Multicenter Evaluation of Fosfomycin MIC Results for *Enterobacterales* Using EUCAST v12.0 Breakpoints (i.v. and oral for uncomplicated UTI only, E. coli) on MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Background: EUCAST V12.0 fosfomycin breakpoints for *Enterobacterales* (i.v. and oral for uncomplicated UTI only, E. coli) were evaluated against data from a multicenter clinical study on a MicroScan Dried Gram Negative MIC (MSDGN) Panel. MIC results were compared to results obtained with agar dilution reference prepared according to CLSI methodology.

Materials/Methods: A total of 344 Gram negative clinical isolates including 191 *Enterobacterales* were tested using the turbidity and Prompt® methods of inoculation during the efficacy phase at three clinical sites. An evaluation was conducted by comparing MIC values obtained using the MSDGN panels to MICs utilizing the CLSI agar dilution reference. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and read visually at 16-20 hours. Agar dilution plates were prepared according to CLSI methodology, incubated for 16-20 hours and read visually. EUCAST v12.0 fosfomycin breakpoints (mg/L) used for interpretation of MIC results were: i.v. for *Enterobacterales* ≤ 32 S, > 32 R and oral for uncomplicated UTI only, *E. coli* ≤ 8 S, > 8 R.

Results: Essential agreement, categorical agreement and categorical errors were calculated compared to MIC results from agar dilution plates. Results for all efficacy isolates with turbidity inoculation and manual read are found in the following table.

Organism Group	Essential Agreement* (EA) %	Categorical Agreement** (CA) %	Very Major Error (VMJ) %	Major Error (MAJ) %
Enterobacterales, iv	94.2* (324/344)	100** (67/67)	0.0 (0/1)	0.0 (0/66)
Enterobacterales, oral (Uncomplicated UTI only), *E. coli*	100 (67/67)	100 (67/67)	0.0 (0/2)	0.0 (0/65)

* EA is calculated for all organisms
** CA is calculated for *E. coli* only

Conclusion: This multicenter study showed that fosfomycin MIC results for i.v. for *Enterobacterales* and *E. coli* (oral, for uncomplicated UTI only) obtained with the MSDGN panel correlate well with MICs obtained using CLSI agar dilution reference using EUCAST interpretive criteria.

INTRODUCTION

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

METHODS

Study Design: A total of 344 Gram negative clinical isolates on MicroScan Dried Gram Negative MIC panels were tested panel at three sites using both the turbidity and Prompt Inoculation methods. MIC results were compared to results obtained with agar dilution reference tested at one site and prepared according to CLSI methodology.

Quality Control Expected Results

Escherichia coli ATCC 25922:

≤ 4 – 16 µg/ml (MicroScan range, dried panel)

0.5 – 2 µg/ml (CLSI M100-ED32 range, agar dilution reference)

Pseudomonas aeruginosa ATCC 27853:

≤ 4 – 16 µg/ml (MicroScan range, dried panel)

2 – 8 µg/ml (CLSI M100-ED32 range, agar dilution reference)

METHODS (Continued)

Panels

MicroScan Dried Gram Negative MIC panels contained two-fold doubling dilutions of fosfomycin 2-64µg/ml in cation-adjusted Mueller-Hinton broth.

Agar Dilution Reference

Agar dilution reference plates were tested at one site with concentrations of 0.25 - 256 µg/ml. Agar dilution reference plates were prepared following CLSI M07-ED11:2018.

Quality Control

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

Panel & Reference 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. 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.

Inoculum suspensions for each strain were prepared using a 0.5 McFarland standard for agar dilution reference plates. Plates were inoculated using an inoculum replicator and incubated at 35±1ºC. All plates were read visually. All reference testing was performed at one site.

Data Analysis

Essential Agreement (EA) = MSDGN panel MIC within +/- 1 dilution of the agar dilution reference result MIC.

Categorical Agreement (CA) = MSDGN panel and reference categorical results (S, I, R) agree using EUCAST breakpoints for Gram negative reporting groups. (Table 1).

Table 1. Fosfomycin EUCAST v12.0 Interpretive Breakpoints (µg/ml)

Organism Group	Susceptible (S)	Resistant (R)
Enterobacterales, iv	32	> 32
Enterobacterales, oral (uncomplicated UTI only), *E. coli*	8	> 8

Major Errors = Agar dilution 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 = Agar dilution reference MIC is R and MSDGN panel MIC is S; calculated for resistant strains only.

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

RESULTS

Efficacy (Tables 2 and 3, iv breakpoints; Tables 4 and 5, oral breakpoints)

A total of 344 Gram negative clinical isolates were tested among three sites. The 344 isolates consisted of 32 *Acinetobacter* spp., 1 *Aeromonas* spp., 8 *B. cepacia* complex, 191 Enterobacterales, 41 PK/PD, and 71 *Pseudomonas* species. Do not report therapy for *Enterobacterales* species, other than *E. coli* and do not report drug, therapy, or MIC for *Klebsiella* spp. and *S. maltophilia* for EUCAST iv breakpoints. Overall EA (97%) for AS4 read method with Prompt inoculation method was considered due to only 2 resistant isolates tested for *E. coli* for EUCAST oral breakpoints.

