

Multicenter Assessment of the Accuracy of MIC Results for Piperacillin/Tazobactam with MicroScan Dried Gram Negative MIC Panels using CLSI Breakpoints

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ABSTRACT

Background: MIC data from MicroScan Dried Gram-negative MIC (MSDGN) Panels with piperacillin/tazobactam were evaluated with CLSI M100 ED33 breakpoints for *Acinetobacter* spp., Enterobacterales, Other-Non-Enterobacterales, and *Pseudomonas aeruginosa* from a multicenter clinical study. MIC results were compared to results obtained with frozen broth microdilution panels prepared according to CLSI methodology.

Material/methods: The study included a total of 681/683 clinical isolates tested using the Prompt and turbidity methods of inoculation during the combined phases of efficacy and challenge. 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. 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. Frozen reference panels were prepared according to CLSI/ISO methodology, incubated for 16-20 hours for Enterobacterales, Other Non-Enterobacterales, and *Pseudomonas aeruginosa* and for 20-24 hours for *Acinetobacter* spp. and read visually. CLSI breakpoints (mg/L) used for interpretation of MIC results were: ≤16/4 S, 32/4-64/4 I, ≥128/4 R for *Acinetobacter* spp. and Other Non-Enterobacterales, ≤8/4 S, 16/4 SDD, ≥32/4 R for Enterobacterales, and ≤16/4 S, 32/4 I, >64/4 R for *Pseudomonas aeruginosa*.

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 elevated major error rates for *S. marcescens* and piperacillin/tazobactam with WalkAway read method with Prompt inoculation, results should be confirmed by a manual read prior to reporting. Due to the performance with *P. rettgeri*, *S. liquefaciens*, and *S. liquefaciens* complex with all read and inoculation methods, do not report drug, therapy, or MIC.

Organism Group	Prompt Essential Agreement %		Prompt Categorical Agreement %		Prompt Major Errors %		Prompt Very Major Errors %	
	WalkAway	Manual	WalkAway	Manual	WalkAway	Manual	WalkAway	Manual
<i>Acinetobacter</i> spp.	96.0 (48/50)	96.0 (48/50)	96.0 (48/50)	100 (50/50)	0 (0/33)	0 (0/33)	0 (0/15)	0 (0/15)
Enterobacterales	93.4 (510/546)	96.5 (527/546)	92.5 (505/546)	95.6 (522/546)	3.8 (17/452)	0.4 (2/452)	1.3 (1/75)	1.3 (1/75)
Other Non-Enterobacterales	100 (15/15)	100 (15/15)	100 (15/15)	100 (15/15)	0 (0/14)	0 (0/14)	--	--
<i>Pseudomonas aeruginosa</i>	92.9 (65/70)	94.3 (66/70)	95.7 (67/70)	95.7 (67/70)	4.1 (2/49)	2.0 (1/49)	0 (0/19)	0 (0/19)
All Organisms	93.7 (638/681)	96.3 (656/681)	93.3 (635/681)	96.0 (654/681)	3.5 (19/548)	0.6 (3/548)	0.9 (1/109)	0.9 (1/109)

Conclusions: Piperacillin/tazobactam MIC results for gram-negative clinical isolates obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels with updated CLSI interpretive criteria in this multicenter study.

INTRODUCTION

A multicenter study was performed to evaluate the performance of a MicroScan Dried Gram Negative MIC panel with piperacillin/tazobactam using *Acinetobacter* spp., Enterobacterales, Other Non-Enterobacterales, and *Pseudomonas aeruginosa* isolates with CLSI interpretive breakpoints.

METHODS

Study Design: MSDGN MIC panels were tested concurrently with a CLSI frozen broth microdilution reference panel at three sites on a total of 681/683 *Acinetobacter* spp., Enterobacterales, Other Non-Enterobacterales, and *Pseudomonas aeruginosa* clinical isolates.

METHODS (Continued)

Quality Control Expected Results (CLSI M100 ED33)

Escherichia coli ATCC 25922: 1/4 - 8/4 µg/ml
Pseudomonas aeruginosa ATCC 27853: 1/4 - 8/4 µg/ml
Escherichia coli ATCC 35218: 0.5/4 - 2/4 µg/ml

Panels

•Frozen reference and MSDGN MIC panels contained two-fold doubling dilutions of piperacillin/tazobactam 8/4-256/4 µg/ml in cation-adjusted Mueller-Hinton broth. Reference panels were prepared and frozen following CLSI/ISO recommendations.

