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MAJOR ARTICLE

It's Not You, It's SOSA: A Case Study on Breaking Up With an FDA-Cleared Susceptibility Testing System's Oxacillin Results for *Staphylococcus* spp. Other Than *S. aureus* and *S. lugdunensis*

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Background. In 2021, the Clinical and Laboratory Standards Institute revised its susceptible oxacillin minimum inhibitory concentration (MIC) breakpoint for *Staphylococcus* spp. other than *S. aureus* and *S. lugdunensis* (SOSA) from ≤0.25 to ≤0.5 μg/mL. Here, we describe the response to this breakpoint change, which at the time of this study was not yet recognized by the US Food and Drug Administration (FDA), in our laboratory, where the primary method for antimicrobial susceptibility testing (AST) of SOSA is VITEK 2. VITEK 2 uses the Automated Expert System (AES) to integrate the results of oxacillin MIC and cefoxitin screen tests into a final interpretation; our laboratory also adjudicates discordant oxacillin and cefoxitin results using a PBP2a test.

Methods. We retrospectively reviewed and assessed the yield of PBP2a testing for 189 SOSA isolates with discordant (when applying the FDA susceptible oxacillin breakpoint of ≤0.25 μg/mL) VITEK 2 oxacillin and cefoxitin results, and then prospectively incorporated PBP2a testing for isolates with oxacillin MICs of 0.5 μg/mL and positive cefoxitin screens into our algorithm.

Results. Compared with accepting the VITEK 2 AES interpretation, PBP2a testing substantially improved the accuracy of *mecA*-mediated resistance classification in both scenarios, especially for the ~4.7% of isolates with oxacillin MICs ≤0.5 μg/mL and positive cefoxitin screens.

Conclusions. Although detection of *mecA* or PBP2a is the gold standard for assessment of β-lactam resistance in staphylococci, targeting a subset of isolates for *mecA* or PBP2a testing based on phenotypic AST results that predict an increased risk of misclassification may be a pragmatic, labor- and cost-saving approach.

Keywords. oxacillin; VITEK 2; antimicrobial susceptibility testing; breakpoint; coagulase-negative *Staphylococcus* spp.

Although *Staphylococcus* spp. other than *Staphylococcus aureus* and *Staphylococcus lugdunensis* (SOSA; traditionally referred to as coagulase-negative staphylococci despite not all species being coagulase negative) are commonly interpreted as contaminants when isolated in the clinical laboratory, the frequency of bona fide SOSA infections has increased in step with medical advances, such as in the care of preterm neonates and individuals with indwelling medical devices [1–4]. When susceptibility is demonstrated, treatment of such infections with β-lactam antibiotics, rather than alternatives like vancomycin, is generally preferred [1]. The primary mechanism of β-lactam resistance in staphylococci is production of an alternative penicillin binding protein, PBP2a (or PBP2'), encoded for by the *mecA* gene [5–7]. Determining whether an isolate harbors *mecA*, therefore, is the chief aim of antimicrobial susceptibility testing (AST) of SOSA.

Laboratories may opt to utilize tests that directly assay for *mecA* or may test SOSA isolates for PBP2a, given that PBP2a test results have been shown to have excellent concordance to *mecA* polymerase chain reaction (PCR) results [8–11]. Many laboratories instead routinely employ phenotypic minimum inhibitory concentration (MIC) or disk diffusion tests because of cost and workflow considerations, including the ease with which susceptibility to additional agents can be tested concurrently. However, heterogeneous expression of *mecA* by staphylococci can challenge the performance of phenotypic AST [7].

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Furthermore, as widespread implementation of matrix-assisted laser desorption/ionization–time of flight mass spectrometry (MALDI-TOF MS) has made identification of staphylococci to the species level more common, it has been increasingly appreciated that the various phenotypic methods used for characterization of β-lactam susceptibility perform unequally across species historically considered together as coagulase-negative staphylococci [8–10, 12]. In recent years, this understanding has led to updated testing recommendations from the Clinical and Laboratory Standards Institute (CLSI), including revision in 2021 of the SOSA oxacillin MIC susceptible breakpoint from ≤0.25 μg/mL to ≤0.5 μg/mL in an effort to reduce major errors (false resistant calls) when compared with *mecA* PCR. CLSI acknowledges that no single oxacillin MIC breakpoint consistently distinguishes between susceptible and resistant isolates across all species within this organism group and recommends that some SOSA isolates with oxacillin MICs in the resistant range undergo *mecA* or PBP2a testing with modification of the oxacillin result to susceptible if the *mecA* or PBP2a test is negative [8, 13].

The revised CLSI SOSA oxacillin MIC breakpoint has only very recently, in June 2022, been recognized by the US Food and Drug Administration (FDA); as such, it has not yet been incorporated by AST device manufacturers into the FDA-cleared systems widely used by clinical laboratories. However, CLIA-approved accreditation organizations permit the use of either FDA or CLSI breakpoints, and individual clinical laboratories might choose to internally validate whether their FDA-cleared systems correctly classify oxacillin resistance among staphylococci when applying, off-label, the new CLSI breakpoint [14–16]. More broadly, revision of the breakpoint by the CLSI based on contemporary data should prompt laboratories to assess whether any modification to their current approach for identifying *mecA*-mediated resistance in SOSA is indicated.

