

# Detection of *mecA*-mediated Resistance Using Cefoxitin Disk Diffusion (DD) in a Collection of *Staphylococcus aureus* (SA) Expressing Borderline Oxacillin MICs

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## REVISED ABSTRACT

**BACKGROUND:** A study was undertaken to investigate the ability of the new CLSI (formerly NCCLS) cefoxitin (FOX) DD test to accurately detect *mecA*-mediated oxacillin (OX) resistance in 135 SA isolates that either expressed borderline OX MICs (1-4 µg/mL) or were *mecA*-positive and had not been detected using CLSI reference methods (OX DD or OX broth microdilution [BMD]).

**METHODS:** The strains were tested against OX and FOX using BMD and DD, CLSI OX agar screen using 2 different lots (BBL and Remel), and four automated methods (MicroScan, Vitek, Vitek2, and Phoenix). All strains were examined for *mecA* using real-time PCR. *MecA*-positive strains not detected by more than one method were retested using induced growth (suspension made from growth on a blood agar plate around a FOX disk) to see if detection was improved.

**RESULTS:** Methods with >95% sensitivity (SN) and specificity (SP) (SN/SP) were FOX DD (99/100), Etest FOX MIC using ≤ 6 µg/mL as susceptible (99/98), and Phoenix FOX MIC using ≤ 4 µg/mL as susceptible (98/100). SN/SP for the other methods were OX agar screen (BBL, 80/86; Remel, 85/50), OX DD (91/59), OX BMD (85/88), FOX BMD using ≤ 4 µg/mL as susceptible (99/77), Etest FOX using ≤ 4 µg/mL as susceptible (99/34), MicroScan (89/96), Vitek (82/93), Vitek2 (91/75), Phoenix OX, (67/96), and Phoenix FOX using ≤ 8 µg/mL as susceptible (91/100). Repeat testing of SN errors using induced growth resulted in only slight improvement of *mecA* detection.

**CONCLUSIONS:** Methods using FOX performed much better for detection of *mecA*-mediated resistance in SA than those using OX. For DD, SN increased slightly from 91% for OX to 99% for FOX; however, SP increased from 59% for OX to 100% for FOX. The SN of the automated methods varied from 75 to 85%. For CLSI BMD, the SN using OX was slightly better than that using FOX. However, since the SN of Etest increased when using a breakpoint of ≤ 6 µg/mL for susceptible rather than ≤ 4 µg/mL, BMD tests using a concentration of 6 µg/mL should be investigated.

## INTRODUCTION

Within the past several years, there have been many reports of the ability of cefoxitin to substitute for oxacillin for phenotypic detection of *mecA*-mediated resistance to oxacillin and other penicillinase-stable penicillins. Cefoxitin has been shown to be more accurate for detection of this kind of resistance because it is a more powerful inducer of the system that regulates *mecA* (2). Initial studies looking at the use of cefoxitin, showed it to be better for detection in heteroresistant strains (1); however, these studies used different parameters than those now recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly NCCLS). In a CLSI multi-laboratory study, we have shown that cefoxitin works well as a replacement for oxacillin in disk diffusion testing if alternate breakpoints are used (3). However, we feel that further validation using strains with borderline oxacillin MICs was warranted. Therefore using strains selected from the culture collection of the Anti-infectives Investigation Team at CDC, we evaluated reference methods (broth microdilution, disk diffusion, oxacillin salt-agar screen) and several commercial methods (Etest, MicroScan, Vitek, Vitek2, Phoenix) for their ability to detect *mecA*-mediated resistance.

