Updated CLSI Levofloxacin Breakpoints from a Multicenter Assessment for *Enterobacterales*, *Salmonella* spp., and *Pseudomonas aeruginosa* Using MicroScan Dried Gram Negative MIC Panels

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ABSTRACT

Background: Data from a multicenter clinical study with *Enterobacterales*, *Salmonella* spp. and *P. aeruginosa* on a MicroScan Dried Gram-negative MIC (MSDGN) Panel was evaluated with updated US FDA/CLSI levofloxacin breakpoints. MIC results were compared to results obtained with frozen broth microdilution panels prepared according to CLSI methodology.

Materials/Methods: A total of 839 *Enterobacterales*, *Salmonella* spp. and *P. aeruginosa* clinical isolates were tested at three clinical sites in efficacy and challenge combined. MSDGN panels were evaluated with turbidity and Prompt® methods of inoculation. MIC values obtained from the MSDGN panels were compared to MICs utilizing a CLSI broth microdilution reference panel. To assess reproducibility, a subset of 15 organisms were tested on MSDGN panels at each site. MSDGN panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and visually. Read times for the MSDGN panels were at 16-20 hours. Frozen reference panels were prepared and read according to CLSI methodology. FDA and CLSI breakpoints (µg/mL) used for interpretation of MIC results were: *Enterobacterales* ≤ 0. 5 S, 1 I, ≥ 2 R; *Salmonella* spp. ≤ 0.12 S, 0.25-1 I, ≥ 2 R; *P. aeruginosa* ≤ 1 S, 2 I, ≥ 4 R.

Results: Essential and categorical agreement was calculated compared to frozen reference panel results. Results for isolates tested during efficacy and challenge with Prompt inoculation and manual read are as follows:

Reporting Group	Essential Agreement (EA) %	Categorical Agreement (CA) %	Very Major Error (VMJ) %	Major Error (MAJ) %
Enterobacterales	96.2 (638/663)	96.7 (641/663)	1.5 (2/133)	0.0 (0/515)
P. aeruginosa	94.6 (88/93)	93.6 (87/93)	0.0 (0/34)	1.9 (1/54)
Salmonella spp.	100 (83/83)	98.8 (82/83)	0.0 (0/19)	0.0 (0/38)

Reproducibility among the three sites was greater than 95% for all read methods for both the turbidity and Prompt inoculation methods.

Conclusion: Levofloxacin MIC results for *Enterobacterales*, *Salmonella* spp., and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using updated FDA/CLSI 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 *Enterobacterales, Salmonella* spp. and *P. aeruginosa* isolates with FDA/CLSI interpretive breakpoints.

METHODS

Study Design: MicroScan Dried Gram Negative 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 839 *Enterobacterales, Salmonella* spp. and *P. aeruginosa* clinical isolates were tested among the three sites.

Quality Control Expected Results, CLSI M100-ED30

Escherichia coli ATCC 25922: ≤0.008 – 0.06 µg/ml*

Pseudomonas aeruginosa ATCC 27853: 0.5 - 4 µg/ml

*extrapolated to validation panel dilutions

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.

Quality Control

Quality control (QC) testing was performed daily using ATCC 25922 *E. coli* and ATCC 27853 *P. aeruginosa* (CLSI M100-ED30).

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 34-37ºC 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. Frozen reference panels were incubated in an off-line incubator. All dried panels were read by the WalkAway, autoSCAN-4, and manually.

Reproducibility

Reproducibility organisms with known results on-scale for levofloxacin were tested in triplicate (for each inoculation method) on the MicroScan Dried Gram Negative MIC panels and singly on the frozen reference panel on three different days at each site.

MicroScan Dried Gram Negative MIC panels were tested using both the turbidity and Prompt inoculation methods and read on the WalkAway system, autoSCAN-4 instrument and manually.

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 FDA/CLSI breakpoints for *Enterobacterales, Salmonella* spp. and *P. aeruginosa*. (Table 1).

Table 1. Levofloxacin FDA/CLSI Interpretive Breakpoints (µg/ml) (https://www.fda.gov/STIC)

Organism Group	Susceptible	Intermediate	Resistant
Enterobacterales	≤ 0.5	1	≥ 2
Salmonella spp	≤ 0.12	0.25-1	≥ 2
P. aeruginosa	≤ 1	2	≥ 4

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 & Challenge Combined (Tables 2-4)

A total of 839 *Enterobacterales, Salmonella* spp and *P. aeruginosa* clinical isolates were tested among three sites.

