Updated CLSI Meropenem Breakpoints for MicroScan Dried Gram Negative MIC Panels from a Multicenter Assessment of *Acinetobacter* species

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ABSTRACT

Background: Updated US FDA/CLSI meropenem breakpoints were evaluated against data from a multicenter clinical study with *Acinetobacter* species 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 four clinical sites by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI broth microdilution reference panel. The study included a total of 56 *Acinetobacter* spp. clinical isolates tested using the turbidity and Prompt® methods of inoculation during the combined phases of efficacy and challenge. MSDGN panels were incubated at 35 ± 1ºC and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels were prepared according to CLSI/ISO methodology, incubated for 20-24 hours and read visually. CLSI breakpoints (µg/mL) used for interpretation of MIC results were: ≤ 2 S, 4 I, ≥ 8 R for *Acinetobacter* species.

Results: Essential agreement, categorical agreement, and categorical errors were calculated compared to MIC results from frozen reference panels for all isolates tested in efficacy and challenge and found in the following table.

Read Method	Essential Agreement %		Categorical Agreement %		Very Major Errors %		Major Errors %		Minor Errors%	
	P	T	P	T	P	T	P	T	P	T
WalkAway	91.1 (51/56)	96.4 (54/56)	98.2 (55/56)	92.9 (52/56)	0.0 (0/37)	0.0 (0/37)	0.0 (0/19)	0.0 (0/19)	1.8 (1/56)	7.1 (4/56)
autoSCAN-4	92.9 (52/56)	96.4 (54/56)	98.2 (55/56)	92.9 (52/56)	0.0 (0/37)	0.0 (0/37)	0.0 (0/19)	0.0 (0/19)	1.8 (1/56)	7.1 (4/56)
Manual	92.9 (52/56)	96.4 (54/56)	98.2 (55/56)	91.1 (51/56)	0.0 (0/37)	0.0 (0/37)	0.0 (0/19)	0.0 (0/19)	1.8 (1/56)	8.9 (5/56)

P = Prompt inoculation method, T = Turbidity inoculation method

Conclusion: Meropenem MIC results for Gram Negative bacteria obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI interpretive criteria in this multicenter study.

INTRODUCTION

Data from a multicenter study was evaluated the performance of a MicroScan Dried Gram Negative MIC panel with meropenem using *Acinetobacter* species with CLSI interpretive breakpoints.

METHODS

Study Design: MicroScan Dried Gram Negative MIC panels were tested concurrently with a CLSI frozen broth microdilution reference panel at four sites using both the turbidity and Prompt Inoculation methods.
A total of 56 *Acinetobacter* species clinical isolates were tested among the four sites.

Quality Control Expected Results

Escherichia coli ATCC 25922:
0.008 – 0.06 µg/ml (MicroScan range, dried panel)
0.008 – 0.06 µg/ml (CLSI M100-ED32 range, frozen reference)

Pseudomonas aeruginosa ATCC 27853:
0.12 – 1 µg/ml (MicroScan range, dried panel)
0.12 – 1 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 tobramycin 0.004 - 32 µg/ml in cation-adjusted Mueller-Hinton broth.
Reference panels (0.004 - 32 µg/ml dilutions) were prepared and frozen following CLSI recommendations.

Quality Control
Quality control (QC) testing was performed daily using 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 breakpoints for Gram negative reporting groups. (Table 1).

Table 1. Meropenem CLSI Interpretive Breakpoints (µg/ml) (CLSI M100-ED32)

Organism Group	Susceptible (S)	Intermediate (I)	Resistant (R)
Acinetobacter spp.	≤ 2	4	≥ 8

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.

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

RESULTS

Efficacy (Tables 2 and 3)
A total of 56 *Acinetobacter* species clinical isolates were tested among four sites. The 56 isolates consisted of 50 *Acinetobacter baumannii* complex, 5 *Acinetobacter lwoffii*, and 1 *Acinetobacter* species. Meropenem MIC values tended to be one or more doubling dilution lower when compared to the reference broth microdilution method for *Acinetobacter* species with manual reads when using the turbidity Inoculation system.

Efficacy - Prompt
Essential Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 91.1% (51/56) for WalkAway System method, 92.9% (52/56) for autoSCAN-4 instrument, and 92.9% (52/56) for manual read method using the Prompt inoculation method.
Categorical Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 98.2% (55/56) for WalkAway System method, 98.2% (55/56) for autoSCAN-4 instrument, and 98.2% (55/56) 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 96.4% (54/56) for WalkAway System method, 96.4% (54/56) for autoSCAN-4 instrument, and 96.4% (54/56) for manual read method using the turbidity inoculation method.
Categorical Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 92.9% (52/56) for WalkAway System method, 92.9% (52/56) for autoSCAN-4 instrument, and 91.1% (51/56) 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	51/56	91.1	55/56	98.2	1/56	1.8	0/19	0.0	0/37	0.0
autoSCAN-4	52/56	92.9	55/56	98.2	1/56	1.8	0/19	0.0	0/37	0.0
Manual	52/56	92.9	55/56	98.2	1/56	1.8	0/19	0.0	0/37	0.0

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	54/56	96.4	52/56	92.9	4/56	7.1	0/19	0.0	0/37	0.0
autoSCAN-4	54/56	96.4	52/56	92.9	4/56	7.1	0/19	0.0	0/37	0.0
Manual	54/56	96.4	51/56	91.1	5/56	8.9	0/19	0.0	0/37	0.0

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**	**0.008 – 0.06 (frozen)**	98.4% (186/189)
***P. aeruginosa* ATCC 27853**	**0.12 – 1 (frozen)**	99.5% (188/189)

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**	**0.008 – 0.06 (dried)**	97.5% (195/200)	98.9% (188/190)	97.5% (195/200)	98.9% (186/188)	98.5% (197/200)	98.9% (187/189)
***P. aeruginosa* ATCC 27853**	**0.12 – 1 (dried)**	100% (202/202)	99.5% (185/186)	100% (200/200)	99.5% (184/185)	100% (201/201)	99.5% (186/187)

CONCLUSION

This multicenter study showed that meropenem MIC results for *Acinetobacter* species obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI interpretive criteria.

† Deceased