Reproducibility

•Reproducibility organisms with known results on-scale for piperacillin/tazobactam were tested in triplicate (for each inoculation method) on the MSDGN MIC panels and singly on the frozen reference panel on three different days at each site.

•MSDGN MIC panels were tested using both the turbidity and Prompt inoculation methods and read on the WalkAway system, autoSCAN-4 instrument, and visually.

Quality Control

•Quality control (QC) testing was performed daily using ATCC 25922 *Escherichia coli*, ATCC 27853 *Pseudomonas aeruginosa*, ATCC 35218 *Escherichia coli* with CLSI QC ranges.

Panel Inoculation, Incubation, and Reading

•All isolates were subcultured into trypticase soy agar (TSA) with 5% sheep blood and incubated for 18-24 hours at 35-37°C 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 also incubated at 35±1°C in the WalkAway system for 18±2 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/SDD, and R) agree using CLSI M100 ED33 breakpoints.

Table 1. Piperacillin/Tazobactam CLSI Breakpoints (µg/ml)
(CLSI M100 ED33)

Organism Group	S	I/SDD	R
<i>Aeromonas</i> spp.	≤ 16/4	32/4-64/4	≥ 128/4
<i>Acinetobacter</i> spp.	≤ 16/4	32/4-64/4	≥ 128/4
Enterobacterales	≤ 8/4	16/4 (SDD)	≥ 32/4
Other Non-Enterobacterales	≤ 16/4	32/4-64/4	≥ 128/4
<i>Pseudomonas aeruginosa</i>	≤ 16/4	32/4	≥ 64/4
<i>Vibrio</i> Spp.	≤ 16/4	32/4-64/4	≥ 128/4

•Major Errors = Frozen reference MIC is S and MSDGN panel MIC is R; calculated for susceptible strains only. No. Major Errors

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

•Very Major Errors = Frozen reference is R and MSDGN panel MIC is S; calculated for resistant strains only. No. Very Major Errors

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

•Minor Errors = Frozen reference is S or R when MSDGN panel MIC is I or MSDGN panel MIC is S or R when frozen reference is I; calculated for all isolates tested. No. Minor Errors

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

RESULTS

Efficacy (Tables 2 and 3)

•A total of 683 *Acinetobacter* spp., Enterobacterales, Other Non-Enterobacterales, and *Pseudomonas aeruginosa* clinical isolates were tested among three sites. MSDGN panels were inoculated using the turbidity inoculation method. Essential Agreement for all isolates combined between MSDGN panel and frozen reference panel was 98.0% (669/683) for manual read method, 96.1% (656/683) for WalkAway System, 96.6% (660/683) for autoSCAN-4 instrument using the turbidity inoculation method.

•Categorical Agreement for all isolates combined between MSDGN panel and frozen reference panel was 95.3% (651/683) for manual read method, 94.1% (643/683) for WalkAway System, 94.4% (645/683) for autoSCAN-4 instrument using the turbidity inoculation method.

Table 2. Efficacy – Turbidity Inoculation Method

Read Method	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
Manual	669/683	98.0	651/683	95.3	30/683	4.4	0/550	0.0	2/109	1.8
WalkAway	656/683	96.1	643/683	94.1	35/683	5.1	3/550	0.6	2/109	1.8
autoSCAN-4	660/683	96.6	645/683	94.4	35/683	5.1	1/550	0.2	2/109	1.8

•A total of 681 *Acinetobacter* spp., Enterobacterales, Other Non-Enterobacterales, and *Pseudomonas aeruginosa* clinical isolates were tested among three sites. MSDGN panels were inoculated using the Prompt inoculation method. 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.

•Essential Agreement for all isolates combined between MSDGN panel and frozen reference panel was 96.3% (656/681) for manual read method, 93.7% (638/681) for WalkAway System, 95.9% (653/681) for autoSCAN-4 instrument using the Prompt inoculation method.

•Categorical Agreement for all isolates combined between MSDGN panel and frozen reference panel was 96.0% (654/681) for manual read method, 93.3% (635/681) for WalkAway System, 95.3% (649/681) for autoScan-4 instrument using the Prompt inoculation method.