Table 2. Efficacy & Challenge - *Enterobacterales*

Performance for *Enterobacterales* in the combined phases of efficacy and challenge are as follows with the Prompt inoculation (P) or turbidity inoculation (T):

Read Method	Inoc Method	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
		No.	%	No.	%	No.	%	No.	%	No.	%
WalkAway	P	636/663	95.9	641/663	96.7	20/663	3.0	0/515	0.0	2/133	1.5
autoSCAN-4	P	624/663	94.1	639/663	96.4	19/663	2.9	1/515	0.2	4/133	3.0
Manual	P	638/663	96.2	641/663	96.7	20/663	3.0	0/515	0.0	2/133	1.5
WalkAway	T	646/663	97.4	645/663	97.3	16/663	2.4	0/515	0.0	2/133	1.5
autoSCAN-4	T	635/663	95.8	643/663	97.0	16/663	2.4	0/515	0.0	4/133	3.0
Manual	T	645/663	97.3	645/663	97.3	16/663	2.4	0/515	0.0	2/133	1.5

Table 3. Efficacy & Challenge – *Salmonella* spp

Performance for *Salmonella* spp in the combined phases of efficacy and challenge are as follows with the Prompt inoculation (P) or turbidity inoculation (T):

Read Method	Inoc Method	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
		No.	%	No.	%	No.	%	No.	%	No.	%
WalkAway	P	83/83	100	82/83	98.8	1/83	1.2	0/38	0.0	0/19	0.0
autoSCAN-4	P	83/83	100	82/83	98.8	1/83	1.2	0/38	0.0	0/19	0.0
Manual	P	83/83	100	82/83	98.8	1/83	1.2	0/38	0.0	0/19	0.0
WalkAway	T	83/83	100	83/83	100	0/83	0.0	0/38	0.0	0/19	0.0
autoSCAN-4	T	83/83	100	82/83	98.8	1/83	1.2	0/38	0.0	0/19	0.0
Manual	T	83/83	100	80/83	96.4	3/83	3.6	0/38	0.0	0/19	0.0

Table 4. Efficacy & Challenge – *P. aeruginosa*

Performance for *P. aeruginosa* in the combined phases of efficacy and challenge are as follows with the Prompt inoculation (P) or turbidity inoculation (T):

Read Method	Inoc Method	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
		No.	%	No.	%	No.	%	No.	%	No.	%
WalkAway	P	87/93	93.6	85/93	91.4	6/93	6.5	1/54	1.9	1/34	2.9
autoSCAN-4	P	85/93	91.4	83/93	89.3	5/93	5.4	1/54	1.9	4/34	11.8
Manual	P	88/93	94.6	87/93	93.6	5/93	5.4	1/54	1.9	0/34	0.0
WalkAway	T	91/93	97.9	88/93	94.6	5/93	5.4	0/54	0.0	0/34	0.0
autoSCAN-4	T	86/93	92.5	84/93	90.3	7/93	7.5	0/54	0.0	2/34	5.9
Manual	T	89/93	95.7	89/93	95.7	3/93	3.2	0/54	0.0	1/34	2.9

Reproducibility (Table 5)

A total of 15 isolates were tested for reproducibility at all three sites in triplicate over three days.

Overall agreement (within ± one two-fold dilution) between all sites for the reproducibility phase was ≥ 95% for all combinations.

Table 5. Reproducibility Testing with Lvx – All Sites Combined

Read Method	Inoculation Method	No. (%) Agreement All Sites Combined
WalkAway	Prompt	402/405 (99.3)
autoSCAN-4	Prompt	403/405 (99.5)
Manual	Prompt	402/405 (99.3)
WalkAway	Turbidity	401/405 (99.0)
autoSCAN-4	Turbidity	403/405 (99.5)
Manual	Turbidity	402/405 (99.3)

Quality Control (Table 6)

QC results for the frozen reference panel were 100% in range for ATCC 25922 *E. coli* and 100% in range for ATCC 27853 *P. aeruginosa*.

Table 6. Quality Control

Organism	QC Range (mg/L)	WalkAway		autoSCAN-4		Manual	
		Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
***E. coli* ATCC 25922**	**≤0.008-0.06**	99.5%	100%	99.5%	100%	99.5%	100%
***P. aeruginosa* ATCC 27853**	**0.5-4**	99.5%	100%	100%	100%	100%	100%

The ability of the MicroScan Dried Gram Negative Panels to detect resistance to levofloxacin is unknown for the following species because an insufficient number of resistant strains were available at the time of comparative testing: *C. koseri*, *P. vulgaris* and *P. agglomerans*. Isolates yielding MIC results suggestive of a resistant interpretive category should be submitted to a reference laboratory.

Due to the occurrence of very major errors with levofloxacin and the autoSCAN-4 with both turbidity and Prompt inoculation methods, isolates of *P. aeruginosa* that provide an MIC of 1 µg/mL should be interpreted manually prior to reporting.

CONCLUSION

Levofloxacin MIC results for *Enterobacterales, Salmonella* spp. and *P. aeruginosa* obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels using updated FDA/CLSI interpretive criteria in this multicenter study. FDA cleared 03/MAR/2020.

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