Our laboratory uses the VITEK 2 AST-GP75 card (bioMérieux, Inc., Durham, NC, USA) as our primary AST method for staphylococci. This card utilizes a combination of 2 tests—(1) oxacillin MIC, developed by the manufacturer against agar dilution, and (2) a cefoxitin screen, developed against cefoxitin disk diffusion with additional comparison with *mecA* PCR—to assess *Staphylococcus* spp. for β-lactam susceptibility; the 2 results are integrated into a single interpretation after analysis by the proprietary Automated Expert System (AES) software (Figure 1*A*) [17]. Although the performance characteristics described within the manufacturer's label are excellent, including 97.8% categorical agreement (CA) when compared with *mecA* PCR, with 1.2% very major errors (VMEs) and 3.2% major errors (MEs), the species breakdown among the collection of isolates for which this level of performance was observed is not disclosed [17]. Taken together with recognition of the challenges inherent to detection of *mecA*-mediated resistance among staphylococci, including when using VITEK 2 [18], our laboratory's standard procedure has in recent years been to reflex isolates to a PBP2a test and report the PBP2a test result when either (1) requested by a clinician or (2) the oxacillin and cefoxitin results on VITEK 2 disagree (ie, for SOSA, when the cefoxitin screen is positive but the oxacillin MIC is ≤0.25 μg/mL, or when the cefoxitin screen is negative but the oxacillin MIC is ≥0.5 μg/mL) (Figure 1*B*).

In response to revision of the CLSI oxacillin MIC breakpoint in 2021, we sought to retrospectively assess the impact of our current strategy (ie, PBP2a testing as a tiebreaker between discordant VITEK 2 oxacillin and cefoxitin results for SOSA, rather than accepting the VITEK 2 AES interpretation) on accuracy, labor, and reagent costs. Next, given that the CLSI breakpoint change would affect not only isolates with discordant oxacillin and cefoxitin results, but also isolates with oxacillin MICs of 0.5 μg/mL and positive cefoxitin screens, which have historically not undergone PBP2a testing in our laboratory, we prospectively evaluated the impact on accuracy, labor, and reagent costs of adding routine PBP2a testing for these isolates (Figure 1*C*).

METHODS

Bacterial Isolates, Identification, and VITEK 2 AST

The retrospective assessment included unique SOSA isolates from clinical cultures in the Massachusetts General Hospital Clinical Microbiology Laboratory that had undergone AST per routine standard operating procedures between September 20, 2019, and May 13, 2021, using the VITEK 2 AST-GP75 card (bioMérieux, Inc., Durham, NC, USA). The prospective evaluation included unique SOSA isolates from clinical cultures in the Massachusetts General Hospital Clinical Microbiology Laboratory undergoing AST per routine standard operating procedures between May 14, 2021, and March 1, 2022, for which either (1) the VITEK 2 AST-GP75 oxacillin and cefoxitin results were discordant when applying a susceptible oxacillin breakpoint of ≤0.25 μg/mL (ie, cefoxitin screen positive and oxacillin MIC ≤0.25 μg/mL, or cefoxitin screen negative and oxacillin MIC ≥0.5 μg/mL) or (2) the oxacillin MIC was 0.5 μg/mL and the cefoxitin screen was positive. More than 1 isolate from a single patient could be included if the isolates were determined to be distinct (ie, differing species-level identifications and/or major differences in the phenotypic AST profiles) by routine clinical laboratory procedures.

VITEK 2 AST was performed according to the manufacturer's instructions; from the beginning of the study until September 19, 2020, software version 8.01 was used, and from September 20, 2020, onward, the software version was 9.02. Because the performance of VITEK 2 for oxacillin AST of *S. saprophyticus* is known to be a limitation of the test and is disclaimed in the manufacturer's instructions for use, *S. saprophyticus* isolates were excluded from both the retrospective and prospective studies.

