Multicenter Evaluation of the Accuracy of MIC Results for Colistin with MicroScan Dried Gram Negative MIC Panels using CLSI Breakpoints

S. Riedel[1], M. Traczewski[2], D. Beasley[2], R.K. Brookman[3], J.E. Diaz[3], Z.C. Lockett[3], and J.Y. Chau[3]

[1]Beth Israel Deaconess Medical Center, Boston, MA,[2]Clinical Microbiology Institute, Wilsonville, OR, [3]Beckman Coulter Microbiology, West Sacramento, CA

ABSTRACT

Background: A multicenter study was performed to evaluate the accuracy of colistin on a MicroScan Dried Gram Negative MIC (MSDGN) Panel when compared to a frozen CLSI/EUCAST broth microdilution reference panel.

Materials/Methods: MSDGN panels were evaluated at three clinical sites (including Beckman Coulter) by comparing MIC values obtained using the MSDGN panels to MICs obtained utilizing a CLSI/EUCAST broth microdilution reference panel. The study included 407 clinical isolates tested using the turbidity and Prompt® methods of inoculation. MSDGN panels were incubated in the WalkAway System (35±1°C) and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 16-20 hours. Frozen reference panels were prepared and inoculated according to CLSI/EUCAST Joint Working Group Recommendations for MIC determination of Colistin (Polymyxin E) and incubated for 16-20 hours and read visually. CLSI M100 ED32 breakpoints (µg/mL) used for interpretation of MIC results were: Enterobacterales ≤ 2 I, ≥ 4 R; *P. aeruginosa* ≤ 2 I, ≥ 4 R.

Results: Essential, categorical agreement, and categorical errors were calculated using MIC results from WalkAway reads for MSDGN panels. All other read methods yielded similar results. Categorical agreement was not calculated for *Acinetobacter* spp. as MIC only is reported.

Organism Group	WA Essential Agreement* (EA) %		WA Categorical Agreement (CA) %		WA Potential Very Major Error** (VMJ) %		WA Potential Major Error** (MAJ) %	
	P	T	P	T	P	T	P	T
Enterobacterales	99.2 (258/260)	99.2 (258/260)	98.1 (255/260)	98.5 (256/260)	1.1 (1/93)	0.0 (0/93)	0.6 (1/167)	1.2 (2/167)
Acinetobacter spp.	100 (19/19)	89.5 (17/19)	-	-	-	-	-	-
P. aeruginosa	96.4 (53/55)	98.2 (54/55)	90.9 (50/55)	96.4 (53/55)	33.3 (1/3)	33.3 (1/3)	1.9 (1/52)	0.0 (0/52)
All Organisms***	98.0 (392/400)	97.3 (396/407)	96.8 (305/315)	98.1 (309/315)	2.1 (2/96)	1.0 (1/96)	0.9 (2/219)	0.9 (2/219)

P = Prompt inoculation method, T = Turbidity inoculation method
* Overall EA is calculated for all organisms and overall CA is calculated for all organisms with interpretive criteria.
** Calculation excluding 1 well errors
*** All organisms include *Acinetobacter* spp., *Aeromonas* spp., *B. cepacia* complex, *Enterobacterales*, Other non-*Enterobacterales*, *P. aeruginosa*, and *S. maltophilia* organism groups

Conclusion: This multicenter study showed colistin MIC results for Gram Negative bacteria obtained with MSDGN panels correlate well with MICs obtained using frozen reference panels with CLSI interpretive criteria.

INTRODUCTION

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

METHODS

Study Design: A total of 407 Gram negative clinical isolates on MicroScan Dried Gram Negative MIC panels were tested concurrently with a CLSI/EUCAST frozen broth microdilution reference panel at three sites using both the turbidity and Prompt Inoculation methods.

Quality Control Expected Results

Escherichia coli ATCC 25922:
- ≤ 2 – 4 µg/ml (MicroScan range, dried panel)
- 0.25 – 2 µg/ml (CLSI M100-ED32 range, frozen reference)

Pseudomonas aeruginosa ATCC 27853:
- ≤ 2 – 4 µg/ml (MicroScan range, dried panel)
- 0.5 – 4 µg/ml (CLSI M100-ED32 range, frozen reference)

Escherichia coli NCTC 13846:
- 2 – 8 µg/ml (EUCAST v12.0 range, frozen reference)

METHODS (Continued)

Panels

Frozen reference and MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of colistin 2 - 4 µg/ml (0.12 - 8 µg/ml on the frozen reference panel) in cation-adjusted Mueller-Hinton broth. Reference panels were prepared and frozen following "Recommendations for MIC determination of colistin (polymyxin E) As recommended by the joint CLSI-EUCAST Polymyxin Breakpoints Working Group".