RESULTS (Continued)

Efficacy - Prompt (EUCAST iv)

Essential Agreement for Gram negative isolates between MSDGN panel and agar dilution reference plate was 92.7% (319/344) for WalkAway System method, 91.6% (315/344) for autoSCAN-4 instrument, and 93.9% (323/344) for manual read method using Prompt inoculation.

Categorical Agreement for Gram negative isolates between MSDGN panel and agar dilution reference plate was 100% (67/67) for WalkAway System method, 98.5% (66/67) for autoSCAN-4 instrument, and 100% (67/67) for manual read method using the Prompt inoculation method.

Table 2. Clinical Isolates - Prompt Inoculation Method (EUCAST iv)

Read Method	Essential Agreement		Categorical Agreement*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%
WalkAway	319/344	92.7	67/67	100	0/66	0.0	0/1	0.0
autoSCAN-4	315/344	91.6	66/67	98.5	0/66	0.0	0/1	0.0
Manual	323/344	93.9	67/67	100	0/66	0.0	0/1	0.0

Efficacy - Prompt (EUCAST oral)

Essential Agreement for Gram negative isolates between MSDGN panel and agar dilution reference plate was 97.0% (65/67) for WalkAway System method, 97.0% (65/67) for autoSCAN-4 instrument, and 98.5% (66/67) for manual read method using Prompt inoculation.

Categorical Agreement for Gram negative isolates between MSDGN panel and agar dilution reference plate was 97.0% (65/67) for WalkAway System method, 97.0% (65/67) for autoSCAN-4 instrument, and 98.5% (66/67) for manual read method using the Prompt inoculation method.

Table 4. Clinical Isolates - Prompt Inoculation Method (EUCAST oral)

Read Method	Essential Agreement		Categorical Agreement*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%
WalkAway	65/67	97.0	65/67	97.0	2/65	3.1	0/2	0.0
autoSCAN-4	65/67	97.0	65/67	97.0	1/65	1.5	1/2	50.0
Manual	66/67	98.5	66/67	98.5	1/65	1.5	0/2	0.0

Efficacy - Turbidity (EUCAST iv)

Essential Agreement for Gram negative isolates between MSDGN panel and agar dilution reference plate was 93.9% (323/344) for WalkAway System method, 91.9% (316/344) for autoSCAN-4 instrument, and 94.2% (324/344) for manual read method using turbidity inoculation.

Categorical Agreement for Gram negative isolates between MSDGN panel and agar dilution reference plate was 100% (67/67) for WalkAway System method, 100% (67/67) for autoSCAN-4 instrument, and 100% (67/67) for manual read method using the turbidity inoculation method.

Table 3. Clinical Isolates – Turbidity Inoculation Method (EUCAST iv)

Read Method	Essential Agreement		Categorical Agreement*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%
WalkAway	323/344	93.9	67/67	100	0/66	0.0	0/1	0.0
autoSCAN-4	316/344	91.9	67/67	100	0/66	0.0	0/1	0.0
Manual	324/344	94.2	67/67	100	0/66	0.0	0/1	0.0

Efficacy - Turbidity (EUCAST oral)

Essential Agreement for Gram negative isolates between MSDGN panel and agar dilution reference plate was 100% (67/67) for WalkAway System method, 100% (67/67) for autoSCAN-4 instrument, and 100% (67/67) for manual read method using turbidity inoculation.

Categorical Agreement for Gram negative isolates between MSDGN panel and agar dilution reference plate was 100% (67/67) for WalkAway System method, 100% (67/67) for autoSCAN-4 instrument, and 100% (67/67) for manual read method using the turbidity inoculation method.

Table 5. Clinical Isolates – Turbidity Inoculation Method (EUCAST oral)

Read Method	Essential Agreement		Categorical Agreement*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%
WalkAway	67/67	100	67/67	100	0/65	0.0	0/2	0.0
autoSCAN-4	67/67	100	67/67	100	0/65	0.0	0/2	0.0
Manual	67/67	100	67/67	100	0/65	0.0	0/2	0.0

Quality Control (Tables 6 and 7)

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 agar dilution reference plate for ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa,* which were read manually with turbidity inoculation method. The number of replicates and percentage within range are indicated in Tables 6 and 7. Variations in total number tested for each read method are due to technical error elimination.

Table 6. Quality Control – Agar Dilution Reference Results

Organism	QC Range (µg/mL)	Manual
		Turbidity
ATCC 25922 *E. coli*	0.5 – 2 (Agar Dilution)	100% (23/23)
ATCC 27853 *P. Aeruginosa*	2 – 8 (Agar Dilution)	100% (23/23)

Table 7. Quality Control – Dried Test Results

Organism	QC Range (µg/mL)	WalkAway		autoSCAN-4		Manual	
		Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
ATCC 25922 *E. coli*	≤ 4 – 16 (dried)	100% (73/73)	100% (74/74)	100% (74/74)	100% (73/73)	100% (74/74)	100% (74/74)
ATCC 27853 *P. aeruginosa*	≤ 4 – 16 (dried)	95% (69/73)	98% (73/74)	96% (71/74)	100% (74/74)	100% (73/73)	100% (73/73)

CONCLUSION

This multicenter study showed that fosfomycin MIC results, with EUCAST iv and oral breakpoints, for Gram negative isolates obtained with the MSDGN panel correlate well with results obtained using agar dilution reference method prepared according to CLSI methodology.