Multicenter Evaluation of *Pseudomonas* species with Updated EUCAST v12.0 Levofloxacin Breakpoints for MicroScan Dried Gram-Negative MIC Panels

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ABSTRACT

Objectives: Levofloxacin with updated EUCAST v12.0 breakpoints was evaluated against data from a multicenter clinical study for *Pseudomonas* 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 three clinical sites by comparing MIC values obtained using the MSDGN panels to MICs utilizing a CLSI broth microdilution reference panel. A total of 902 Gram-negative clinical isolates were tested using the turbidity and Prompt methods of inoculation, including 124 *Pseudomonas* spp. clinical isolates. MSDGN panels were incubated at 35 ± 1ºC and read on the WalkAway System, the autoSCAN-4 instrument, and read visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels, prepared according to ISO/CLSI methodology, were inoculated using the turbidity inoculation method. All frozen reference panels were incubated at 35 ± 2ºC and read visually at 16-18 hours for *Pseudomonas* spp. EUCAST v12.0 breakpoints (µg/ml) used for interpretation of MIC results were: *Pseudomonas* spp. ≤ 0.001 S and > 2 R.

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) with the following recommendation: Due to the occurrence of potential very major errors with levofloxacin and WA reads with Prompt inoculation method, *P. aeruginosa* isolate MIC results ≤ 2mg/L should be confirmed manually.

Organism Group	Prompt Essential Agreement* %		Prompt Categorical Agreement* %		Prompt Major Errors** %		Prompt Very Major Error** %	
	WalkAway	Manual	WalkAway	Manual	WalkAway	Manual	WalkAway	Manual
***Pseudomonas* species**	91.9 (114/124)	94.4 (117/124)	94.4 (117/124)	96.0 (119/124)	2.7 (2/74)	2.7 (2/74)	4.0 (2/50)	2.0 (1/50)
All Organisms***	95.7 (861/902)	95.9 (865/902)	96.5 (819/849)	96.7 (821/849)	0.3 (2/630)	0.3 (2/630)	2.0 (4/203)	1.5 (3/203)

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

Conclusion: Levofloxacin MIC results for *Pseudomonas* species obtained with the MSDGN panel, which included and extended dilutions series, correlate well with MICs obtained using frozen reference panels with updated EUCAST v12.0 interpretive criteria in this multicenter study.

INTRODUCTION

Data from a multicenter study evaluated the performance of a MicroScan Dried Gram Negative MIC panel with levofloxacin using *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 902 Gram negative clinical isolates, including 124 *Pseudomonas* spp., were tested.

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-ED33 range, frozen reference)

Pseudomonas aeruginosa ATCC 27853:

0.5 – 4 µg/ml (MicroScan range, dried panel)

0.5 – 4 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 levofloxacin 0.008 - 16 µg/mL in cation-adjusted Mueller-Hinton broth. Reference panels were prepared and frozen following CLSI/ISO recommendations. Supplemental testing was performed internally at Beckman Coulter to include 28 *Pseudomonas* spp. tested on dried commercial panels with dilutions of 0.5 – 4 µg/ml and reference panels with dilutions of 0.25 – 8 µg/ml.

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 *Pseudomonas* species, in this analysis, S=I Susceptible, increased exposure (I), therefore all errors are calculated as potential major errors and potential very major errors.

Table 1. Levofloxacin EUCAST Interpretive Breakpoints (µg/ml)
(EUCAST v12.0 & EUCAST v13.0 interpretive criteria are equivalent)

Organism Group	Susceptible (S)	Resistant (R)
Acinetobacter spp.	≤ 0.5	> 1
Aeromonas spp.	≤ 0.5	> 1
B. cepacia complex	-	-
Enterobacterales	≤ 0.5	> 1
Pseudomonas spp.	≤ 0.001	> 2
PK/PD	≤ 0.5	> 1
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 (Tables 2 and 3)

A total of 902 Gram negative clinical isolates, were tested among three sites, including one internal site. The 902 isolates consisted of 38 *Acinetobacter* spp., 3 *Aeromonas* spp., 6 *B. cepacia* complex, 684 Enterobacterales, 124 *Pseudomonas* spp, (96 *Pseudomonas* isolates from three external clinical trial sites and 28 *Pseudomonas* isolates, including 15 *P. aeruginosa* isolates, tested internally at Beckman Coulter), 25 PK/PD spp., and 22 *S. maltophilia* spp. Due to the occurrence of potential very major errors with levofloxacin and WA and AS-4 reads with Prompt inoculation method and AS-4 reads with turbidity, *P. aeruginosa* isolate MIC results ≤ 2mg/L should be confirmed manually. Levofloxacin MIC values tended to be one or more doubling dilution lower when compared to the reference broth microdilution method for all organisms with all read and inoculation methods

Efficacy & Challenge - Prompt

Essential Agreement for *Pseudomonas* species between MSDGN panel and frozen reference panel was 91.9% (114/124) for WalkAway System method, 91.1% (113/124) for autoSCAN-4 instrument, and 94.4% (117/124) for manual read method using the Prompt inoculation method. Categorical Agreement for *Pseudomonas* species between MSDGN panel and frozen reference panel was 94.4% (117/124) for WalkAway System method, 94.4% (117/124) for autoSCAN-4 instrument, and 96.0% (119/124) for manual read method using the Prompt inoculation method. Overall essential and categorical agreement and error rates for overall performance (all isolates combined) are listed in table below (Table 2).

Efficacy & Challenge - Turbidity

Essential Agreement for *Pseudomonas* species between MSDGN panel and frozen reference panel was 96.0% (119/124) for WalkAway System method, 91.1% (113/124) for autoSCAN-4 instrument, and 95.2% (118/124) for manual read method using the turbidity inoculation method. Categorical Agreement for *Pseudomonas* species between MSDGN panel and frozen reference panel was 96.8% (120/124) for WalkAway System method, 95.2% (118/124) for autoSCAN-4 instrument, and 97.6% (121/124) for manual read method using the turbidity inoculation method. Overall essential and categorical agreement and error rates for overall performance (all isolates combined) are listed in the table below (Table 3).

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	861/902	95.7	819/849	96.5	21/849	2.5	2/630	0.3	4/203	2.0
autoSCAN-4	841/902	93.2	817/849	96.2	20/849	2.4	2/630	0.3	8/203	3.9
Manual	865/902	95.9	821/849	96.7	21/849	2.5	2/630	0.3	3/203	1.5

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	878/902	97.3	826/849	97.3	17/849	2.0	0/630	0.0	3/203	1.5
autoSCAN-4	856/902	94.9	822/849	96.8	17/849	2.0	0/630	0.0	7/203	3.4
Manual	875/902	97.0	827/849	97.4	17/849	2.0	0/630	0.0	3/203	1.5

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% 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)**	100% (189/189)
***P. aeruginosa* ATCC 27853**	**0.5 – 4 (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**	**≤ 0.008 – 0.06 (dried)**	99.5% (185/186)	100% (189/189)	99.5% (187/188)	100% (188/188)	99.5% (188/189)	100% (189/189)
***P. aeruginosa* ATCC 27853**	**0.5 – 4 (dried)**	99.5% (184/185)	100% (189/189)	100% (189/189)	100% (187/187)	100% (189/189)	100% (189/189)

CONCLUSION

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