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Results of Disk Diffusion Testing with Cefoxitin Correlate with Presence of *mecA* in *Staphylococcus* spp.

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The cefoxitin disk diffusion (DD) test for predicting *mecA*-mediated oxacillin resistance in staphylococci was assessed during a three-phase study. In phase 1, one laboratory tested 62 and 53 strains of *Staphylococcus aureus* and coagulase-negative staphylococci (CoNS), respectively. These data were used to choose the provisional cefoxitin DD breakpoints (resistant/susceptible) of ≤19 mm/≥20 mm for *S. aureus* and ≤24 mm/≥25 mm for CoNS for the next phase of testing. In phase 2, 10 laboratories each tested approximately 40 in-house strains of staphylococci (half of which were *S. aureus*) using Mueller-Hinton agar from different manufacturers. In this phase, the sensitivity and specificity, respectively, of the cefoxitin disk test were 98 and 100% for *S. aureus* and 99 and 96% for CoNS. The cefoxitin DD test performed equivalently to oxacillin broth microdilution (BMD) and to oxacillin DD tests among *S. aureus* and *mecA*-positive CoNS strains but gave better results than oxacillin BMD or oxacillin DD for *mecA*-negative strains of CoNS. The cefoxitin DD test also was much easier to read and did not require the use of transmitted light for detection of resistance. Based on data from the first two phases, the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) adopted the use of the cefoxitin DD test for predicting *mecA*-mediated oxacillin resistance in staphylococci and revised Table 2C in CLSI document M100-S14 to reflect the change. In the third phase, an additional 61 challenge strains of CoNS for which the oxacillin MICs were 0.5 to 2 μg/ml were tested in a single laboratory to determine the effectiveness of the cefoxitin DD test for this group of borderline-resistant isolates. These data were used to refine the description of the test in CLSI document M100-S15. The cefoxitin DD test is preferred over the oxacillin DD test for predicting *mecA*-mediated oxacillin resistance in *S. aureus* and CoNS.

Accurate detection of *mecA*-mediated resistance to oxacillin and other penicillinase-stable penicillins (PSPs), i.e., methicillin, nafcillin, cloxacillin, dicloxacillin, and flucloxacillin, is necessary to ensure appropriate antimicrobial chemotherapy of staphylococcal infections, particularly those from community-associated infections (8, 14). Oxacillin has been the agent recommended by the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS) for phenotypic tests to predict resistance to PSPs because of its stability and superior sensitivity over other PSPs for susceptibility tests (2, 6). However, antimicrobial susceptibility tests using oxacillin are often difficult to read despite changes in techniques to improve the discrimination between oxacillin-susceptible and -resistant results. Both MIC panels and disk diffusion plates where oxacillin is tested must be examined carefully to detect any growth that may be indicative of resistance (2, 11, 12). The oxacillin salt agar screen has been recommended by CLSI as an additional test that can be used to confirm either dilution tests or disk diffusion tests that are indeterminate. However, the agar screen test is recommended only for *S. aureus* and also can be hard to read, especially for strains that are very heteroresistant (5, 17).

Cefoxitin, a cephamycin, is a more potent inducer of the *mecA* regulatory system than are the penicillins (9). Several groups of investigators have reported that the results of cefoxitin disk diffusion (DD) tests correlate better with the presence of *mecA* than do the results of disk diffusion tests using oxacillin (3, 4, 7, 10, 15, 21). To assess the usefulness of cefoxitin for predicting *mecA*-mediated oxacillin resistance in staphylococci, a three-phase study was undertaken. Based on data described in phases 1 and 2 of the study, the CLSI Subcommittee on Antimicrobial Susceptibility Testing (CLSI-AST) adopted the use of the cefoxitin DD test for predicting *mecA*-mediated oxacillin resistance in staphylococci and added a section for the new test to Table 2C (the staphylococcal table) in both the disk diffusion (M2) and MIC (M7) sections of CLSI document M100-S14 (13). Additional data were requested by the CLSI-AST to clarify the utility of the cefoxitin disk test for predicting oxacillin resistance in isolates of coagulase-negative staphylococci (CoNS) for which the oxacillin MICs were 0.5 to 2 μg/ml, and, thus, phase 3 of the study was undertaken. Results from all three phases are presented here.