## MATERIALS AND METHODS

### ORGANISMS

- from approximately 2800 *S. aureus* (SA) strains received at CDC since 1994, those meeting the following criteria were selected:
  - all strains with oxacillin MICs of 1-4 µg/mL, and/or
  - all strains with oxacillin MICs = S and oxacillin zone diameters = I or R, and/or
  - all strains with oxacillin MICs = S and *mecA* = positive
- total number selected = 135
  - 79 *mecA* positive
  - 56 *mecA* negative

### METHODS AND AGENTS TESTED, INITIAL TESTING

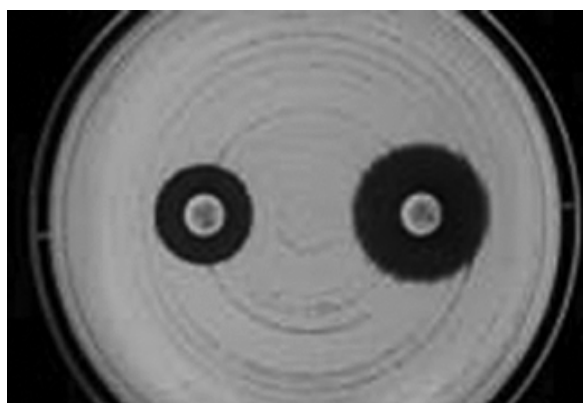
- **Reference methods** (Table 1)
  - Broth microdilution (BMD, CLSI method), oxacillin and ceftioxin
  - Disk diffusion (DD, CLSI method), oxacillin and ceftioxin
  - Oxacillin salt-agar screen, 2 manufacturers (BBL and Remel), inoculated using both a 1 µL loop and a swab
- **Commercial methods** (cards or panels used (Table 2))
  - Etest, ceftioxin
  - MicroScan (POS MIC Type 20A), oxacillin
  - Vitek (GPS 109), oxacillin
  - Vitek2 (AST-GP55 or AST-GP61), oxacillin
  - Phoenix (PMIC/ID-25), oxacillin and ceftioxin
- **Molecular methods**
  - *mecA* (Realtime PCR, SmartCycler)

### REPEAT TESTING

- **Sensitivity Errors:** strains for which there was a very major error for oxacillin susceptibility by BMD and one or more of the following, DD, MicroScan, Phoenix, Vitek, and Vitek2 (n=18), were repeated using both induced (i.e., growth from around a ceftioxin disk) and non-induced growth. *MecA*-negative strains were included as controls (n=7). (Table 3)
- **Specificity Errors:** strains for which there were major errors for oxacillin susceptibility by BMD and one or more of the methods listed above (n=16) were repeated in duplicate. (Table 4)

### INCUBATION AND READING

- Reference methods, 35°C, 18 and 24 h
- Commercial methods, as required by system



*mecA* positive *S. aureus*  
FOX (ceftioxin = 16mm 'R'  
OX (oxacillin) = 20 mm 'S'

Table 1. Category of Susceptibility and Sensitivity and Specificity of 135 *S. aureus* isolates using CLSI Reference Methods

Method	Antimicrobial Agent (Breakpoints)	Time	<i>mecA</i> positive (n = 79) No. of results with category of:				<i>mecA</i> negative (n = 56) No. of results with category of:			
			S	I	R	SENS	S	I	R	SPEC
Broth microdilution	Oxacillin	18 h	23	–	56	70.9	55	–	1	98.2
		24 h	12	–	67	84.8	49	–	7	87.5
	Ceftioxin (≤ 4µg/mL=S, ≥ 8µg/mL=R)	18 h	1	–	78	98.7	53	–	3	94.6
		24 h	1	–	78	98.7	43	–	13	76.8
Salt Agar Screen										
BBL/1 µL Loop	Oxacillin	24 h	16	–	63	79.7	48	–	8	86
BBL/swab	Oxacillin	24 h	30	–	49	62	51	–	5	91.1
Remel/1 µL Loop	Oxacillin	24 h	12	–	67	84.8	28	–	28	50
Remel/swab	Oxacillin	24 h	22	–	57	73.2	47	–	9	83.9
Disk diffusion	Oxacillin	18 h	7	1	71	91.1	32	9	15	57.1
		24 h	7	1	71	91.1	33	6	17	58.9
	Ceftioxin	18 h	2	–	77	97.5	56	–	0	100
		24 h	1	–	78	98.7	56	–	0	100

Table 2. Category of Susceptibility and Sensitivity and Specificity of 135 *S. aureus* isolates using Commercial Methods

