

EUCAST Oxacillin in Combination with Cefoxitin Screen Breakpoints for MicroScan Dried Gram Positive MIC Panels from a Multicenter Assessment of Gram Positive Bacteria

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ABSTRACT

Background: EUCAST v12.0 breakpoints for oxacillin combined with cefoxitin screen performance were evaluated against data from a multicenter clinical study with *Staphylococcus* species on a MicroScan Dried Gram Positive MIC (MSDGP) Panel. MIC results were compared to the oxacillin results obtained with frozen broth microdilution panels prepared according to CLSI/ISO methodology. Cefoxitin is tested as a surrogate for oxacillin for some species of *Staphylococcus*. To evaluate methicillin (oxacillin) susceptible or methicillin (oxacillin) resistant isolates, oxacillin and cefoxitin EUCAST categorization rules were applied.

Materials/Methods: MSDGP panels were evaluated at three clinical sites by comparing MIC values obtained using the MSDGP panels to MICs utilizing a CLSI/ISO broth microdilution reference panel. The study included 349 clinical isolates tested using the turbidity and Prompt[®] methods of inoculation during efficacy. MSDGP panels were incubated at 35 ± 1°C and read on the WalkAway System, the autoSCAN-4 instrument, and visually. Read times for the MSDGP panels were at 16-20 hours. Frozen reference panels were prepared and read according to CLSI/ISO methodology. EUCAST breakpoints (µg/mL) used for interpretation of MIC results were: ≤ 2 S and > 2 R for oxacillin with *Staphylococcus aureus*, *Staphylococcus lugdunensis*, and *Staphylococcus saprophyticus* and ≤ 0.25 S and > 0.25 R for all other *Staphylococcus* spp. and with ≤ 4 S and > 4 R for cefoxitin screen with *Staphylococcus aureus*, *Staphylococcus lugdunensis* and *Staphylococcus saprophyticus* (EUCAST 12.0 guidelines applied to all *Staphylococcus* spp., except *Staphylococcus saprophyticus* which uses the cefoxitin (screen only) breakpoint according to EUCAST 4.0).

Results: Essential and categorical agreement were calculated compared to frozen reference panel results. Results for isolates tested during efficacy are found in the following table. Due to the occurrence of very major errors and the low sensitivity with oxacillin and AS-4 reads with turbidity inoculation method, results from Coagulase Negative *Staphylococcus* species should be confirmed using manual read.

Read Method	Organism Group	Oxacillin Essential Agreement %		Categorical Agreement %		Very Major Errors* %		Major Errors* %	
		P	T	P	T	P	T	P	T
Walk Away	<i>S. aureus</i> , <i>S. lugdunensis</i> , & <i>S. saprophyticus</i>	98.7 (219/222)	100 (222/222)	99.1 (220/222)	99.6 (221/222)	0.0 (0/38)	0.0 (0/38)	0.0 (0/184)	0.0 (0/184)
	Other <i>Staphylococcus</i> spp.	87.4 (111/127)	95.3 (121/127)	96.1 (122/127)	96.1 (122/127)	1.7 (1/60)	1.7 (1/60)	3.0 (2/67)	0.0 (0/67)
	<i>S. aureus</i> , <i>S. lugdunensis</i> , & <i>S. saprophyticus</i>	99.6 (221/222)	97.8 (217/222)	99.1 (220/222)	99.1 (220/222)	0.0 (0/38)	0.0 (0/38)	0.0 (0/184)	0.0 (0/184)
Auto SCAN-4	Other <i>Staphylococcus</i> spp.	91.3 (116/127)	92.9 (118/127)	98.4 (125/127)	94.5 (120/127)	0.0 (0/60)	6.7 (4/60)	0.0 (0/67)	0.0 (0/67)
Manual	<i>S. aureus</i> , <i>S. lugdunensis</i> , & <i>S. saprophyticus</i>	98.7 (219/222)	99.6 (221/222)	98.2 (218/222)	99.6 (221/222)	0.0 (0/38)	0.0 (0/38)	0.5 (1/184)	0.0 (0/184)
	Other <i>Staphylococcus</i> spp.	88.2 (112/127)	95.3 (121/127)	96.9 (123/127)	97.6 (124/127)	0.0 (0/60)	1.7 (1/60)	1.5 (1/67)	0.0 (0/67)
	<i>S. aureus</i> , <i>S. lugdunensis</i> , & <i>S. saprophyticus</i>	98.7 (219/222)	99.6 (221/222)	98.2 (218/222)	99.6 (221/222)	0.0 (0/38)	0.0 (0/38)	0.5 (1/184)	0.0 (0/184)

Conclusion: In this multicenter study, oxacillin and cefoxitin screen MIC results for Gram positive bacteria obtained with the MSDGP panel correlate well with MICs obtained using frozen reference panels using EUCAST v12.0 interpretive criteria.