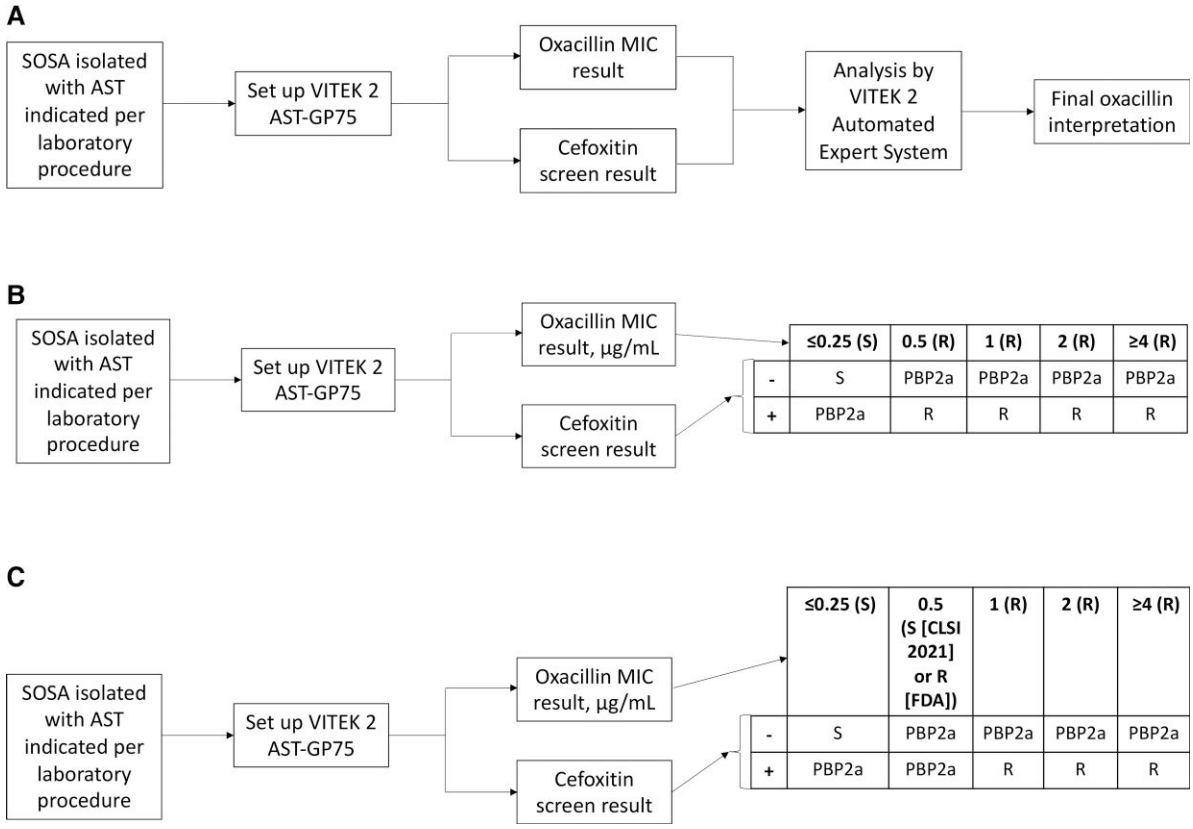

Figure 1. Approaches to oxacillin susceptibility testing of SOSA using the VITEK 2 antimicrobial susceptibility testing system. A, Oxacillin susceptibility testing of SOSA using the VITEK 2 GP-AST75 card according to the manufacturer's instructions. Using proprietary software (the AES), the VITEK 2 system integrates an oxacillin MIC result and a cefoxitin screen result into a single oxacillin interpretation. This FDA-cleared commercial antimicrobial susceptibility testing system uses the FDA oxacillin breakpoints (≤0.25 µg/mL, susceptible; ≥0.5 µg/mL, resistant). B, Existing standard procedure for oxacillin susceptibility testing of SOSA in the study laboratory. Isolates with concordant (using the FDA oxacillin breakpoints) VITEK 2 oxacillin and cefoxitin results are reported as oxacillin susceptible (oxacillin MIC ≤0.25 µg/mL and negative cefoxitin screen) or oxacillin resistant (oxacillin MIC ≥0.5 µg/mL and positive cefoxitin screen). Isolates with discordant VITEK 2 oxacillin and cefoxitin results (oxacillin MIC ≤0.25 µg/mL and positive cefoxitin screen or oxacillin MIC ≥0.5 µg/mL and negative cefoxitin screen) undergo PBP2a testing for adjudication. A single oxacillin interpretation is reported for these isolates based on the PBP2a result. C, Modified approach to susceptibility testing of SOSA in the study laboratory in response to the 2021 CLSI oxacillin breakpoint revision. The procedure outlined in (B) was modified to incorporate PBP2a testing for isolates with VITEK 2 oxacillin MICs of 0.5 µg/mL and positive cefoxitin screens. A single oxacillin interpretation is reported for these isolates based on the PBP2a result. Abbreviations: +, positive; −, negative; AES, Automated Expert System; AST, antimicrobial susceptibility testing; CLSI, Clinical and Laboratory Standards Institute; FDA, Food and Drug Administration; MIC, minimum inhibitory concentration; R, resistant; S, susceptible; SOSA, *Staphylococcus* spp. other than *S. aureus* and *S. lugdunensis*.

Throughout the study, SOSA isolates were identified by VITEK MS matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF MS), version 3.0–3.2, in vitro diagnostic Knowledge Bases (bioMérieux). When VITEK MS generated a species identification for which the performance of the VITEK MS had not yet been verified by the Massachusetts General Hospital Clinical Microbiology Laboratory, a result of coagulase-negative *Staphylococcus* spp. was used.

PBP2a Testing

During the retrospective study period, PBP2a testing was performed and the PBP2a test results were reported when VITEK 2 testing generated oxacillin and cefoxitin results with discordant interpretations when applying the FDA susceptible oxacillin breakpoint of ≤0.25 µg/mL (Figure 1*B*). During the prospective study period, PBP2a testing was performed and the PBP2a test results were reported when either (1) VITEK 2 oxacillin and cefoxitin results were discordant, as for the retrospective isolates, or (2) the oxacillin MIC was 0.5 µg/mL and the cefoxitin screen was positive (Figure 1*C*). PBP2a testing was performed using the FDA-cleared Oxoid Penicillin-Binding Protein (PBP2') Latex Agglutination Test (Oxoid Limited, Hampshire, UK) using overnight growth closest to a 1-µg oxacillin disk (BD BBL Sensi-Disc, Becton, Dickinson, and Company, Sparks, MD, USA) on a trypticase soy agar with 5% sheep blood plate (BD BBL, Becton, Dickinson, and Company) incubated at 35°C. This plate also served to check the VITEK 2 AST inoculum for purity; an oxacillin disk was routinely placed on the purity check plate for all staphylococci undergoing VITEK 2 AST so that organisms would be immediately available for PBP2a testing if indicated.

