Updated EUCAST v12.0 Ciprofloxacin Breakpoints for MicroScan Dried Gram-Negative MIC Panels from a Multicenter Evaluation of *Acinetobacter* species and *Pseudomonas* species

P1584

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ABSTRACT

Objectives: Updated EUCAST v12.0 ciprofloxacin breakpoints were evaluated against data from a multicenter clinical study with *Acinetobacter* spp. and *Pseudomonas* spp. 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 three 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 862 clinical isolates, including 38 *Acinetobacter* spp. and 96 *Pseudomonas* spp., 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 16-18 hours for *Pseudomonas* spp. and 20-24 hours for *Acinetobacter* spp. and read visually. EUCAST v12.0 breakpoints (µg/mL) used for interpretation of MIC results were: ≤0.001 S, >1 R for *Acinetobacter* spp. and ≤0.001 S, >0.5 R for *Pseudomonas* spp.
Results: When compared to frozen reference panel results, essential and categorical agreements for all isolates tested in efficacy and challenge are as follows (AS-4 read method yielded similar results):

Organism Group	Prompt Essential Agreement* %		Prompt Categorical Agreement* %		Prompt Major Errors** %		Prompt Very Major Error** %	
	WalkAway	Manual	WalkAway	Manual	WalkAway	Manual	WalkAway	Manual
Acinetobacter species	97.4 (37/38)	100 (38/38)	100 (38/38)	100 (38/38)	0.0 (0/19)	0.0 (0/19)	0.0 (0/19)	0.0 (0/19)
Pseudomonas species	95.8 (92/96)	94.8 (91/96)	97.9 (94/96)	97.9 (94/96)	1.6 (1/61)	1.6 (1/61)	0.0 (0/35)	0.0 (0/35)
All Organisms***	94.3 (813/862)	94.4 (814/862)	97.9 (804/821)	97.9 (804/821)	0.2 (1/612)	0.2 (1/612)	0.0 (0/201)	0.0 (0/201)

*Overall EA calculated for all organisms, overall CA calculated for all organisms with interpretive criteria.
**Calculation excluding 1 well errors; *Acinetobacter* spp. & *Pseudomonas* spp. errors are potential errors
*** All organisms include *Acinetobacter* spp., *Aeromonas* spp., *B. cepacia* complex, *Enterobacterales*, *Pseudomonas* spp., PK/PD, and *S. maltophilia* organism groups.

Conclusion: In this multicenter study, ciprofloxacin MIC results for *Acinetobacter* species and *Pseudomonas* species obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels with updated EUCAST v12.0 interpretive criteria.

INTRODUCTION

Data from a multicenter study evaluated the performance of a MicroScan Dried Gram Negative MIC panel with ciprofloxacin using *Acinetobacter* species and *Pseudomonas* species with EUCAST v12.0 interpretive breakpoints.

METHODS

Study Design: MSDGN 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 862 Gram negative clinical isolates, including 38 *Acinetobacter* spp. and 96 *Pseudomonas* spp., were tested among the three sites.

Quality Control Expected Results

Escherichia coli ATCC 25922:
≤ 0.004 – 0.016 µg/ml (MicroScan range, dried panel)
≤ 0.004 – 0.016 µg/ml (CLSI M100-ED33 range, frozen reference)

Pseudomonas aeruginosa ATCC 27853:
0.12 – 1 µg/ml (MicroScan range, dried panel)
0.12 – 1 µg/ml (CLSI M100-ED33 range, frozen reference)

METHODS (Continued)

Panels
Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of ciprofloxacin 0.004 - 8 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,* 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 EUCAST v12.0 breakpoints for Gram negative reporting groups. (Table 1). Due to arbitrary, "off-scale" breakpoint of ≤ 0.001 for *Acinetobacter* species and *Pseudomonas* species, in this analysis, S = I (Susceptible, increased exposure" (I)), so those errors are calculated as potential major errors and potential very major errors.

Table 1. Ciprofloxacin EUCAST Interpretive Breakpoints (µg/ml)
(EUCAST v12.0 is equivalent to EUCAST v13.0, other than Enterobacterales indications for meningitis)

Organism Group	Susceptible (S)	Resistant (R)
Acinetobacter spp.	≤ 0.001	> 1
Aeromonas spp.	≤ 0.25	> 0.5
B. cepacia complex	-	-
Enterobacterales	≤ 0.25	> 0.5
Salmonella spp.	≤ 0.06	> 0.06
Pseudomonas spp.	≤ 0.001	> 0.5
PK/PD	≤ 0.25	> 0.5
S. maltophilia	-	-
Vibrio spp.	≤ 0.25	> 0.25

