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BD Phoenix and Vitek 2 Detection of *mecA*-Mediated Resistance in *Staphylococcus aureus* with Cefoxitin[▽]

Alan D. Junkins, Shawn R. Lockhart, Kristopher P. Heilmann, Cassie L. Dohrn, Diana L. Von Stein, Patricia L. Winokur, Gary V. Doern, and Sandra S. Richter*

University of Iowa Carver College of Medicine, Iowa City, Iowa

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The BD Phoenix (BD Diagnostics, Sparks, MD) and Vitek 2 (bioMérieux, Durham, NC) automated susceptibility testing systems have implemented the use of cefoxitin to enhance the detection of methicillin (meticillin)-resistant *Staphylococcus aureus* (MRSA). To assess the impact of this change, 620 clinically significant *S. aureus* isolates were tested in parallel on Phoenix PMIC/ID-102 panels and Vitek 2 AST-GP66 cards. The results for oxacillin and cefoxitin generated by the automated systems were compared to those generated by two reference methods: *mecA* gene detection and MICs of oxacillin previously determined by broth microdilution according to CLSI guidelines. Testing of isolates with discordant results was repeated to attain a majority or consensus final result. There was 100% final agreement between the results of the two reference methods. For the 448 MRSA and 172 methicillin-susceptible *S. aureus* isolates tested, the rates of categorical agreement of the results obtained with the automated systems with those obtained by the reference methods were 99.8% for the Phoenix panels and 99.7% for the Vitek 2 cards. A single very major error occurred on each instrument (0.2%) with different MRSA isolates. The only major error was attributed to the Vitek 2 system overcalling oxacillin resistance. In 16 instances (9 on the Phoenix system, 7 on the Vitek 2 system), an oxacillin MIC in the susceptible range was correctly changed to resistant by the expert system on the basis of the cefoxitin result. The inclusion of cefoxitin in the Phoenix and Vitek 2 panels has optimized the detection of MRSA by both systems.

The accurate detection of *mecA*-mediated ß-lactam resistance in *Staphylococcus aureus* is essential for the treatment of overt infections and the implementation of infection control practices. Although FDA-cleared PCR assays for the rapid detection of methicillin (meticillin)-resistant *S. aureus* (MRSA) are available for use for surveillance and testing of clinical specimens, isolates causing infections continue to require susceptibility testing to guide therapy.

The phenotypic detection of *mecA*-mediated resistance has presented ongoing challenges due to variable gene expression that is modulated by many factors (1). Variables such as temperature, incubation time, growth medium, and sodium chloride concentrations have been considered in the development of Clinical Laboratory Standards Institute (CLSI) reference susceptibility test methods (3). Among the penicillinase-resistant penicillins, oxacillin is the most stable and sensitive for the detection of *mecA*-mediated resistance. However, heterogeneously resistant populations may have oxacillin test results indicating susceptibility (1).

Recognition that cefoxitin is a stronger inducer of *mecA* expression than oxacillin led to studies that assessed this agent as a surrogate marker for methicillin resistance (2, 6, 9, 15, 16). For disk diffusion testing of staphylococci, cefoxitin (30 μg) provides more accurate results than oxacillin and zones that are easier to read (2, 3, 6). While cefoxitin has replaced oxacillin in the CLSI disk diffusion test, laboratories may use oxacillin or cefoxitin to predict *mecA*-mediated resistance by use of the CLSI broth microdilution (BMD) method (4). A resistant oxacillin or cefoxitin MIC test result indicates resistance to penicillins, cephems, carbapenems, and ß-lactams and ß-lactamase inhibitors (4).

Manufacturers of automated susceptibility testing instruments have also adapted their products to optimize the detection of *mecA*-mediated resistance. The BD Phoenix (BD Diagnostics, Sparks, MD) and the Vitek 2 (bioMérieux, Durham, NC) systems now offer panels that include oxacillin and cefoxitin. The instruments' expert systems interpret any *S. aureus* isolate that tests positive by the cefoxitin screen (MIC $>$ 4 μg/ml on the Phoenix system, MIC $>$ 6 μg/ml on the Vitek 2 system) as oxacillin resistant.