Quality Control

Quality control (QC) testing was performed daily at using ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa*, and NCTC 13846 *E. coli* 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. Colistin CLSI Interpretive Breakpoints (µg/ml) (CLSI M100-ED32, CLSI M45-ED3)

Organism Group	Susceptible (S)	Intermediate (I)	Resistant (R)
Acinetobacter spp.	-	≤ 2	≥ 4
Aeromonas spp.	-	-	-
B. cepacia complex	-	-	-
Enterobacterales	-	≤ 2	≥ 4
Other Non-Enterobacterales	-	-	-
P. aeruginosa	-	≤ 2	≥ 4
S. maltophilia	-	-	-

Potential Major Errors = Frozen reference MIC is I and MSDGN panel MIC is R; calculated for intermediate strains only.

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

Potential Very Major Errors = Frozen reference MIC is R and MSDGN panel MIC is I; calculated for resistant strains only.

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

RESULTS

Efficacy (Tables 2 and 3)

A total of 407 Gram negative clinical isolates were tested among three sites. The 407 isolates consisted of 19 *Acinetobacter* spp., 14 *Aeromonas* spp., 15 *B. cepacia* complex, 260 Enterobacterales, 29 Other Non-Enterobacterales, 55 *P. aeruginosa*, and 15 *S. maltophilia*. Differences in Prompt and turbidity totals tested are due to smaller, pin-point colonies not suitable for Prompt inoculation per instructions in the Prompt procedural manual. Do not report therapy for *Acinetobacter* spp. Do not report drug, therapy, or MIC for *Salmonella* species. Due to the occurrence of potential major errors with colistin and AS-4 reads with Prompt inoculation method, results from *Escherichia coli* or *P. aeruginosa* should be confirmed using manual read.

Efficacy - Prompt

Essential Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 98.0% (392/400) for WalkAway System method, 96.3% (385/400) for autoSCAN-4 instrument, and 98.5% (394/400) for manual read method using the Prompt inoculation method.

Categorical Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 96.8% (305/315) for WalkAway System method, 94.9% (299/315) for autoSCAN-4 instrument, and 96.2% (303/315) for manual read method using the Prompt inoculation method.

Table 2. Clinical Isolates - Prompt Inoculation Method

Read Method	Essential Agreement		Categorical Agreement*		Potential Major Errors		Potential Very Major Errors	
	No.	%	No.	%	No.	%	No.	%
WalkAway	392/400	98.0	305/315	96.8	2/219	0.9	2/96	2.1
autoSCAN-4	385/400	96.3	299/315	94.9	8/219	3.7	2/96	2.1
Manual	394/400	98.5	303/315	96.2	1/219	0.5	2/96	2.1

Efficacy - Turbidity

Essential Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 97.3% (396/407) for WalkAway System method, 97.1% (395/407) for autoSCAN-4 instrument, and 97.3% (396/407) for manual read method using the turbidity inoculation method.

Categorical Agreement for Gram negative isolates between MSDGN panel and frozen reference panel was 98.1% (309/315) for WalkAway System method, 96.8% (305/315) for autoSCAN-4 instrument, and 97.8% (308/315) for manual read method using the turbidity inoculation method.

Table 3. Clinical Isolates – Turbidity Inoculation Method

Read Method	Essential Agreement		Categorical Agreement*		Potential Major Errors		Very Potential Major Errors	
	No.	%	No.	%	No.	%	No.	%
WalkAway	396/407	97.3	309/315	98.1	2/219	0.9	1/96	1.0
autoSCAN-4	395/407	97.1	305/315	96.8	4/219	1.8	1/96	1.0
Manual	396/407	97.3	308/315	97.8	1/219	0.5	1/96	1.0

Quality Control (Tables 4 and 5)

Overall quality control results were ≥95% within range 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% within range for the frozen reference panel for ATCC 25922 *E. coli,* ATCC 27853 *P. aeruginosa*, and NCTC 13846 *E. coli,* 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**	**0.25 – 2 (frozen)**	100% (63/63)
***P. aeruginosa* ATCC 27853**	**0.5 – 4 (frozen)**	100% (63/63)
***E. coli* NCTC 13846**	**2 – 8 (frozen)**	100% (63/63)

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**	**≤ 2 – 4 (dried)**	98.4% (62/63)	98.4% (61/62)	98.4% (62/63)	100% (62/62)	98.4% (62/63)	100% (62/62)
***P. aeruginosa* ATCC 27853**	**≤ 2 – 4 (dried)**	100% (63/63)	100% (63/63)	100% (63/63)	100% (63/63)	100% (63/63)	100% (63/63)

CONCLUSION

This multicenter study showed that colistin MIC results for Gram negative isolates obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using CLSI interpretive criteria.