The PBP2a test was performed according to the manufacturer's instructions.

Retrospective Data Analysis

The percentage of retrospective isolates with each combination of VITEK 2 oxacillin and cefoxitin results was calculated. Among the subset of retrospective isolates that underwent PBP2a testing because of discordant VITEK 2 oxacillin and cefoxitin results, the rates of CA, VMEs, and MEs when using the VITEK 2 AES interpretation, compared with the PBP2a test result as the gold standard, were calculated. Where an individual combination of VITEK 2 oxacillin MIC and cefoxitin screen results was observed for ≥15 isolates, CA between the VITEK 2 AES interpretations and PBP2a results for that specific combination was determined; PBP2a testing was classified as higher yield (ie, more likely to result in a change in the reported oxacillin interpretation) when CA was <90% and lower yield (ie, less likely to result in a change in the reported oxacillin interpretation) when CA was ≥90%. The laboratory reagent costs, based on list price, and hands-on time associated with our current laboratory practice (performing a PBP2a test for isolates with discordant VITEK 2 oxacillin and cefoxitin results using a susceptible oxacillin breakpoint of ≤0.25 μg/mL) were estimated.

Prospective Data Analysis

For the set of prospective isolates that underwent PBP2a testing, because of either discordant VITEK 2 oxacillin and cefoxitin results or the combination of an oxacillin MIC 0.5 μg/mL and a positive cefoxitin screen, the rates of CA, VMEs, and MEs when using the VITEK 2 AES interpretation, compared with the PBP2a test result as the gold standard, were calculated. Where an individual combination of VITEK 2 oxacillin MIC and cefoxitin screen results was observed for ≥15 isolates, CA between the VITEK 2 AES interpretations and PBP2a results for that specific combination was determined; PBP2a testing was classified as higher yield when CA was <90% and lower yield when CA was ≥90%. The added laboratory reagent costs, based on list price, and hands-on time involved in incorporating PBP2a testing for isolates with oxacillin MICs of 0.5 μg/mL and positive cefoxitin screens were estimated.

RESULTS

Retrospective Study

There were 3451 clinical SOSA isolates included in the retrospective assessment; the percentages of isolates with each combination of VITEK 2 oxacillin and cefoxitin results are shown in Figure 2. The VITEK 2 oxacillin and cefoxitin results were discordant for 189 (5.5%) isolates, including 50 *S. hominis*, 37 *S. epidermidis*, 33 *S. capitis*, 26 *S. simulans*, 11 *S. haemolyticus*, 5 *S. cohnii*, 2 *S. intermedius* group, 2 *S. sciuri*, and 23 coagulase-negative staphylococci for which species-level identifications were not reported because the accuracy of the MALDI-TOF MS identifications in these cases, primarily *S. pettenkoferi* and *S. pasteuri,* had not been studied in our laboratory. The proportion of all isolates tested by VITEK 2 for which oxacillin and cefoxitin results were discordant varied by species (Table 1). The specimen sources from which the isolates with discordant results were cultured included blood (43%), wound (20%), urine (18%), tissue (15%), fluid (3%), and cerebrospinal fluid (2%); this distribution was similar to that of all 3451 SOSA isolates that underwent AST during the same time period, the specimen sources for which included blood (37%), wound (26%), urine (19%), tissue (11%), fluid (7%), respiratory (1%), cerebrospinal fluid (0.9%), and other (0.2%). The 189 isolates with discordant VITEK 2 oxacillin and cefoxitin results underwent PBP2a testing before result reporting; the oxacillin MIC results, cefoxitin screen results, VITEK 2 AES interpretations, and PBP2a results are shown in Table 1. The overall rate of CA between the VITEK 2 AES interpretations and PBP2a results for these 189 isolates was 58.7% (111 of 189 isolates), with a VME rate of 11.6% (8 of 69 PBP2a-positive isolates) and ME rate of 58.3% (70 of 120 PBP2a-negative isolates).

There were 3 combinations of VITEK 2 oxacillin and cefoxitin results observed in >15 isolates and that, together, comprised 90% of isolates that underwent PBP2a testing (Table 1): (1) oxacillin MIC ≤0.25 μg/mL and cefoxitin screen positive (n = 92), (2) oxacillin MIC 0.5 μg/mL and cefoxitin screen negative (n = 56), and (3) oxacillin MIC ≥4 μg/mL and cefoxitin screen negative (n = 22). In all 3 of these groups, CA between the VITEK 2 AES interpretation and the PBP2a results was <90%. Among the isolates with oxacillin MICs ≤0.25 μg/mL and positive cefoxitin screens, the VITEK 2 AES interpretation was oxacillin resistant for all 92 isolates, of which just 37 proved to be PBP2a-positive, for a CA rate of 40.2%. Among the 56 isolates with oxacillin MICs of 0.5 μg/mL and negative cefoxitin screens, the VITEK 2 AES interpretation was oxacillin resistant for 4 isolates, 1 of which was PBP2a positive and 3 of which were PBP2a negative, and oxacillin susceptible for 52 isolates, 4 of which were PBP2a positive and 48 of which were PBP2a negative. The rate of CA between the VITEK 2 AES interpretation and the PBP2a result in this category was 87.5% (49 of 56 isolates). Among the isolates with oxacillin MICs of ≥4 μg/mL and negative cefoxitin screens, the VITEK 2 AES interpretation was oxacillin resistant for all 22 isolates, 18 of which were PBP2a positive, for a CA rate of 81.8%.