Method	Antimicrobial Agent (Breakpoints)	Time	<i>mecA</i> positive (n = 79) No. of results with category of:			<i>mecA</i> negative (n = 56) No. of results with category of:		
			S	R	SENS	S	R	SPEC
MicroScan	Oxacillin		9	70	88.6	54	2	96.4
Vitek	Oxacillin		20 <sup>a</sup>	59	75	52	4	92.9
Vitek2	Oxacillin		7	72	91.1	42	14	75
Phoenix	Oxacillin		26 <sup>b</sup>	53	67.1	54	2	96.4
	Cefoxitin (≤ 4=S, ≥ 8=R)		2	77	97.5	56	0	100
	Cefoxitin (≤ 8=S, ≥ 6=R) <sup>c</sup>		7	72	91.1	56	0	100
Etest	Cefoxitin (≤ 4=S, ≥ 6=R)	18 h	1	78	98.7	14	42	25
		24 h	1	78	98.7	19	37	33.9
	Cefoxitin (≤ 6=S, ≥ 8=R)	18 h	1	78	98.7	55	1	98.2
		24 h	1	78	98.7	56	0	100

<sup>a</sup> 6 flagged as possibly resistant, but recommended supplemental testing may have missed them.

<sup>b</sup> 19 flagged as possible resistant based on cefoxitin MIC of ≥ 6 µg/mL.

<sup>c</sup> BDxpert™ software breakpoints “for research use only”.

Table 3. Repeat Testing of Sensitivity (Very Major) Errors

	Number of isolates Retested	Number correct in repeat test after	
		no induction	induction*
<b>Oxacillin</b>			
Broth microdilution	12	5	9
Disk diffusion	5	0	2
MicroScan	8	2	4
Vitek	14	1	4
Vitek	27	0	2
Phoenix	7	1	4
<b>Cefoxitin</b>			
Broth microdilution ≤ 4=S	1	0	0
Disk diffusion	1	0	0
Phoenix ≤ 4=S	2	0	1
Phoenix ≤ 8=S**	7	5	1

\* Induction = growth taken from around a 30-µg cefoxitin disk.

\*\* BDxpert™ software breakpoints “for research use only”

## RESULTS

- Results are reported as sensitivity and specificity compared to *mecA*
  - **Sensitivity:** the percent of *mecA*-positive strains correctly classified
  - **Specificity:** the percent of *mecA*-negative strains correctly classified
- Initial Testing
  - Table 1 Reference methods
  - Table 2 Commercial methods
- Repeat Testing of Discrepant Results
  - Table 3 Sensitivity Errors
  - Table 4 Specificity Errors

Table 4. Repeat Testing of Specificity (Major) Errors

	Number of isolates Retested	Number correct in repeat duplicate test	
		Replicate 1	Replicate 2
<b>Oxacillin</b>			
Broth microdilution	6	3	2
Disk diffusion	11	5	4
MicroScan	2	1	1
Vitek	3	1	1
Vitek2	12	7	6
Phoenix	2	0	0
<b>Cefoxitin</b>			
Broth microdilution ≤ 4=S	7	5	6

## REFERENCES

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### CONCLUSIONS

- The only CLSI reference method that had >95% sensitivity and specificity for detection of *mecA* in this collection of *S. aureus* with borderline oxacillin MICs was disk diffusion testing using cefoxitin.
- Broth microdilution testing using cefoxitin had good sensitivity but specificity decreased from 95% at 18 h to 77% at 24 h because of MICs increasing from 4 µg/mL to 8 µg/mL. Preliminary data (not shown) suggests that the use of a susceptible breakpoint for cefoxitin of  $\leq 6$  µg/mL rather than  $\leq 4$  µg/mL may increase specificity.
- The only commercial systems that showed excellent sensitivity and specificity (>95%) in this study were those using cefoxitin as a surrogate for oxacillin.
- For tests using oxacillin, the use of an inoculum made from growth induced with cefoxitin decreased the number of sensitivity errors but did not eliminate them.

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