MATERIALS AND METHODS

Study design. The study format and organisms used are outlined in Table 1. The first phase of the study was performed in a single laboratory (Centers for Disease Control and Prevention [CDC]) to determine the utility of the cefoxitin DD test for predicting oxacillin resistance in 62 challenge strains of *S. aureus* and

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TABLE 1. Study format and organisms used to evaluate the use of cefoxitin to predict *mecA*-mediated resistance

Study phase	Format	No. of *S. aureus* strains (no. of results[a])		No. of CoNS strains (no. of results)	
		mecA positive	*mecA* negative	*mecA* positive	*mecA* negative
1	Single laboratory, challenge organisms, 5 MHA lots	21 (105)	41 (205)	31 (155)	22 (110)
2	10 laboratories, mainly unselected organisms, in-house MHA lot	97	104	126	70
3	Single laboratory, selected organisms, 2 MHA lots			13 (26)	48 (96)

[a] Maximum number of results possible when including testing of all different manufacturers' media.

53 strains of CoNS that had been collected previously for (i) evaluating the oxacillin agar screen test for *S. aureus* (16) and (ii) validating oxacillin disk diffusion testing of CoNS (18). The *S. aureus* isolates included 21 *mecA*-positive strains of expression class 1 or 2 (i.e., their expression of resistance makes them very heteroresistant) (20) and 41 *mecA*-negative strains, including five *mecA*-negative strains with resistance due to modification of existing penicillin-binding proteins for which the oxacillin MICs were 4 to 16 μg/ml (the so-called MOD strains) (19). The CoNS in phase 1 included 31 *mecA*-positive and 22 *mecA*-negative isolates that had previously demonstrated oxacillin MICs ranging from 0.25 to 4 μg/ml. All strains in this phase had previously been characterized for the presence of *mecA*.

In phase 2, 10 hospital laboratories (the Cefoxitin Disk Study Group) selected 40 clinical strains (20 *S. aureus* strains and 20 CoNS strains) from their institutions for testing. The laboratories were not asked to select strains for any particular characteristic; however, only one isolate from each patient could be included. Each laboratory tested the strains using their current in-house lot of Mueller-Hinton agar (MHA) and frozen broth microdilution (BMD) panels that had been prepared at CDC. After testing, the strains were shipped to CDC for additional susceptibility testing (BMD for all and DD for discrepant strains) and characterization using the PBP2′ latex agglutination test (Oxoid Ltd., Basingstoke, Hampshire, England) for all strains and *mecA* characterization by PCR for discrepant strains. The total number of strains tested in phase 2 was 397: 201 strains of *S. aureus*, of which 97 were *mecA* positive, and 196 strains of CoNS, of which 126 were *mecA* positive. Only strains for which a discrepancy occurred were identified to the species level, using standard biochemical methods (1).

In phase 3, an additional 61 isolates that were predominantly non-*S. epidermidis* CoNS were selected from the CDC culture collection for testing in a single laboratory (CDC) to evaluate the ability of the cefoxitin DD test to correctly classify strains of CoNS for which the oxacillin MICs were between 0.5 and 2 μg/ml. Finally, to further investigate sensitivity issues discovered in phase 3, 24 additional isolates of *S. simulans* (10 containing *mecA*) were tested after phase 3 was completed.

Quality control strains. Four *S. aureus* strains, ATCC 25923 and ATCC 29213, both oxacillin susceptible, and ATCC 43300 and UCLA 8076, both heteroresistant, and *Escherichia coli* ATCC 25922 were used as controls on each day of testing.

Broth microdilution. The CLSI BMD reference method was used in all phases of the study (11). All panels were prepared in-house at CDC using cation-adjusted Mueller-Hinton broth (CAMHB; Difco or BD [BBL], Sparks, MD) and frozen at −70°C. In phases 1 and 3, panels were prepared using Difco CAMHB; oxacillin wells were supplemented with 2% NaCl (Sigma, St. Louis, MO). Cefoxitin wells received no NaCl supplementation. In phase 2, MIC panels were prepared using both Difco and BBL CAMHB and oxacillin and cefoxitin (Sigma). The wells containing oxacillin were supplemented with 2% NaCl, and the wells containing cefoxitin were either not supplemented or supplemented with either 2% or 4% NaCl. In phase 3, MIC testing was done using in-house-prepared reference diagnostic panels (Difco; CAMHB) that contained oxacillin but not cefoxitin. All panels were incubated at 35°C and read at both 18 h and 24 h.