The current list price for the reagents for 1 PBP2a test in our laboratory is $10.75, making the total cost of reagents to perform the 189 PBP2a tests $2031.75, which represented $23.63 per week over the retrospective study period. The hands-on time for a technologist to perform 1 PBP2a test in our laboratory is ~12 minutes, making the total hands-on time involved in performing these 189 PBP2a tests 37.8 hours, or 27 minutes per week. Per changed oxacillin interpretation, the cost was

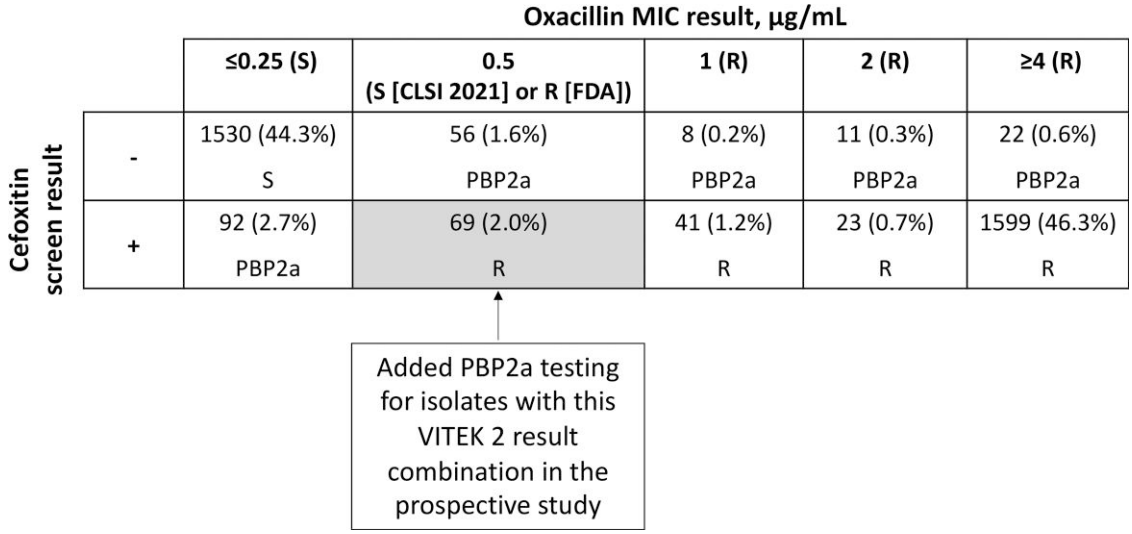

Figure 2. Distribution of VITEK 2 oxacillin MIC and cefoxitin screen results among retrospective SOSA isolates. During the retrospective study period, 3451 unique clinical SOSA isolates underwent susceptibility testing. The percentages of isolates with each possible combination of VITEK 2 oxacillin MIC and cefoxitin screen results are shown, along with the action prompted by each result combination in the study laboratory (ie, report as oxacillin susceptible, report as oxacillin resistant, or perform PBP2a testing before reporting an oxacillin result) during the retrospective study period. For isolates with VITEK 2 oxacillin MICs of 0.5 µg/mL and positive cefoxitin screens (shaded box), during the retrospective study, oxacillin was reported as resistant, while during the prospective study, PBP2a testing was performed before reporting an oxacillin result. Abbreviations: +, positive; −, negative; CLSI, Clinical and Laboratory Standards Institute; FDA, Food and Drug Administration; MIC, minimum inhibitory concentration; R, resistant; S, susceptible; SOSA, *Staphylococcus* spp. other than *S. aureus* and *S. lugdunensis*.

$26.05, calculated by dividing the total cost of reagents to perform the PBP2a tests by the number of PBP2a results that led us to report a different oxacillin interpretation than had been suggested by the VITEK 2 AES. Per changed oxacillin interpretation, the hands-on time to perform PBP2a testing was 29 minutes.

Prospective Study

There were 123 SOSA isolates that underwent PBP2a testing during the prospective study period, including 26 *S. hominis*, 17 *S. epidermidis*, 12 *S. simulans*, 8 *S. haemolyticus*, 7 *S. capitis*, 7 *S. cohnii*, 3 *S. intermedius* group, 2 *S. caprae*, 2 *S. sciuri*, and 39 coagulase-negative staphylococci for which species-level identifications were not reported because the accuracy of the MALDI-TOF MS identifications in these cases, primarily *S. pettenkoferi* and *S. pasteuri*, had not been studied in our laboratory. The specimen sources from which these isolates were cultured included blood (54.5%), wound (13.8%), tissue (13.0%), urine (12.2%), fluid (5.7%), and respiratory (0.8%). For 90 isolates, PBP2a testing was performed because the VITEK 2 oxacillin and cefoxitin results were discordant when applying a susceptible oxacillin breakpoint of ≤0.25 µg/mL; the remaining 33 isolates had oxacillin MICs of 0.5 µg/mL and positive cefoxitin screens and would not have routinely undergone PBP2a testing using our historical standard operating procedure.