Major Errors = Frozen reference MIC is S or S, increased exposure" (I) 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 or S, increased exposure" (I); 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 and MSGDN panel MIC is I or MSDGP panel MIC is S or R and 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 Data (Tables 2 and 3)
A total of 862 Gram negative clinical isolates, were tested among three sites. The 862 isolates consisted of 38 *Acinetobacter* spp., 3 *Aeromonas* spp., 6 *B. cepacia* complex, 663 Enterobacterales, 21 *Salmonella* spp., 96 *Pseudomonas* spp, 13 PK/PD spp., and 22 *S. maltophilia* spp. Due to the occurrence of potential very major errors with ciprofloxacin and AS-4 reads with turbidity and Prompt inoculation methods, *P. aeruginosa* isolate AS4 MIC results should be confirmed manually.

Efficacy & Challenge - Prompt
Essential Agreement for *Acinetobacter* species between MSDGN panel and frozen reference panel was 97.4% (37/38) for WalkAway System method, 94.7% (36/38) for autoSCAN-4 instrument, and 100% (38/38) for manual read method using the Prompt inoculation method. Essential Agreement for *Pseudomonas* species between MSDGN panel and frozen reference panel was 95.8% (92/96) for WalkAway System method, 92.7% (89/96) for autoSCAN-4 instrument, and 94.8% (91/96) for manual read method using the Prompt inoculation method. Categorical Agreement for *Acinetobacter* species between MSDGN panel and frozen reference panel was 100% (38/38) for WalkAway System method, 100% (38/38) for autoSCAN-4 instrument, and 100% (38/38) for manual read method using the Prompt inoculation method. Categorical Agreement for *Pseudomonas* species between MSDGN panel and frozen reference panel was 97.9% (94/96) for WalkAway System method, 93.8% (90/96) for autoSCAN-4 instrument, and 97.9% (94/96) for manual read method using the Prompt inoculation method. Overall essential and categorical agreement for all isolates combined are listed in table 2 below.

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	813/862	94.3	804/821	97.9	14/821	1.7	1/612	0.2	0/201	0.0
autoSCAN-4	808/862	93.7	801/821	97.6	14/821	1.7	1/612	0.2	3/201	1.5
Manual	814/862	94.4	804/821	97.9	14/821	1.7	1/612	0.2	0/201	0.0

Efficacy & Challenge - Turbidity
Essential Agreement for *Acinetobacter* species between MSDGN panel and frozen reference panel was 94.7% (36/38) for WalkAway System method, 92.1% (35/38) for autoSCAN-4 instrument, and 97.4% (37/38) for manual read method using the turbidity inoculation method. Essential Agreement for *Pseudomonas* species between MSDGN panel and frozen reference panel was 97.9% (94/96) for WalkAway System method, 94.8% (91/96) for autoSCAN-4 instrument, and 99.0% (95/96) for manual read method using the turbidity inoculation method. Categorical Agreement for *Acinetobacter* species between MSDGN panel and frozen reference panel was 100% (38/38) for WalkAway System method, 100% (38/38) for autoSCAN-4 instrument, and 100% (38/38) for manual read method using the turbidity inoculation method. Categorical Agreement for *Pseudomonas* species between MSDGN panel and frozen reference panel was 97.9% (94/96) for WalkAway System method, 96.9% (93/96) for autoSCAN-4 instrument, and 99.0% (95/96) for manual read method using the turbidity inoculation method. Overall essential and categorical agreement for all isolates combined are listed in the table 3 below.

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	831/862	96.4	805/821	98.1	13/821	1.6	1/612	0.2	0/201	0.0
autoSCAN-4	827/862	95.9	804/821	97.9	13/821	1.6	0/612	0.0	2/201	1.0
Manual	832/862	96.5	806/821	98.2	13/821	1.6	0/612	0.0	0/201	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 100% 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**	**≤ 0.004 – 0.016 (frozen)**	100% (189/189)
***P. aeruginosa* ATCC 27853**	**0.12 – 1 (frozen)**	100% (189/189)

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**	**≤ 0.004 – 0.016 (dried)**	98.9% (184/186)	99.5% (188/189)	99.5% (187/188)	100% (188/188)	99.5% (188/189)	99.5% (188/189)
***P. aeruginosa* ATCC 27853**	**0.12 – 1 (dried)**	100% (185/185)	100% (189/189)	100% (189/189)	100% (187/187)	100% (189/189)	100% (189/189)

CONCLUSION

This multicenter study showed that ciprofloxacin MIC results for Gram negative clinical isolates, including *Acinetobacter* spp. and *Pseudomonas* spp., obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using EUCAST v12.0 interpretive criteria.