INTRODUCTION

Data from a multicenter study was evaluated for the performance of a MicroScan Dried Gram Positive MIC panel with oxacillin and cefoxitin screen using Gram positive isolates with EUCAST v12.0 interpretive breakpoints.

METHODS

Study Design: MicroScan Dried Gram Positive 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 349 Gram positive clinical isolates were tested at three sites.

Quality Control Expected Results

E. faecalis ATCC 29212:

- 4 – > 4 µg/ml (Oxacillin MicroScan range, dried panel)
- 8 – 32 µg/ml (Oxacillin CLSI M100 ED32 range, frozen reference)

S. aureus ATCC 29213:

- ≤ 0.25 – 0.5 µg/ml (Oxacillin MicroScan range, dried panel)
- 0.12–0.5 µg/ml (Oxacillin CLSI M100 ED32 range, frozen reference)
- ≤ 4 µg/ml (Cefoxitin MicroScan range, dried panel)
- 1 - 4 µg/ml (Cefoxitin CLSI M100 ED32 range, frozen reference)

S. aureus ATCC 43300

- > 4 µg/ml (Cefoxitin MicroScan range & CLSI M100 ED32 range)

Quality control (QC) testing was performed daily using ATCC 29212 *E. faecalis*, ATCC 29213 *S. aureus*, and ATCC 43300 *S. aureus* for a minimum of 20 replicates per site.

Panels

Frozen reference and MSDGP MIC panels contained two-fold doubling dilutions of oxacillin 0.25-4 µg/ml (0.06-32 µg/ml on the frozen reference panel) in cation-adjusted Mueller-Hinton broth. Reference panels were prepared and frozen following CLSI recommendations.

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±2°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 MSDGP MIC and frozen reference panels. MSDGP MIC panels were also inoculated using the Prompt Inoculation method. Following inoculation, MSDGP MIC panels were incubated at 35±1°C in the WalkAway system for 18 hours. All panels were read by the WalkAway, autoSCAN-4, and visually.

Data Analysis

Essential Agreement (EA) = MSDGP panel MIC within +/- 1 dilution of the frozen reference result MIC. Categorical Agreement (CA)=MSDGP panel and reference categorical results (S,R) agree using EUCAST breakpoints for *Staphylococcus* species. EUCAST v12.0 guidelines applied to all *Staphylococcus* spp., except *S. saprophyticus* which uses the cefoxitin (screen only) breakpoint according to EUCAST 4.0 (Table 1).

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

Drug	Organism Group	Susceptible (S)	Resistant (R)
Oxacillin	<i>S. aureus</i> , <i>S. lugdunensis</i> , & <i>S. saprophyticus</i>	≤ 2	> 2
	CNS other than <i>S. lugdunensis</i> and <i>S. saprophyticus</i>	≤ 0.25	> 0.25
	<i>S. aureus</i> & <i>S. lugdunensis</i>	≤ 4	> 4
Cefoxitin	<i>Staphylococcus</i> spp.	≤ 4	> 8
	(<i>S. saprophyticus</i>)	MS	MR

*MS=Methicillin Susceptible, MR=Methicillin Resistant

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.

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

METHODS (continued)

Data Analysis (continued)

Per EUCAST v12.0, “*S. aureus* (including *S. intermedius*, *S. pseudintermedius*, and *S. schleiferi* subsp. *coagulans*) and *S. lugdunensis* with cefoxitin MIC values >4 mg/L and *S. saprophyticus* with cefoxitin MIC values >8mg/L are methicillin resistant”. Combined oxacillin and cefoxitin screen MIC results were applied to *S. aureus*, *S. lugdunensis*, and *S. saprophyticus* in order to determine methicillin interpretations, as described in Table 2. (EUCAST v12.0 and EUCAST v13.0 are equivalent.)

Table 2. Oxacillin & Cefoxitin Screen Analysis

CfxS	MIC		Oxacillin Interpretation	
	Oxacillin MIC	<i>S. aureus</i> , <i>S. lugdunensis</i> , or <i>S. saprophyticus</i>	CNS other than <i>S. lugdunensis</i> and <i>S. saprophyticus</i>	
≤ 4 Neg	≤ 0.25	S	S	
	0.5, 1, or 2	S	R	
	> 2	R	R	
> 4 Pos	≤ 0.25	R	S	
	0.5, 1, 2 or > 2	R	R	

RESULTS

Efficacy (Tables 2 and 3)