This purpose of this study was to examine the accuracies of the Phoenix and the Vitek 2 instruments for the detection of *mecA*-mediated resistance in *S. aureus*. The oxacillin, cefoxitin, and expert system results generated by the Phoenix and Vitek 2 instruments were compared to the results generated by two reference methods: the oxacillin MICs determined by the CLSI BMD method and *mecA* gene detection by PCR.

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MATERIALS AND METHODS

Isolate characteristics. A total of 620 clinically significant unique *S. aureus* isolates collected from 2001 to 2007 were included in the study. The majority of methicillin-susceptible *S. aureus* (MSSA) isolates (>90%) and approximately 50% of the MRSA strains were obtained from laboratories throughout the Unites States during 2001 and 2003. The remaining isolates were collected from patients throughout Iowa from 2005 to 2007 as part of statewide surveillance for invasive MRSA disease. The latter collection included 55 community-acquired MRSA isolates previously characterized as being of the USA300 (n = 51) or the USA400 (n = 4) genotype by pulsed-field gel electrophoresis (8).

* Corresponding author. Mailing address: Department of Pathology, C606 GH, University of Iowa Roy J. and Lucille A. Carver College of Medicine, 200 Hawkins Drive, Iowa City, IA 52242-1009. Phone: (319) 356-2990. Fax: (319) 356-4916. E-mail: sandra-richter@uiowa.edu.

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TABLE 1. Comparison of Phoenix and Vitek 2 results to reference BMD oxacillin MICs

System tested[a]	% Categorical agreement	No. of resistant isolates[b]	No. (%) VM errors[c]	No. of susceptible isolates[b]	No. (%) major errors[d]	% Essential agreement[e]	No. of instrument oxacillin MIC results within the following $\log_2$ dilution of reference method MIC						
							>−2	−2	−1	Same	+1	+2	>+2
Phoenix	99.8	448	1 (0.2)	172	0 (0)	91.6	5	3	10	491	67	42	2
Vitek 2	99.7	448	1 (0.2)	172	1 (0.6)	94.7	6	2	10	511	66	25	0

[a] A total of 620 isolates were tested with each system.
[b] As determined by the reference BMD method.
[c] A VM error was resistance by the reference method but susceptibility by the automated method; percentages are based on the number of resistant isolates.
[d] A major error is susceptibility by the reference method but resistance by the automated method; percentages are based on the number of susceptible isolates.
[e] Essential agreement is an MIC determined with the Phoenix or Vitek 2 system that is ±1 $\log_2$ dilution of the MIC determined by the reference method.

The isolates were stored at −70°C by use of a bead system. Susceptibility testing was performed following two subcultures on Trypticase soy agar with 5% sheep blood and 18 to 24 h of incubation at 35°C in an atmosphere of 5 to 7% CO_2. A single colony was selected when the second subculture was performed.

The oxacillin MICs had previously been determined in the central laboratory by the CLSI BMD method (4). All isolates with oxacillin MICs close to the CLSI breakpoint were chosen for inclusion in this study. The identity of isolates as *S. aureus* was confirmed by coagulase testing with the Staphaurex (Remel, Lenexa, KS) or Staphyloslide (BD Diagnostics) system.

Susceptibility testing with Phoenix and Vitek 2 systems. Phoenix PMIC/ID-102 panels (cefoxitin concentrations, 4, 8, and 16 μg/ml; oxacillin concentrations, 0.25, 0.5, 1, and 2 μg/ml) and Vitek 2 AST-GP66 cards (cefoxitin concentration, 6 μg/ml; oxacillin concentrations, 0.5, 1, and 2 μg/ml) were inoculated and run on each instrument, according to the manufacturers' instructions. For each isolate, a Phoenix panel and a Vitek 2 card were inoculated on the same day from a single culture plate.

Quality control. Quality control was performed according to the manufacturers' recommendations. The results were used only when the quality control values were in acceptable ranges.

Reference methods. PCR for detection of the *mecA* gene was performed as described by Oliveira and Lencastré (10). Testing was performed with organisms taken from the same plate used for testing on the Phoenix and Vitek 2 systems (within 1 week of inoculation).

The CLSI BMD method (3, 4) for the determination of oxacillin MICs was performed in cation-adjusted Mueller-Hinton broth with 2% NaCl. The trays were incubated in ambient air for 24 h at 35°C prior to visual reading of the end points. *Staphylococcus aureus* ATCC 29213 was used as the quality control strain.