The oxacillin MICs, cefoxitin screen results, VITEK 2 AES interpretations, and PBP2a results for all 123 prospective isolates are shown in Table 2. The overall rate of CA between the VITEK 2 AES interpretations and PBP2a results was 48.8% (60 of 123 isolates), with a VME rate of 13.0% (6 of 46 PBP2a-positive isolates) and ME rate of 74.0% (57 of 77 PBP2a-negative isolates).

There were 3 combinations of VITEK 2 oxacillin and cefoxitin results observed in >15 isolates and that, together, comprised 79.0% of isolates that underwent PBP2a testing in the prospective study: (1) oxacillin MIC ≤0.25 µg/mL and cefoxitin screen positive (n = 45), (2) oxacillin MIC 0.5 µg/mL and cefoxitin screen positive (n = 33), and (3) oxacillin MIC 0.5 µg/mL and cefoxitin screen negative (n = 19). The rate of CA between the VITEK 2 AES interpretation and PBP2a results was <90% for each of these 3 combinations (Table 2). Among the isolates with oxacillin MICs ≤0.25 µg/mL and positive cefoxitin screens, the VITEK 2 AES interpretation was oxacillin resistant for all 45 isolates, of which just 9 proved to be PBP2a positive, for a CA rate of 20%. Among the 33 isolates with oxacillin MICs of 0.5 µg/mL and positive cefoxitin screens, all of which were called oxacillin resistant by the VITEK 2 AES, 14 proved to be PBP2a positive and 19 PBP2a negative, for a CA rate of 42.4%. Among the 19 isolates with oxacillin MICs of 0.5 µg/mL and negative cefoxitin screens, the VITEK 2 AES interpretation was oxacillin susceptible for all 19 isolates, 4 of which proved to be PBP2a positive and 15 PBP2a negative, for a CA rate of 78.9%.

The 33 PBP2a tests performed for isolates with oxacillin MICs of 0.5 µg/mL and positive cefoxitin screens, which would not have been done if we had been following our historical

Table 1. Retrospective Study VITEK 2 AST and PBP2a Results for SOSA Isolates With Discordant Oxacillin MIC and Cefoxitin Screen Results[a]

		Original VITEK 2 FOX screen and OXA MIC results (n)																			
		FOX + (92)				FOX – (97)															
		OXA MIC ≤ 0.25 µg/mL (92)				OXA MIC 0.5 µg/mL (56)				OXA MIC 1 µg/mL (8)				OXA MIC 2 µg/mL (11)				OXA MIC ≥ 4 µg/mL (22)			
		VITEK 2 AES interpretation (n)																			
		S (0)		R (92)		S (52)		R (4)		S (1)		R (7)		S (5)		R (6)		S (0)		R (22)	
		PBP2a result																			
Species	Rate of discordance between OXA MIC and FOX screen results among all isolates tested by VITEK 2 AST[b]	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+
CoNS	23/127 = 18.1%	…	…	10	…	11	…	…	…	…	…	…	…	…	…	…	…	…	…	…	2
S. capitis	33/284 = 11.6%	…	…	4	1	25	2	…	…	…	…	…	…	…	…	1	…	…	…	…	…
S. cohnii	5/31 = 6.1%	…	…	…	…	5	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
S. epidermidis	37/2124 = 1.7%	…	…	16	7	…	…	3	1	…	…	2	2	…	…	…	1	…	…	2	3
S. haemolyticus	11/272 = 4.0%	…	…	1	5	4	…	…	…	…	…	…	…	…	…	…	…	…	…	1	…
S. hominis	50/359 = 13.9%	…	…	22	24	1	…	…	…	…	…	2	…	…	…	1	…	…	…	…	…
S. intermedius group	2/15 = 13.3%	…	…	1	…	…	1	…	…	…	…	…	…	…	…	…	…	…	…	…	…
S. sciuri	2/3 = 66.7%	…	…	…	…	…	…	…	…	…	…	…	…	2	…	…	…	…	…	…	…
S. simulans	26/117 = 22.2%	…	…	1	…	2	1	…	…	…	1	1	…	…	3	1	2	…	…	1	13
Total	189/3451 = 5.5%	0	0	55	37	48	4	3	1	0	1	5	2	2	3	3	3	0	0	4	18
		CA between VITEK 2 AES and PBP2a (111/189 = 58.7% overall)																			
		37/92 = 40.2%				49/56 = 87.5%				ND				ND				18/22 = 81.8%			
		VME rate (8/69 = 11.6% overall)																			
		0/37 = 0%				4/5 = 80%				ND				ND				0/18 = 0%			
		ME rate (70/120 = 58.3% overall)																			
		55/55 = 100%				3/51 = 5.9%				ND				ND				4/4 = 100%			

AST, antimicrobial susceptibility testing; SOSA, *Staphylococcus* spp. other than *S. aureus* and *S. lugdunensis*; MIC, minimum inhibitory concentration; FOX, cefoxitin screen; OXA, oxacillin; +, positive; –, negative; S, susceptible; R, resistant; CoNS, coagulase-negative *Staphylococcus*; CA, categorical agreement; AES, Automated Expert System; VME, very major error; ME, major error; ND, not determined due to small n.