A total of 349 Gram positive clinical isolates were tested among three sites. The 349 isolates consisted of 222 *S. aureus*, *S. lugdunensis*, and *S. saprophyticus* (including *S. intermedius*, *S. pseudintermedius*, and *S. schleiferi* subsp. *coagulans*) and 127 Other *Staphylococcus* species (excluding *S. aureus*, *S. lugdunensis*, *S. saprophyticus*, *S. intermedius*, *S. pseudintermedius*, and *S. schleiferi* subsp. *coagulans*). Combined oxacillin and cefoxitin screen MIC results were applied to *S. aureus*, *S. lugdunensis*, and *S. saprophyticus* in order to determine methicillin interpretations. Offline *mecA* testing was performed per CLSI M100 ED32 for all *Staphylococcus* spp (except *S. aureus*, *S. lugdunensis*, *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*) on all isolates with dried and/or frozen MIC values of 1 or 2 µg/ml. Due to the occurrence of very major errors and the low sensitivity with oxacillin and AS-4 reads with turbidity inoculation method, results from Coagulase Negative *Staphylococcus* species should be confirmed using manual read. Essential Agreement and Categorical Agreement for Gram positive isolates between MSDGP panel and frozen reference panel using the Prompt and turbidity inoculation methods are listed in Table 2 and 3.

Table 2. Clinical Isolates - Prompt Inoculation Method

Read Method	Essential Agreement		Categorical Agreement*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%
WalkAway	330/349	94.6	342/349	98.0	2/251	0.8	1/98	1.0
autoSCAN-4	337/349	96.6	345/349	98.9	0/251	0.0	0/98	0.0
Manual	331/349	94.8	341/349	97.7	2/251	0.8	0/98	0.0

Table 3. Clinical Isolates – Turbidity Inoculation Method

Read Method	Essential Agreement		Categorical Agreement*		Major Errors		Very Major Errors	
	No.	%	No.	%	No.	%	No.	%
WalkAway	343/349	98.3	343/349	98.3	0/251	0.0	1/98	1.0
autoSCAN-4	335/349	96.0	340/349	97.4	0/251	0.0	4/98	4.1
Manual	342/349	98.0	345/349	98.9	0/251	0.0	1/98	1.0

Quality Control (Tables 4 and 5)

Overall quality control results were within range (≥95%) for each read and inoculation method on the dried test panel for oxacillin with ATCC 29212 *E. faecalis* and ATCC 29213 *S. aureus* (read at 18 hours), and for cefoxitin screen with ATCC 29213 *S. aureus* and ATCC 43300 *S. aureus*. QC results were within range (≥95%) for the frozen reference panel, which were read manually with turbidity inoculation method, for oxacillin with ATCC 29212 *E. faecalis* and ATCC 29213 *S. aureus* (read at 24 hours) and for cefoxitin screen with ATCC 29213 *S. aureus* and ATCC 43300 *S. aureus*. 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

Drug	Organism	QC Range (µg/mL)	Manual
			Turbidity
Oxacillin (18 hr read)	<i>E. faecalis</i> ATCC 29212	8 – 32 (frozen)	100% (75/75)
Oxacillin (24 hr read)	<i>S. aureus</i> ATCC 29213	0.12 – 0.5 (frozen)	98.7% (74/75)
Cefoxitin Screen (18 hr read)	<i>S. aureus</i> ATCC 29213	1 – 4 (frozen)	100% (75/75)
	<i>S. aureus</i> ATCC 43300	> 4 (frozen)	100% (75/75)

Table 5. Quality Control – Dried Test Results

Drug	Organism	QC Range (µg/mL)	WalkAway		autoSCAN-4		Manual	
			Prompt	Turbidity	Prompt	Turbidity	Prompt	Turbidity
Oxacillin	<i>E. faecalis</i> ATCC 29212	4 – > 4 (dried)	100% (74/74)	100% (75/75)	100% (74/74)	100% (75/75)	100% (74/74)	100% (75/75)
	<i>S. aureus</i> ATCC 29213	≤ 0.25 – 0.5 (dried)	98.7% (74/75)	98.6% (73/74)	100% (75/75)	100% (74/74)	100% (75/75)	100% (74/74)
Cefoxitin Screen	<i>S. aureus</i> ATCC 29213	≤ 4 (dried)	100% (75/75)	100% (74/74)	100% (75/75)	100% (74/74)	98.7% (74/75)	100% (74/74)
	<i>S. aureus</i> ATCC 43300	> 4 (dried)	100% (75/75)	100% (74/74)	100% (75/75)	100% (74/74)	100% (75/75)	100% (74/74)

CONCLUSION

This multicenter study showed that oxacillin with cefoxitin screen MIC results for *Staphylococcus* species with the MSDGP panel correlate well with MICs obtained using frozen reference panels using EUCAST v12.0 interpretive criteria.