Table 3. Efficacy – Prompt Inoculation Method

Read Method	Essential Agreement		Categorical Agreement		Minor Errors		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%	No.	%
Manual	656/681	96.3	654/681	96.0	23/681	3.4	3/548	0.6	1/109	0.9
WalkAway	638/681	93.7	635/681	93.3	26/681	3.8	19/548	3.5	1/109	0.9
autoSCAN-4	653/681	95.9	649/681	95.3	28/681	4.1	3/548	0.6	1/109	0.9

Performance issues with *P. rettgeri*, *S. liquefaciens* and *S. liquefaciens* complex resulted in decisions to not report the drug, therapy, or MIC. Results for *S. marcescens* using WalkAway read method with Prompt inoculation should be confirmed by a manual read prior to reporting due to elevated major error rates.

Reproducibility (Tables 4 & 5)

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

Table 4. Reproducibility Best Case – All Sites combined

Read Method	Inoculation Method	No. (%) Agreement Best Case All Sites Combined
Manual	Turbidity	262/270 (97.0)
WalkAway		258/270 (95.6)
autoSCAN-4		258/270 (95.6)
Manual	Prompt	260/270 (96.3)
WalkAway		263/270 (97.4)
autoSCAN-4		263/270 (97.4)

Table 5. Reproducibility Worst Case – All Sites combined

Read Method	Inoculation Method	No. (%) Agreement Worst Case All Sites Combined
Manual	Turbidity	262/270 (97.0)
WalkAway		258/270 (95.6)
autoSCAN-4		258/270 (95.6)
Manual	Prompt	260/270 (96.3)
WalkAway		263/270 (97.4)
autoSCAN-4		263/270 (97.4)

Quality Control (Tables 6 and 7)

Overall quality control results were >95% for each read and inoculation method on the dried test panel for ATCC 25922 *E. coli*, ATCC 27853 *P. aeruginosa* and ATCC 35218 *E. coli*. Quality control results were >95% 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 6, 7, and 8. Variations in total number tested for each read method are due to technical error elimination.

Table 6. Quality Control – Dried Test Results

Organism	QC Range (µg/mL)	Percent (%) in Range					
		Manual		WalkAway		autoSCAN-4	
		Turbidity	Prompt	Turbidity	Prompt	Turbidity	Prompt
<i>E. coli</i> ATCC 25922	1/4 - 8/4	98/98 100%	98/98 100%	98/98 100%	98/98 100%	98/98 100%	98/98 100%
<i>P. aeruginosa</i> ATCC 27853	1/4 - 8/4	95/98 96.9%	95/97 97.9%	95/98 96.9%	95/97 97.9%	96/98 98.0%	95/97 97.9%
<i>E. coli</i> ATCC 35218	0.5/4 - 2/4	98/98 100%	96/96 100%	98/98 100%	96/96 100%	98/98 100%	96/96 100%

Table 7. Quality Control – Frozen Reference Results

Organism	QC Range (µg/mL)	Manual		Quality control performance of piperacillin on the frozen reference panel confirmed integrity of <i>E. coli</i> ATCC 35218 at all sites.	
		Turbidity	Prompt	Organism	QC Range (µg/mL)
<i>E. coli</i> ATCC 25922	1/4 - 8/4 (frozen)	100% (98/98)	100%	<i>E. coli</i> ATCC 35218	> 256 (frozen)
<i>P. aeruginosa</i> ATCC 27853	1/4 - 8/4 (frozen)	98.0% (96/98)	100%		
<i>E. coli</i> ATCC 35218	0.5/4 - 2/4 (frozen)	100% (98/98)	100%		

Table 8. QC Performance with Piperacillin

Organism	QC Range (µg/mL)	Manual Turbidity
<i>E. coli</i> ATCC 35218	> 256 (frozen)	100% (98/98)

CONCLUSION

There is a strong correlation between the MIC results obtained using MicroScan Dried Gram Negative panel and MICs obtained using a CLSI broth microdilution frozen reference panel for susceptibility testing of piperacillin/tazobactam and *Acinetobacter* spp., Enterobacterales, Other Non-Enterobacterales, and *Pseudomonas aeruginosa* in a multicenter study using CLSI M100 ED33 interpretive criteria.

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