[a]The VITEK 2 AST-GP75 card was used; this FDA-cleared commercial AST system uses the FDA oxacillin susceptible breakpoint of ≤0.25 µg/mL.

[b]The sum of the denominators for species listed in this table (3332) does not equal the total denominator of isolates tested by VITEK 2 AST in the retrospective study (3451) because there were some species, including *S. caprae*, *S. schleiferi*, and *S. warneri*, for which the VITEK 2 oxacillin MIC and cefoxitin screen results were concordant for all isolates.

algorithm, accounted for an added cost of $7.71 and added hands-on time of 9 minutes per week during the prospective study period above and beyond the cost and hands-on time already spent on PBP2a testing in our laboratory. Among these 33 tests, the cost was $18.67, and the hands-on time 21 minutes, per changed oxacillin interpretation.

DISCUSSION

Revision of the CLSI oxacillin susceptible breakpoint for SOSA raises questions for the many clinical microbiology laboratories using FDA-cleared AST systems, which do not yet incorporate this new breakpoint, about whether to implement this change or make other modifications to their AST procedure for this group of organisms. As we describe here, exploring these questions within our laboratory reinforced the fact that accurate delineation of the presence or absence of *mecA*-mediated oxacillin resistance among SOSA using phenotypic AST can be challenging, including when using the automated VITEK 2 AST system, which involves an oxacillin MIC test, a cefoxitin screen, and an AES interpretation. Review of our retrospective data set showed that our existing procedure of performing PBP2a testing to adjudicate discordant VITEK 2 oxacillin and cefoxitin results led us to report a different oxacillin result than would have been reported if we had accepted the VITEK 2 AES interpretation for 41.3% of such isolates. In the retrospective study, PBP2a testing of isolates with oxacillin MICs of ≤0.25 µg/mL but positive cefoxitin screens had the highest yield, as there were substantial percentages of both PBP2a-positive isolates (40.2%) and PBP2a-negative isolates (59.8%) among this group. Because the VITEK 2 AES interpretation was oxacillin resistant for all of these isolates, PBP2a testing markedly reduced the ME rate, facilitating administration of first-line β-lactam antimicrobials rather than alternative agents, such as vancomycin, when these isolates warranted treatment.

Particularly salient to the 2021 CLSI breakpoint change was the observation that the majority of isolates with VITEK 2 oxacillin MICs of 0.5 µg/mL and negative cefoxitin screens proved to be PBP2a negative (91.1% and 78.9% in the retrospective and prospective study periods, respectively). However, if we had simply implemented the revised CLSI

Table 2. Prospective Study VITEK 2 AST and PBP2a Results for SOSA Isolates With Either Discordant Oxacillin MIC and Cefoxitin Screen Results or Oxacillin MIC 0.5 µg/mL and Positive Cefoxitin Screens[a]

	Original VITEK 2 FOX screen and OXA MIC results (n)																							
	FOX + (78)								FOX – (45)															
	OXA MIC ≤ 0.25 µg/mL (45)				OXA MIC 0.5 µg/mL (33)				OXA MIC 0.5 µg/mL (19)				OXA MIC 1 µg/mL (2)				OXA MIC 2 µg/mL (10)				OXA MIC ≥ 4 µg/mL (14)			
	VITEK 2 AES interpretation (n)																							
	S (0)		R (45)		S (0)		R (33)		S (19)		R (0)		S (2)		R (0)		S (5)		R (5)		S (0)		R (14)	
	PBP2a result (n)																							
Species (n)	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+	–	+
CoNS (39)	…	…	15	1	…	…	16	1	5	…	…	…	…	…	…	…	1	…	…	…	…	…	…	…
S. capitis (7)	…	…	2	…	…	…	…	…	4	1	…	…	…	…	…	…	…	…	…	…	…	…	…	…
S. caprae (2)	…	…	…	1	…	…	…	…	1	…	…	…	…	…	…	…	…	…	…	…	…	…	…	…
S. cohnii (7)	…	…	…	…	…	…	2	…	3	…	…	…	…	…	…	…	2	…	…	…	…	…	…	…
S. epidermidis (17)	…	…	6	1	…	…	…	3	…	…	…	…	…	…	…	…	…	…	…	2	…	…	…	5
S. haemolyticus (8)	…	…		2	…	…	1	3	1	…	…	…	…	…	…	…	…	…	1	…	…	…	…	…
S. intermedius group (3)	…	…	…	…	…	…	…	…	…	1	…	…	…	1	…	…	…	…	…	…	…	…	…	1
S. hominis (26)	…	…	13	4	…	…	…	7	…	…	…	…	…	…	…	…	…	…	…	1	…	…	1	
S. sciuri (2)	…	…	…	…	…	…	…	…	…	…	…	…	1	…	…	…	1	…	…	…	…	…	…	…
S. simulans (12)	…	…	…	…	…	…	…	…	1	2	…	…	…	…	…	…	…	1	…	1	…	…	…	7
Total (123)	0	0	36	9	0	0	19	14	15	4	0	0	1	1	0	0	4	1	1	4	0	0	1	13
	CA between VITEK 2 AES and PBP2a (60/123 = 48.8% overall)																							
	9/45 = 20%				14/33 = 42.4%				15/19 = 78.9%				ND				ND				ND			
	VME rate (6/46 = 13.0% overall)																							
	0/9 = 0%				0/14 = 0%				4/4 = 100%				ND				ND				ND			
	ME rate (57/77 = 74.0% overall)																							
	36/36 = 100%				19/19 = 100%				0/15 = 0%				ND				ND				ND			