Disk diffusion. The CLSI reference method for disk diffusion was used in all phases of the study (12). In phase 1, three commercially prepared lots of MHA plates (BD [BBL], Sparks, MD; Remel, Lenexa, KS; and Hardy Diagnostics, Santa Maria, CA) and two in-house-prepared lots of MHA (Acumedia, Baltimore, MD, and Oxoid, Hampshire, England) were used. In phase 2, the 10 laboratories performed disk diffusion testing using a single lot from their current supply of MHA. Ten different lots of MHA were used: one from Acumedia, five from BD (BBL), one from Hardy Diagnostics, and three from Remel. One lot each of MHA from BD (BBL) and Remel was used in phase 3. A single lot of 1-μg oxacillin disks and 30-μg cefoxitin disks from BD were used in the study. MHA plates were incubated at 35°C, and zone diameters were read at both 18 h and 24 h.

In phase 1, oxacillin zone diameters were read as recommended by CLSI using transmitted light. Cefoxitin zone diameters were read with both transmitted and reflected light; no differences were noted in the sizes of the zones. In phase 2, the method of reading the cefoxitin zone diameters was not specified in the protocol since the previous phase of the study had shown no difference in the zone diameters read with reflected or transmitted light. To further investigate sensitivity problems with testing *S. simulans* isolates during phase 3, additional strains of that species were tested and zones were read with both transmitted and reflected light.

***mecA* and PBP2a status.** All isolates in phases 1 and 3 were tested for *mecA* using PCR as previously described (18). In phase 2, isolates were screened using the Oxoid PBP2′ latex agglutination test kit; isolates for which the PBP2a result was discrepant with the phenotypic result were tested for *mecA* by PCR. The *mecA* result was used as the "gold standard" in all phases.

Data analysis. A strain was designated as oxacillin resistant when either an intermediate (where appropriate) or a resistant category was obtained. Sensitivity was defined as the percentage of *mecA*-positive strains determined to be resistant by phenotypic testing, and specificity was defined as the percentage of *mecA*-negative strains determined to be susceptible by phenotypic testing.

RESULTS

Cefoxitin disk diffusion. Results of disk diffusion testing of cefoxitin for all phases of the study using the presence of *mecA* or PBP2a as the gold standard are shown in Table 2 for *S.*

TABLE 2. Cefoxitin zone diameters from phases 1 and 2 for *Staphylococcus aureus* using a 30-μg cefoxitin disk read at 24 h of incubation

mecA result	Phase[a]	*n*	Cumulative percentage of results at a zone diam (mm) of:													
			≤14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Positive	1	105	63	79	88	96	98	100									
	2	97	93	97	98	98	98			99							100
Negative	1	205									2	11	28	52	76	96	97
	2	104									1	10	14	43	65	86	94

[a] Results from phase 1 combine the results from five Mueller-Hinton agar medium manufacturers.

TABLE 3. Cefoxitin zone diameters from phases 1, 2, and 3 for CoNS using a 30-μg cefoxitin disk read at 24 h of incubation

mecA result	Phase[a]	*n*	Cumulative percentage of results at a zone diam (mm) of:														
			≤14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Positive	1	155	23	27	31	38	49	57	65	76	81	86	90	94	97	99	99
	2	125	59	65	71	78	87	90	94	96	99			100			
	3	26	8				15		27	38	58	65	69	73	77	89	96
Negative	1	109										1	4	8	12	21	33
	2	70								1.4		3	4	10	14	31	49
	3	95								2	4		7	14	22	32	56

[a] Results from phase 1 combine the results from five Mueller-Hinton agar medium manufacturers. Results from phase 3 combine the results from two Mueller-Hinton agar medium manufacturers.

aureus and in Table 3 for CoNS. Using breakpoints of ≤19 mm for *mecA*-positive and ≥20 mm for *mecA*-negative isolates of *S. aureus*, the sensitivity and specificity at 24 h were 98 to 100% and 100%, respectively. For CoNS, using breakpoints of ≤24 mm for *mecA*-positive and ≥25 mm for *mecA*-negative isolates, the sensitivity and specificity at 24 h in phase 2 for unselected organisms were 99% and 96%, respectively. However, the sensitivities in phases 1 and 3 with challenge organisms were 90 and 69%, respectively. The lower sensitivity in phase 3 was due primarily to the influence of the *S. simulans* isolates. Specificities for CoNS in the three phases ranged from 93 to 96%. Sensitivity and specificity values for 18-h readings were equivalent to the 24-h readings for *S. aureus* in both phases; however, 24-h readings for CoNS increased the sensitivity of the test in phase 1 from 86% at 18 h to 90% at 24 h; in phase 2, sensitivity was 99% at both 18 and 24 h. Specificity, however, did not change significantly for 18-h versus 24-h readings for CoNS.

