EUCAST v12.0 Breakpoints for Piperacillin/Tazobactam on MicroScan Dried Gram Negative MIC Panels for Enterobacterales species and *Pseudomonas* species

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ABSTRACT

Background: MIC data from MicroScan Dried Gram-negative MIC (MSDGN) Panels with piperacillin/tazobactam were evaluated with EUCAST v12.0 breakpoints from a multicenter clinical study. MIC results from Enterobacterales and *Pseudomonas* spp were compared to results obtained with CLSI frozen broth microdilution panels.

Material/methods: The study included a total of 547 Enterobacterales and 85 *Pseudomonas* spp. clinical isolates tested using the turbidity and Prompt 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 ± 1ºC 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 and *Pseudomonas* spp. and read visually. EUCAST v12.0 breakpoints (mg/L) used for interpretation of MIC results were: ≤ 8/4 S, > 8/4 R for Enterobacterales and ≤ 0.001/4 S, > 16/4 R for *Pseudomonas* spp.

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 low performance 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 and 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 Error** %	
	WalkAway	Manual	WalkAway	Manual	WalkAway	Manual	WalkAway	Manual
Enterobacterales	93.4 (510/546)	96.5 (527/546)	94.5 (516/546)	97.6 (533/546)	3.8 (17/452)	0.4 (2/452)	1.1 (1/94)	1.1 (1/94)
Pseudomonas species	94.1 (80/85)	95.3 (81/85)	96.5 (82/85)	96.5 (82/85)	3.2 (2/63)	1.6 (1/63)	4.6 (1/22)	4.6 (1/22)
All Organisms***	93.7 (638/681)	96.3 (656/681)	94.8 (598/631)	97.5 (615/631)	3.7 (19/515)	0.6 (3/515)	1.7 (2/116)	1.7 (2/116)

*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 errors
*** All organisms include *Acinetobacter* spp., Enterobacterales, and *Pseudomonas* spp. organism groups.

Conclusions: Piperacillin/tazobactam MIC results for Enterobacterales and *Pseudomonas* spp. obtained with the MSDGN panel correlate well with MICs obtained using frozen reference panels with updated EUCAST v12.0 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 Enterobacterales and *Pseudomonas* spp. isolates with EUCAST 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 on a total of 683 *Acinetobacter* spp., Enterobacterales, and *Pseudomonas* spp. clinical isolates.

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

METHODS (Continued)

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 35-37ºC 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 also incubated at 35±1ºC 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 and R) agree using EUCAST v12.0 breakpoints for Enterobacterales and *Pseudomonas* species. *Acinetobacter* species were evaluated for MIC reporting only (Table 1). Due to arbitrary, "off-scale" breakpoint of ≤ 0.001 for *Pseudomonas* species, in this analysis, S = I (Susceptible, increased exposure" (I)), so all errors are calculated as potential major errors and potential very major errors.

Table 1. Piperacillin/Tazobactam EUCAST Breakpoints (µg/ml)

EUCAST v12.0 is equivalent to EUCAST v13.0 interpretive criteria

Organism Group	S	R
Acinetobacter spp.	IE	IE
Enterobacterales	≤ 8/4	> 8/4
Pseudomonas spp.	≤ 0.001/4	> 16/4
PK/PD	≤ 8/4	> 16/4
Vibrio Spp.	≤ 1/4	> 1/4

•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 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 is S or R when MSDGN panel MIC is I or MSDGP panel MIC is S or R when 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 (Tables 2 and 3)

•A total of 683 *Acinetobacter* spp., Enterobacterales and *Pseudomonas* spp. clinical isolates were tested among three sites. MSDGN panels were inoculated using the turbidity inoculation method. Essential Agreement for *Acinetobacter* spp., Enterobacterales and *Pseudomonas* spp. 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 Enterobacterales and *Pseudomonas* spp. between MSDGN panel and frozen reference panel was 96.8% (613/633) for manual read method, 95.9% (607/633) for WalkAway System, 96.7% (612/633) for autoSCAN-4 instrument using the turbidity inoculation method.

Table 2. Efficacy – Turbidity Inoculation Method

	Essential Agreement		Categorical Agreement		Major Errors		Very Major Errors	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	669/683	98.0	613/633	96.8	0/517	0.0	2/116	1.7
WalkAway	656/683	96.1	607/633	95.9	4/517	0.8	2/116	1.7
autoSCAN-4	660/683	96.6	612/633	96.7	0/517	0.0	2/116	1.7

•A total of 681 *Acinetobacter* spp., Enterobacterales and *Pseudomonas* spp. 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 *Acinetobacter* spp., Enterobacterales and *Pseudomonas* spp. 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 Enterobacterales and *Pseudomonas* spp. between MSDGN panel and frozen reference panel was 97.5% (615/631) for manual read method, 94.8% (598/631) for WalkAway System, 97.0% (612/631) for autoScan-4 instrument using the Prompt inoculation method.

Table 3. Efficacy – Prompt Inoculation Method

	Essential Agreement		Categorical Agreement		Major Errors		Very Major Errors	
Read Method	No.	%	No.	%	No.	%	No.	%
Manual	656/681	96.3	615/631	97.5	3/515	0.6	2/116	1.7
WalkAway	638/681	93.7	598/631	94.8	19/515	3.7	2/116	1.7
autoSCAN-4	653/681	95.9	612/631	97.0	3/515	0.6	2/116	1.7

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

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 and 7. 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
E. coli ATCC 25922	1/4 – 8/4	98/98 100%	98/98 100%	98/98 100%	98/98 100%	98/98 100%	98/98 100%
P. aeruginosa 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%
E. coli 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
		Turbidity
E. coli ATCC 25922	1/4 – 8/4 (frozen)	100% (98/98)
P. aeruginosa ATCC 27853	1/4 – 8/4 (frozen)	98.0% (96/98)
E. coli ATCC 35218	0.5/4 – 2/4 (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 *Acientobacter* spp., Enterobacterales and *Pseudomonas* spp. in a multicenter study using EUCAST v12.0 interpretive criteria.