Evaluation of results. In the event of discordant results, all four methods were repeated as needed to attain a majority or consensus result. The results of both the *mecA* PCR and the BMD method with oxacillin were considered reference results for the evaluation of instrument performance. The accuracy of each automated system was measured as essential agreement (oxacillin MIC ± 1 $\log_2$ dilution of the reference method oxacillin MIC) and categorical agreement (same interpretative category assignment of MSSA or MRSA by the automated instruments and the reference methods). Categorical discrepancies were classified as very major (VM) errors (false susceptible; rates were determined with the number of resistant organisms as the denominator) and major errors (false resistance; rates were calculated with the number of susceptible isolates as denominator).

RESULTS

Of the 620 *S. aureus* isolates tested, 448 (72.3%) were classified as MRSA by the CLSI BMD method (oxacillin MICs ≥ 4 μg/ml). The final rate of agreement between the reference methods (*mecA* PCR and the oxacillin BMD method) was 100%.

Comparison of the Phoenix and Vitek 2 results with the reference method oxacillin MICs is shown in Table 1. The VM error rate for both instruments was 0.2%, which represents the failure to detect one oxacillin-resistant isolate. The Phoenix system VM error result was for a *mecA*-positive isolate with an oxacillin MIC of 8 μg/ml by the BMD method (the isolate was tested three times). The Vitek 2 system VM error occurred with a different *mecA*-positive isolate (oxacillin MIC, 8 μg/ml by the BMD method) that was tested five times (three oxacillin-susceptible and five cefoxitin-susceptible results with the Vitek 2 system).

The only major error occurred with an MSSA isolate that repeatedly tested resistant to oxacillin on the Vitek 2 system (oxacillin MIC, 1 μg/ml by the BMD method). The Vitek 2 cefoxitin screen was negative for this organism, but the expert system interpreted the result as MRSA on the basis of the result obtained with oxacillin.

The rates of essential agreement with the oxacillin MIC determined by the reference method were 91.6% for the Phoenix system and 94.7% for the Vitek 2 system. For both instruments, most discordant results were for isolates with reference method MICs of 0.12 or 0.25 μg/ml and instrument MICs of 0.5 or 1 μg/ml (MIC values well below the CLSI breakpoint that defines oxacillin susceptibility).

Discordance between the oxacillin and cefoxitin results for 19 isolates led to interpretative changes by the instrument expert systems (Table 2). There were nine instances on the Phoenix system and seven instances on the Vitek 2 system in which an MIC interpretation indicating oxacillin susceptibility was correctly changed to a resistance interpretation by the expert system on the basis of a cefoxitin-resistant result. Additionally, for four isolates that tested oxacillin resistant on the Vitek 2 system, a negative cefoxitin screen was changed to positive. One of these four isolates represented the only major error in the study.

Both instruments correctly detected resistance in the 55 community-acquired MRSA strains. A Phoenix system oxacillin MIC of 2 μg/ml for one of these isolates was interpreted as resistant by the expert system on the basis of a cefoxitin MIC of 16 μg/ml.

The sensitivity and specificity for the detection of MRSA according to each instrument's oxacillin, cefoxitin, and expert system results are shown in Tables 3 and 4. The cefoxitin result was a better indicator that an isolate was MRSA than the oxacillin result for both instruments and had 100% specificity and >99% sensitivity.

DISCUSSION

This study demonstrated improved detection of *mecA*-mediated resistance in *S. aureus* by both systems with addition of cefoxitin to panels. On the Phoenix system, the sensitivity for the detection of MRSA improved from 97.8% when the oxacillin result alone was used to 99.8% when both the oxacillin and the cefoxitin results combined were used (Table 4). Similarly, the sensitivity of the Vitek 2 system increased from 98.2% to 99.8%. The addition of cefoxitin did not reduce the specificity of detec-

TABLE 2. Expert system interpretation for *S. aureus* isolates with discordant results with cefoxitin and oxacillin[a]