In phase 1, the sensitivity and specificity results for the oxacillin DD test varied depending on the source of Mueller-Hinton agar used (data not shown). However, the source of Mueller-Hinton agar did not affect either the sensitivity or specificity results of the cefoxitin DD test, which gave much more reproducible results. For example, the sensitivity of the oxacillin DD test varied from 62 to 100% for *S. aureus* and 90 to 97% for CoNS; specificities of oxacillin DD ranged from 56 to 85% for *S. aureus* and 73 to 86% for CoNS depending on which manufacturer of Mueller-Hinton agar was used. For cefoxitin DD among the five media, sensitivity and specificity were both 100% for *S. aureus* and 85 to 90% and 95 to 100%, respectively, for CoNS.

Cefoxitin broth microdilution. Cefoxitin MICs are shown in Table 4 for phases 1 and 2 for testing in CAMHB with no NaCl supplementation. If breakpoints of ≤4 μg/ml for susceptible and ≥8 μg/ml for resistant were used, the sensitivities and specificities of the 24-h readings were 99 to 100% and 90 to 100% for *S. aureus* and 81 to 98% and 95 to 97% for CoNS, respectively. In phase 2, the addition of 2% and 4% NaCl was examined to see if it improved the accuracy of the cefoxitin MIC test (Table 5). Although the addition of NaCl increased the cefoxitin MICs for *mecA*-negative *S. aureus* isolates by at least 1 dilution, the MICs remained ≤4 μg/ml. Thus, using the phase 2 organisms, Difco CAMHB, and breakpoints of ≤4 μg/ml for susceptible and ≥8 μg/ml for resistant, the sensitivities for *S. aureus* and CoNS at all salt concentrations were 98 to 99% and the specificities were 97 to 99% for *S. aureus* and 94 to 97% for CoNS. While no benefit of added salt was noted, there was a slight trend towards decreased specificity in both groups of organisms.

Overall comparison of cefoxitin versus oxacillin testing. In Table 6, the sensitivities and specificities of MIC and DD testing for oxacillin and cefoxitin are given for all phases of the study along with the results of PPB2a testing in phases 2 and 3. Phases 1 and 3 both used challenge organisms, while phase 2 used nonselected organisms and was more representative of isolates tested in routine laboratory situations. The results of

TABLE 4. Cefoxitin MICs for phases 1 and 2 using CAMHB without salt supplementation read at 24 h of incubation[a]

Organism group	*mecA* result	Phase	CAMHB manufacturer	*n*	% of results at MIC (μg/ml) of:				
					≤2	4	8	16	≥32
S. aureus	Positive	1	Difco	21			14	14	72
		2	Difco	97	1		3	27	69
		2	BD	97	1		3	19	77
	Negative	1	Difco	41	2	88	10		
		2	Difco	104	82	17	1		
		2	BD	104	77	23			
CoNS	Positive	1	Difco	31		19	36	29	16
		2	Difco	126		2	12	33	53
		2	BD	126	3	10	20	25	42
	Negative	1	Difco	22	64	32	5		
		2	Difco	70	84	13	3		
		2	BD	70	90	6	4		

[a] Current NCCLS interpretive criteria are as follows: ≤8 μg/ml, susceptible; 16 μg/ml, intermediate; ≥32 μg/ml, resistant.

TABLE 5. Effect of salt supplementation on 24-h cefoxitin broth microdilution MICs from phase 2 using Difco CAMHB supplemented with 0, 2, and 4% NaCl

Organism[a]	*mecA* result	% NaCL	Percentage of results at cefoxitin MIC (μg/ml) of:				
			≤2	4	8	16	≥32
S. aureus	Positive	0	1		3	27	69
		2		1	2	21	76
		4		1	3	30	66
	Negative	0	82	17	1		
		2	13	87	1		
		4	2	95	2	1	
CoNS	Positive	0		2	12	33	53
		2		<1	10	25	64
		4		2	13	26	69
	Negative	0	84	13	3		
		2	79	15	6		
		4	73	21	4	1	

[a] Organism numbers are shown in Table 1.

phase 2 demonstrate that oxacillin MIC and DD tests and cefoxitin MIC and DD tests are essentially equivalent in performance for both sensitivity and specificity for *S. aureus*. All four tests also show equivalent sensitivities for CoNS. However, for CoNS the cefoxitin MIC and DD tests showed better specificity than did tests with oxacillin. This is confirmed by the results from phase 3 testing of CoNS, where strains for which the oxacillin MICs were 0.5 to 2 μg/ml (i.e., MICs above the susceptible breakpoint of 0.25 μg/ml for CoNS but below the resistant breakpoint of 4 μg/ml for *S. aureus*) were tested. Although the sensitivity of cefoxitin DD in phase 3 was lower than in phases 1 and 2 (primarily due to the increased number of *S. simulans* isolates in phase 3), the specificity increased greatly (to 93%) when compared to test results using oxacillin (17% for oxacillin MIC and 36% for oxacillin DD).

