Expected Results

Escherichia coli ATCC 25922:
- ≤0.5 µg/ml (MicroScan range, dried panel)
- 0.06 – 0.5 µg/ml (CLSI M100-ED32 range, frozen reference)

Pseudomonas aeruginosa ATCC 27853:
- 1 – 4 µg/ml (MicroScan range, dried panel)
- 1 – 4 µg/ml (CLSI M100-ED32 range, frozen reference)

METHODS (Continued)

Panels
Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of ceftazidime 0.5 - 64 µg/ml in cation-adjusted Mueller-Hinton broth. Reference panels were prepared and frozen following CLSI recommendations.

Quality Control
Quality control (QC) testing was performed daily with ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa* for a minimum of 20 replicates per site.

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 35±2ºC 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±1ºC in the WalkAway system for 18 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, I, R) agree using CLSI/FDA breakpoints for Gram negative reporting groups. (Table 1).

Table 1. Ceftazidime Interpretive Breakpoints (µg/ml)

Organism Group	FDA[1] Breakpoints		
	Susceptible (S)	Intermediate (I)	Resistant (R)
Enterobacterales[2]	≤ 4	8	≥ 16
P. aeruginosa[2]	≤ 8	-	≥ 16
Acinetobacter spp.[2]	≤ 8	16	≥ 32

1- FDA STIC 06/14/2019, https://www.fda.gov/drugs/development-resources/ceftazidime-injection-products
2- Breakpoints equivalent to CLSI M100-ED32

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.

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

Categorical Errors = Due to a lack of intermediate breakpoint for P. aeruginosa, any MIC result that is considered a major or very major error, but is within essential agreement of the reference MIC value, is considered acceptable

RESULTS

Efficacy (Tables 2 and 3)
A total of 1,351 Gram negative clinical isolates were tested among five sites. The 1,351 isolates consisted of 1,080 Enterobacterales, 87 *Acinetobacter* spp., and 184 P. aeruginosa. One well errors were excluded from categorical error calculations for *P. aeruginosa* due to the lack of an intermediate breakpoint. The following limitations apply: Do not report drug, therapy, or MIC for *Providencia* spp. and *M. morganii*. Additionally, due to the occurrence of very major errors with ceftazidime and the autoSCAN-4 with turbidity inoculation method, *Klebsiella* species that provide an MIC of 2 or 4 µg/mL should be interpreted manually prior to reporting. Results obtained with *Enterobacter* spp. and ceftazidime for all read methods with the Prompt inoculation system and manual reads with turbidity inoculation and results obtained with *Proteus vulgaris* and ceftazidime for the WalkAway and Manual read methods with both the Prompt and turbidity inoculation methods were within categorical agreement, but outside of essential agreement when compared to the reference method. If critical to patient care, *Enterobacter* spp. and *Proteus vulgaris* isolates should be retested using alternate methods.

Efficacy - Prompt
Essential Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 92.1% (1244/1351) for WalkAway System method, 95.3% (1287/1351) for autoSCAN-4 instrument, and 93.7% (1266/1351) for manual read method using the Prompt inoculation method.
Categorical Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 95.3% (1287/1351) for WalkAway System method, 96.8% (1308/1351) for autoSCAN-4 instrument, and 96.7% (1306/1351) for manual read method using the Prompt inoculation method.

Efficacy - Turbidity
Essential Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 96.5% (1304/1351) for WalkAway System method, 97.0% (1311/1351) for autoSCAN-4 instrument, and 96.5% (1303/1351) for manual read method using the turbidity inoculation method.
Categorical Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 97.3% (1314/1351) for WalkAway System method, 97.7% (1320/1351) for autoSCAN-4 instrument, and 97.6% (1319/1351) for manual read method using the turbidity inoculation method.

Table 2. Clinical Isolates - Prompt Inoculation Method

Read Method	Essential Agreement		Categorical Agreement*		Minor Errors*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
WalkAway	1244/1351	92.1	1287/1351	95.3	33/1167	2.8	24/1083	2.2	4/248	1.6
autoSCAN-4	1287/1351	95.3	1308/1351	96.8	26/1167	2.2	9/1083	0.8	4/248	1.6
Manual	1266/1351	93.7	1306/1351	96.7	25/1167	2.1	14/1083	1.3	4/248	1.6

Table 3. Clinical Isolates – Turbidity Inoculation Method

Read Method	Essential Agreement		Categorical Agreement*		Minor Errors*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
WalkAway	1304/1351	96.5	1314/1351	97.3	22/1167	1.9	6/1083	0.6	4/248	1.6
autoSCAN-4	1311/1351	97.0	1320/1351	97.7	19/1167	1.6	3/1083	0.3	6/248	2.4
Manual	1303/1351	96.5	1319/1351	97.6	18/1167	1.5	4/1083	0.4	5/248	2.0

Reproducibility
Results for all inoculation methods (turbidity and Prompt) and read methods (WA, AS-4, manual) met acceptance criteria (≥ 95%) for best case scenarios for all sites combined in accordance with FDA guidelines and ISO guidelines.

Quality Control (Tables 4 and 5)
Overall quality control results were ≥95% for each read and inoculation method on the dried test panel for ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa*. Quality control results were ≥95% as well as for the frozen reference panel, which were read manually with turbidity inoculation method. The number of replicates and percentage within range are indicated in Tables 4 and 5. Variations in total number tested for each read method are due to technical error elimination.

Table 4. Quality Control–Frozen Reference Results

Organism	QC Range (µg/mL)	Manual
		Turbidity
***E. coli* ATCC 25922**	**≤ 0.5 (frozen)**	99.7% (365/366)
***P. aeruginosa* ATCC 27853**	**1 – 4 (frozen)**	100% (366/366)

Table 5. Quality Control – Dried Test Results

Organism	QC Range (µg/mL)	WalkAway		autoSCAN-4		Manual	
		Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
***E. coli* ATCC 25922**	**0.06 – 0.5 (dried)**	97.9% (366/374)	99.2% (364/367)	98.1% (369/376)	99.2% (361/364)	98.4% (371/377)	99.2% (363/366)
***P. aeruginosa* ATCC 27853**	**1 – 4 (dried)**	98.4% (369/375)	98.6% (358/363)	97.1% (366/377)	96.7% (348/360)	98.9% (374/378)	98.9% (360/364)

CONCLUSION

This multicenter study showed that ceftazidime MIC results for Enterobacterales, *Acinetobacter* spp., and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using FDA/CLSI interpretive criteria.