Isolate no.	*mecA* PCR result	Oxacillin MIC (μg/ml), category, by reference BMD method	Phoenix system			Vitek 2 system		
			Oxacillin MIC (μg/ml), category	Cefoxitin MIC (μg/ml), category	Expert change	Oxacillin MIC (μg/ml), category	Cefoxitin screen result	Expert change
2	Neg	1, S	0.5, S	≤4, S		≥4, R	Neg	Cfx to Pos[b]
460	Pos	8, R	>2, R	16, R		≥4, R	Neg	Cfx to Pos
583	Pos	8, R	>2, R	>16, R		≥4, R	Neg	Cfx to Pos
172	Pos	4, R	2, S	8, R	Ox to R	≥4, R	Neg	Cfx to Pos
7	Pos	>8, R	2, S	8, R	Ox to R	≥4, R	Pos	
51	Pos	>8, R	2, S	16, R	Ox to R	≥4, R	Pos	
58	Pos	>8, R	0.5, S	16, R	Ox to R	≥4, R	Pos	
98	Pos	8, R	1, S	8, R	Ox to R	1, S	Neg	—[c]
382	Pos	8, R	2, S	16, R	Ox to R	≥4, R	Pos	
408	Pos	8, R	2, S	16, R	Ox to R	≥4, R	Pos	
494	Pos	4, R	2, S	8, R	Ox to R	≥4, R	Pos	
625	Pos	4, R	2, S	8, R	Ox to R	≥4, R	Pos	
50	Pos	8, R	>2, R	>16, R		1, S	Pos	Ox to R
301	Pos	>8, R	>2, R	>16, R		≤0.25, S	Pos	Ox to R
409	Pos	8, R	>2, R	8, R		1, S	Pos	Ox to R
431	Pos	16, R	>2, R	>16, R		0.5, S	Pos	Ox to R
496	Pos	16, R	>2, R	16, R		0.5, S	Pos	Ox to R
546	Pos	4, R	>2, R	8, R		1, S	Pos	Ox to R
588	Pos	4, R	>2, R	8, R		1, S	Pos	Ox to R
693	Pos	8, R	0.5, S	≤4, S	—[c]	≥4, R	Pos	

[a] R, resistant; S, susceptible; Ox, oxacillin; Cfx, cefoxitin; Pos, positive; Neg, negative.
[b] Major error.
[c] —, VM error.

tion of *mecA*-mediated resistance on either instrument. The error rates for the Phoenix system (0.2% VM errors, 0% major errors) and the Vitek 2 system (0.2% VM errors, 0.6% major errors) were within the acceptable ranges established by FDA (≤1.5% VM errors, ≤3% major errors) (7).

Previous studies have noted the existence of MRSA strains with low-level resistance that contain the *mecA* gene but that have oxacillin MICs below the CLSI breakpoint (≤2 μg/ml and sometimes as low as 0.5 μg/ml) (5, 11). Among the 620 *S. aureus* isolates evaluated in this study, no *mecA*-positive strains had oxacillin MICs below 4 μg/ml. Compared to the results obtained by the instruments with oxacillin alone, the results obtained with cefoxitin and oxacillin combined provided the greatest increases in sensitivity for the detection of *mecA*-mediated resistance among strains with oxacillin MICs of 4 μg/ml: 72.7% to 100% for the Phoenix system and 81.8% to 100% for the Vitek 2 system (Table 4).

Expert changes to the cefoxitin result occurred only with the Vitek 2 system: the single concentration of 6 μg/ml failed to detect three MRSA isolates that were oxacillin resistant (Table 2). It is interesting that the nine Phoenix and seven Vitek 2 expert system changes of an oxacillin result from susceptible to resistant did not occur with any of the same isolates (that is, they occurred with 16 different isolates).

The error rates seen in this evaluation were lower than those in previous published studies that used Vitek 2 cards without

TABLE 3. Results for MRSA detection obtained with cefoxitin, oxacillin, and expert systems

mecA PCR result[a]	BMD oxacillin MIC[b] (μg/ml)	No. of isolates with the indicated MIC	No. of isolates identified as MRSA by each method					
			Phoenix system			Vitek 2 system		
			Oxacillin	Cefoxitin	Combined	Oxacillin	Cefoxitin	Combined
Neg	≤0.12	1	0	0	0	0	0	0
Neg	0.12	51	0	0	0	0	0	0
Neg	0.25	78	0	0	0	0	0	0
Neg	0.5	35	0	0	0	0	0	0
Neg	1	6	0	0	0	1[c]	0	1[c]
Neg	2	1	0	0	0	0	0	0
Pos	4	11	8	11	11	9	10	11
Pos	8	47	43	46	46[d]	44	44	46[e]
Pos	>8	390	387	390	390	387	390	390