Strains discrepant by cefoxitin disk diffusion. Table 7 lists the CoNS strains in each phase that were incorrectly categorized by cefoxitin DD (when compared to *mecA* results), showing the oxacillin MICs along with the oxacillin and cefoxitin DD category results. Among *mecA*-positive CoNS, 5 of the 12 discrepant isolates were *S. simulans*; the 5 strains were called susceptible (i.e., all very major sensitivity errors) by cefoxitin DD with all of the Mueller-Hinton agar brands used. Of the other seven sensitivity errors, four were *S. epidermidis*, two were *S. hominis*, and one was *S. saprophyticus*. However, not all of the media lots or manufacturers were in error for these seven organisms. Thus, except for *S. simulans*, no consistent species-specific or medium-specific error was noted in cefoxitin DD testing.

To further investigate the failure of the cefoxitin DD test to detect *mecA*-mediated resistance in *S. simulans*, we collected an additional 14 *mecA*-negative and 10 *mecA*-positive isolates and tested them by BMD and DD. DD tests were read using both transmitted and reflected light searching for resistant colonies that may have been overlooked using reflected light only. Of the 10 *mecA*-positive *S. simulans* strains tested, six were incorrectly called susceptible using both reflected and transmitted light, i.e., no inner colonies or haze was obvious with closer examination. Of the 14 *mecA*-negative strains, all were correctly categorized using reflected light, but 3 of the 14 were incorrectly called resistant when transmitted light was used, i.e., a light haze was read as resistant, but the haze did not correlate with the presence of *mecA*. Therefore, using transmitted light to detect possible resistant colonies did not improve detection in *mecA*-positive strains of *S. simulans* and tended to incorrectly call resistance in some *mecA*-negative strains.

DISCUSSION

During the last several years, the CLSI-AST has attempted to improve the accuracy of detecting *mecA*-positive strains of both *S. aureus* and CoNS. Previous CLSI recommendations for detecting oxacillin resistance in staphylococci included agar dilution (using MHA), BMD (using CAMHB with 2% NaCl), disk diffusion, the oxacillin salt agar screen test (using MHA with 6 μg/ml of oxacillin and 4% NaCl spotted with an undiluted bacterial suspension equivalent to a 0.5 McFarland turbidity), and detection of *mecA* by PCR or of the product of the *mecA* gene, PBP2a, by latex agglutination (11, 12). While all of

TABLE 6. Sensitivity and specificity of broth microdilution MIC and disk diffusion for oxacillin and cefoxitin using modified breakpoints for cefoxitin and 24-h incubation and for PBP2a latex agglutination[b]

Study phase	Organism group	Oxacillin				Cefoxitin				PBP2a	
		MIC[a]		Disk diffusion		MIC[a,b]		Disk diffusion[c]		Sens	Spec
		Sens[d]	Spec[d]	Sens	Spec	Sens	Spec	Sens	Spec		
1	*S. aureus*	100	85	86[e]	74[e]	100	90	100[e]	100[e]	NT	NT
	CoNS	97	82	94[e]	79[e]	81	96	90[e]	96[e]	NT	NT
2	*S. aureus*	98	100	98	99	99	99	98	100	100	97
	CoNS	98	91	99	89	98	97	99	97	100	94
3	CoNS	100	17	89[e]	36[e]	NT[f]	NT	69[e]	93[e]	100	94

[a] Results given for MIC testing performed in Difco CAMHB only.
[b] Cefoxitin MIC breakpoints used: ≤4 μg/ml, susceptible; ≥8 μg/ml, resistant.
[c] Cefoxitin disk diffusion breakpoints used: *S. aureus*, ≤19 mm, resistant, and ≥20 mm, susceptible; CoNS, ≤24 mm, resistant, and ≥25 mm, susceptible.
[d] Sensitivity (Sens), percentage of *mecA*-positive strains correctly categorized; specificity (Spec), percentage of *mecA*-negative strains correctly categorized.
[e] Results for disk diffusion are for five lots of Mueller-Hinton agar combined in phase 1 and for two lots of Mueller-Hinton agar combined in phase 3.
[f] NT, not tested.

TABLE 7. Coagulase-negative staphylococcus strains that were incorrectly characterized by cefoxitin disk diffusion using modified cefoxitin breakpoints[b]

mecA result	Phase	Strain no.	Species	Oxacillin MIC (μg/ml)	DD result[a] for:	
					Oxacillin	Cefoxitin
Positive	1	12	*S. epidermidis*	2	**S**/R/**S**/R/**S**	R/R/R/R/**S**
		28	*S. simulans*	1	**S**/**S**/**S**/**S**/**S**	**S**/**S**/**S**/**S**/**S**
		29	*S. hominis*	**0.12**	**S**/**S**/**S**/**S**/**S**	R/**S**/**S**/**S**/R
		34	*S. saprophyticus*	>64	R/R/R/R/R	R/**S**/**S**/R/R
		42	*S. epidermidis*	**0.25**	R/**S**/**S**/R/**S**	R/R/**S**/R/**S**
		4203	*S. epidermidis*	**0.25**	R/**S**/**S**/I/**S**	**S**/R/**S**/R/R
	2	792	*S. simulans*	>16	R	**S**
	3	140	*S. simulans*	4	**S**/R	**S**/**S**
		429	*S. simulans*	2	R/R	**S**/**S**
		437	*S. simulans*	1	**S**/R	**S**/**S**
		245	*S. epidermidis*	2	R/R	**S**/R
		036329	*S. hominis*	4	**S**/R	**S**/R
Negative	1	3	*S. saprophyticus*	**1**	**R**/**I**/**I**/**I**/**R**	S/S/S/S/**R**
	2	631	*S. capitis*	0.25	S	**R**
		674	*S. auricularis*	0.5	**R**	**R**
		757	*S. sciuri*	**1**	**R**	**R**
	3	82	*S. warneri*	0.5	**R**/**R**	**R**/S
		259	Species[c]	0.5	S/**R**	−/S
		508	*S. capitis*	**1**	**R**/**R**	**R**/**R**
		552	Species	**1**	**R**/**R**	**R**/S
		652	*S. saprophyticus*	**1**	**R**/**R**	**R**/S
		028766	*S. cohnii*	**1**	**R**/**R**	**R**/**R**

[a] For disk diffusion testing, categories are shown for all lots of MHA tested. Phase 1 used five manufacturers of MHA. Phase 2 used one in-house lot of MHA. Phase 3 used two manufacturers of MHA.
[b] Values in boldface represent incorrect categories based on *mecA*.
[c] Species, species was not determined.

these methods can be used for *S. aureus*, the oxacillin salt agar screen test cannot be used for CoNS and CoNS need to be induced for optimal results with the PBP2a test. All of the tests that use oxacillin must be examined carefully by holding the plates up to transmitted light and looking carefully for any growth either in the wells of the MIC plate, in the zone of inhibition, or in the quadrant of the oxacillin salt agar screen plate. All the tests, aside from *mecA* detection via PCR, are prone to errors (17, 18). In addition, laboratories that cannot afford to perform the PBP2a latex agglutination test or do not have access to PCR need alternative methods for detecting *mecA*-mediated resistance. Thus, the CLSI undertook several studies to investigate the utility of cefoxitin DD test originally proposed by Mougeot et al. (10) and further investigated by Felton and colleagues (7), as a potential alternative to *mecA* testing.

In phase 1, the cefoxitin DD zones were distinct and easy to read for both *S. aureus* and CoNS and did not require the use of transmitted light to detect resistance. The sensitivity and specificity of the test were both 100% for *S. aureus*. For CoNS, however, the sensitivity of cefoxitin DD results was not markedly better than that of oxacillin disk diffusion, although the specificity of cefoxitin DD was much improved. After reviewing the phase 1 data, the CLSI-AST decided that additional testing in multiple laboratories was necessary to confirm the promising results and requested additional data on the cefoxitin DD test for CoNS. CLSI-AST also requested data indicating whether a cefoxitin MIC test could be used for *S. aureus* and CoNS.

Phase 2 testing showed that the proposed cefoxitin DD breakpoints for *S.* aureus (≤19 mm for oxacillin susceptible and ≥20 mm for oxacillin resistant) and CoNS (≤24 mm for oxacillin susceptible and ≥25 mm for oxacillin resistant) continued to work well, even in a 10-laboratory study. In this phase, the sensitivity of the cefoxitin DD test for *S. aureus* decreased slightly, with two resistant isolates not detected, but the performance of the test for CoNS improved over phase 1. The list of isolates of CoNS that were incorrectly characterized by cefoxitin DD in all three phases was small and represented 10 different species (Table 7). However, failure of the test to detect some strains of *mecA*-positive *S. simulans* remains puzzling.