[a] Neg, negative; Pos, positive.
[b] Detection of 448 MRSA isolates and 172 MSSA isolates by the CLSI BMD method and the following breakpoints for oxacillin: susceptible, MIC ≤ 2 μg/ml; resistant, MIC ≥ 4 μg/ml.
[c] One MSSA isolate was incorrectly reported as MRSA by the Vitek 2 system on the basis of the results obtained with oxacillin (major error).
[d] One MRSA isolate with a BMD oxacillin MIC of 8 μg/ml was not detected by either the oxacillin or the cefoxitin test on the Phoenix system (VM error).
[e] One MRSA isolate with a BMD oxacillin MIC of 8 μg/ml was not detected by either the oxacillin or the cefoxitin test on the Vitek 2 system (VM error).

TABLE 4. Sensitivity and specificity of cefoxitin, oxacillin, and expert systems for MRSA detection

System and agent tested	Specificity (%)	Sensitivity (%) by oxacillin MIC			
		4 μg/ml	8 μg/ml	>8 μg/ml	All isolates
Phoenix system					
Oxacillin	100	72.70	91.50	99.20	97.80
Cefoxitin	100	100	97.90	100	99.80
Combined	100	100	97.90	100	99.80
Vitek 2 system					
Oxacillin	99.40	81.80	93.60	99.20	98.20
Cefoxitin	100	90.90	93.60	100	99.10
Combined	99.40	100	97.90	100	99.80

cefoxitin. By the use of oxacillin as the sole indicator of *mecA*-mediated resistance, Felten et al. reported a Vitek 2 system VM error rate of 6.0% (no major errors) for 83 MRSA strains that included 26 isolates with low-level resistance (5). A study by Sakoulas et al. using Vitek 2 cards without cefoxitin demonstrated a VM error rate of 0.5% for 203 MRSA isolates and a major error rate of 2.8% for 107 MSSA isolates (12). The report of no oxacillin VM or major errors from a study that used Phoenix panels without cefoxitin was most likely due to the small sample size tested (96 MRSA and 127 MSSA isolates) (13).

Recently, Roisin et al. (11) evaluated the Vitek 2 AST-P549 card that includes a cefoxitin screen, but noted a VM error rate (2.5%) higher than that found in the current study when 157 MRSA isolates from Belgium were tested. The higher VM error rate may be attributed to the inclusion of 29 heterogeneous MRSA strains with oxacillin MICs as low as 0.5 μg/ml that were collected from 1995 to 2005 (10% were not detected as MRSA by the Vitek 2 system).

The results obtained with the Phoenix system with cefoxitin (PMIC/ID-25 panels) were 97.5% sensitive and 100% specific for the detection of *mecA* resistance when a challenge set of 135 *S. aureus* isolates with borderline oxacillin MICs were tested (14). The accuracy of cefoxitin on the Phoenix panel was a marked improvement to the oxacillin results (67.1% sensitive, 96.4% specific) (14). The sensitivity and specificity for the detection of *mecA* resistance in that collection by use of the Vitek 2 system with oxacillin were 91% and 75%, respectively; a Vitek 2 card containing cefoxitin was not available at that time (14).

The 100% agreement between the reference BMD oxacillin MICs and the *mecA* gene PCR results in the current study suggests that there would not be a significant improvement in accuracy by the replacement of oxacillin with cefoxitin in a BMD format for the typical population of *S. aureus* strains encountered in the clinical laboratory. However, Swenson et al. found that cefoxitin MICs were a better predictor of the presence of *mecA* than oxacillin MICs when a challenge set of organisms with oxacillin MICs close to the CLSI BMD breakpoint was tested (14). The benefit of retaining the oxacillin BMD test is the detection of rarely reported oxacillin resistance due to mechanisms other than *mecA*.

Our findings demonstrate the improved accuracy of the Phoenix and Vitek 2 systems with the addition of cefoxitin to the test panels for the detection of *mecA*-mediated resistance among *S. aureus* isolates. Both systems provided reliable detection of MRSA when isolates typically encountered by a clinical laboratory were tested.

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