The one caveat to our study is that not all of the CoNS isolates in phase 2 were identified to the species level. CLSI now groups *S. lugdunensis* together with *S. aureus* for oxacillin and cefoxitin testing based on unpublished data presented to CLSI from other investigators. However, because not all of the isolates were identified to the species level in this study, we do not know the accuracy of the cefoxitin DD test for differentiating *mecA*-positive *S. lugdunensis* from *mecA*-negative isolates. One *mecA*-negative isolate of *S. lugdunensis* was included in phase 1. Oxacillin DD results called that strain resistant using either *S. aureus* or CoNS breakpoints, whereas cefoxitin DD called it susceptible with the media from all five manufacturers' MHA with the *S. aureus* breakpoints, but only two of the five manufacturers with the CoNS breakpoints.

In response to this study, CLSI-AST revised Tables 2C in M2 and M7 indicating that the revised cefoxitin disk test can be used to predict *mecA* carriage in *S. aureus* and CoNS. The traditional cefoxitin DD breakpoints for staphylococci in Table

2C of M2 have been removed. For those laboratories using disk diffusion as their primary test for staphylococci, the substitution of a cefoxitin disk for an oxacillin disk will result in an easier-to-read test and provide equivalent detection (sensitivity and specificity) of oxacillin resistance in *S. aureus* and equal sensitivity but improved specificity in CoNS. Although the cefoxitin DD test continued to call false resistance in some *mecA*-negative strains, no single species accounted for this. The one caution in using this test is that it failed to detect resistance in several strains of *mecA*-positive *S. simulans*.

Although the use of the cefoxitin MIC test has not been recommended by the CLSI-AST, its performance when using modified breakpoints of ≤4 μg/ml for susceptible and ≥8 μg/ml for resistant was essentially equivalent to that for cefoxitin DD. The use of cefoxitin MIC results to predict oxacillin resistance in *S. aureus* has been investigated in a commercial AST system and was superior to oxacillin (C. Nonhoff, G. Mascart, M. J. Struelens, C. Van Den Borre, and O. Denis, Abstr. Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. P1637, 2004). The use of a concentration between 4 and 8 μg/ml, i.e., 6 μg/ml, may improve the performance of such an MIC screening test and warrants further study. Also, others have shown that there is also potential for a cefoxitin agar screen test containing 6 or 8 μg/ml of cefoxitin similar to the oxacillin salt-agar screen test (S. Poutanen, P. Akhavan, S. Ho, S. Pong-Porter, Y. Rzayev, A. Shigayeva, M. Lum, C. Larocque, K. Pike, T. Mazzulli, D. Low, and B. Willey, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. A-4185, 2003). This would be useful for laboratories that normally perform MIC tests as their primary testing method and wish to confirm borderline MIC test results without performing a disk diffusion test.

In summary, the cefoxitin DD test can be used to predict the presence of *mecA* in *S. aureus* and CoNS with a high degree of sensitivity and specificity when compared to *mecA* detection using PCR. The test improved on the specificity of oxacillin disk diffusion and oxacillin MIC testing without sacrificing sensitivity, especially for CoNS. Those using disk diffusion as their routine susceptibility testing method for staphylococci should replace their 1-μg oxacillin disks with 30-μg cefoxitin disks for routine testing.

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REFERENCES

1. **Bannerman, T. L.** 2003. *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci that grow aerobically, p. 384–404. *In* P. R. Murray, E. J. Baron, J. H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.), Manual of clinical microbiology. ASM Press, Washington, D.C.
2. **Barry, A. L., and R. N. Jones.** 1987. Reliability of high-content disks and modified broth dilution tests for detecting staphylococcal resistance to the penicillinase-resistant penicillins. J. Clin. Microbiol. **25:**1897–1901.
3. **Boutiba-Ben Boubaker, I., R. Ben Abbes, H. Ben Abdallah, K. Mamlouk, F. Mahjoubi, A. Kammoun, A. Hammami, and S. Ben Redjeb.** 2004. Evaluation of a cefoxitin disk diffusion test for the routine detection of methicillin-resistant *Staphylococcus aureus*. Clin. Microbiol. Infect. **10:**762–765.
4. **Cauwelier, B., B. Gordts, P. Descheemaecker, and H. Van Landuyt.** 2004. Evaluation of a disk diffusion method with cefoxitin (30 μg) for detection of methicillin-resistant *Staphylococcus aureus*. Eur. J. Clin. Microbiol. Infect. Dis. **23:**389–392.
5. **Cavassini, M., A. Wenger, K. Jaton, D. S. Blanc, and J. Bille.** 1999. Evaluation of MRSA-Screen, a simple anti-PBP 2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. **37:**1591–1594.
6. **Drew, W. L., A. L. Barry, R. O'Toole, and J. C. Sherris.** 1972. Reliability of the Kirby-Bauer disc diffusion method for detecting methicillin-resistant strains of *Staphylococcus aureus*. Appl. Microbiol. **24:**240–247.
7. **Felten, A., B. Grandry, P. H. Lagrange, and I. Casin.** 2002. Evaluation of three techniques for detection of low-level methicillin-resistant *Staphylococcus aureus* (MRSA): a disk diffusion method with cefoxitin and moxalactam, the Vitek 2 system, and the MRSA-Screen latex agglutination test. J. Clin. Microbiol. **40:**2766–2771.
8. **Kazakova, S. V., J. C. Hageman, M. Matava, A. Srinivasan, L. Phelan, B. Garfinkel, T. Boo, S. McAllister, J. Anderson, B. Jensen, D. Dodson, D. Lonsway, L. K. McDougal, M. Arduino, V. J. Fraser, G. Killgore, F. C. Tenover, S. Cody, and D. B. Jernigan.** 2005. A clone of methicillin-resistant *Staphylococcus aureus* among professional football players. N. Engl. J. Med. **352:**468–475.
9. **McKinney, T. K., V. K. Sharma, W. A. Craig, and G. L. Archer.** 2001. Transcription of the gene mediating methicillin resistance in *Staphylococcus aureus* (*mecA*) is corepressed but not coinduced by cognate *mecA* and β-lactamase regulators. J. Bacteriol. **183:**6862–6868.
10. **Mougeot, C., J. Guillaumat-Taillet, and J. M. Libert.** 2001. *Staphylococcus aureus*: nouvelle détection de la résistance intrinsèque par la méthode de diffusion. Pathol. Biol. **49:**199–204.
11. **NCCLS.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M7-A6. NCCLS, Wayne, PA.
12. **NCCLS.** 2003. Performance standards for antimicrobial disk susceptibility tests, 8th ed. Approved standard M2-A8. NCCLS, Wayne, PA.
13. **NCCLS.** 2004. Performance standards for antimicrobial susceptibility testing. Fourteenth informational supplement M100-S14. NCCLS, Wayne, PA.
14. **Palavecino, E.** 2004. Community-acquired methicillin-resistant *Staphylococcus aureus* infections. Clin. Lab. Med. **24:**403–418.
15. **Skov, R., R. Smyth, M. Clausen, A. R. Larsen, N. Frimodt-Moller, B. Olsson-Liljequist, and G. Kahlmeter.** 2003. Evaluation of a cefoxitin 30 μg disc on Iso-Sensitest agar for detection of methicillin-resistant *Staphylococcus aureus*. J. Antimicrob. Chemother. **52:**204–207.
16. **Swenson, J. M., J. Spargo, F. C. Tenover, and M. J. Ferraro.** 2001. Optimal inoculation methods and quality control for the NCCLS oxacillin agar screen test for detection of oxacillin resistance in *Staphylococcus aureus*. J. Clin. Microbiol. **39:**3781–3784.
17. **Swenson, J. M., P. P. Williams, G. Killgore, C. M. O'Hara, and F. C. Tenover.** 2001. Performance of eight methods, including two new rapid methods, for detection of oxacillin resistance in a challenge set of *Staphylococcus aureus* organisms. J. Clin. Microbiol. **39:**3785–3788.
18. **Tenover, F. C., R. N. Jones, J. M. Swenson, B. Zimmer, S. McAllister, J. H. Jorgensen, and the NCCLS Staphylococcus Working Group.** 1999. Methods for improved detection of oxacillin resistance in coagulase-negative staphylococci: results of a multicenter study. J. Clin. Microbiol. **37:**4051–4058.
19. **Tomasz, A., H. B. Drugeon, H. M. de Lencastre, D. Jabes, L. McDougal, and J. Bille.** 1989. New mechanism for methicillin resistance in *Staphylococcus aureus*: clinical isolates that lack the PBP 2a gene and contain modified penicillin-binding proteins with modified penicillin-binding capacity. Antimicrob. Agents Chemother. **33:**1869–1874.
20. **Tomasz, A., S. Nachman, and H. Leaf.** 1991. Stable classes of phenotypic expression in methicillin-resistant clinical isolates of staphylococci. Antimicrob. Agents Chemother. **35:**124–129.
21. **Urbaskova, P., O. Melter, B. Mackova, V. Jakubu, and M. Wunschova.** 2004. Detection of MRSA in a group of 752 strains of *S. aureus* using a cefoxitin disk. Epidemiol. Mikrobiol. Immunol. **53:**62–65.