AST, antimicrobial susceptibility testing; SOSA, *Staphylococcus* spp. other than *S. aureus* and *S. lugdunensis*; MIC, minimum inhibitory concentration; FOX, cefoxitin screen; OXA, oxacillin; +, positive; –, negative; S, susceptible; R, resistant; CoNS, coagulase-negative *Staphylococcus*; CA, categorical agreement; AES, Automated Expert System; VME, very major error; ME, major error; ND, not determined due to small n.

[a]The VITEK 2 AST-GP75 card was used; this FDA-cleared commercial AST system uses the FDA oxacillin susceptible breakpoint of ≤0.25 µg/mL.

susceptible oxacillin breakpoint of ≤0.5 µg/mL, thereby bringing the VITEK 2 oxacillin and cefoxitin results into concordance and precluding the need for PBP2a testing using our laboratory's historical algorithm, we would have reported false-susceptible results for the 12% of isolates with this combination of results that were shown to harbor *mecA*-mediated resistance. On the other hand, prospective PBP2a testing of isolates with oxacillin MICs of 0.5 µg/mL and positive cefoxitin screens, which we estimate based on our retrospective data set to account for ~2.0% of all SOSA undergoing AST in our laboratory (Figure 2), was found to be of even higher yield; 42.4% of such isolates were PBP2a positive and 57.6% were PBP2a negative. Based on these results, balanced against the only modest increases in reagent cost and hands-on time that we observed during the prospective study ($7.71 and 9 minutes per week), we have modified our laboratory's standard operating procedure and now reflex SOSA to a PBP2a test whenever (1) requested by a clinician; (2) the VITEK 2 oxacillin MIC is ≤0.25 µg/mL and the cefoxitin screen is positive (which, in the retrospective study, accounted for 2.7% of isolates); (3) the VITEK 2 oxacillin MIC is 0.5 µg/mL, regardless of the cefoxitin screen result (3.6% of isolates); or (4) the VITEK 2 oxacillin MIC is ≥1 µg/mL and the cefoxitin screen is negative (1.1% of isolates).

While tests that detect the *mecA* gene or its product, PBP2a, are considered the most definitive methods for assessment of oxacillin resistance in staphylococci [13], the cost and labor involved in performing such testing for all SOSA may be prohibitive for clinical laboratories. As many SOSA isolated in the clinical microbiology laboratory are not acting as pathogens and do not warrant administration of targeted antimicrobial therapy, limiting *mecA* or PBP2a testing to only those SOSA for which AST will be of high clinical value may mitigate cost and labor issues; however, it can be difficult for laboratory staff to quickly ascertain whether a given isolate is clinically important, and strategies of either actively reaching out to clinicians to inquire whether AST is needed or passively waiting to perform AST only upon request by clinicians may prolong turnaround time and be associated with their own workflow challenges. Furthermore, *mecA* or PBP2a testing is likely to be added to, rather than replace, phenotypic AST, given that susceptibility

results for additional antimicrobials may be needed. Consequently, a pragmatic approach for laboratories may be to target a subset of isolates for *mecA* or PBP2a testing based on phenotypic AST results that predict an increased risk of misclassification based on phenotype alone, weighing benefits and costs at their institutions; here, we have described 1 version of such an approach.

Although it is possible that PBP2a testing of all SOSA isolates, including those with VITEK 2 oxacillin MICs of ≤0.25 μg/mL and negative cefoxitin screens, as well as those with oxacillin MICs of ≥1 μg/mL and positive cefoxitin screens, would have uncovered additional VITEK 2 AES VMEs or MEs, we believe that errors for isolates with these combinations of results, which comprise the majority (92.5%) (Figure 2) of SOSA isolates in our clinical laboratory, are less likely. Clearance of an automated AST system by the FDA requires that the drug concentrations included in the test have met the FDA's standards for essential agreement (EA), that is, that the MIC results from a reference method and the commercial device agree within a single doubling dilution [14]. The EA between VITEK 2 oxacillin AST and agar dilution is reported by the manufacturer as 97.2% [17]. While the breakdown of *Staphylococcus* spp. that were tested in the trial that supported FDA clearance of VITEK 2 oxacillin AST is unspecified, leading to the potential that certain species for which EA is lower could have been under-represented in the trial, and the trial was undertaken a number of years ago, raising the possibility of different results if contemporary isolates were studied, this high EA suggests that most categorical disagreement between VITEK 2 oxacillin AST and either *mecA* PCR or PBP2a testing likely occurs among isolates within 1 dilution of the breakpoint.

Another limitation of our study is that we did not test isolates for *mecC*, which is also a known cause of oxacillin resistance in staphylococci [19]. However, *mecC* is currently considered to be rare among SOSA and generally has not been detected from clinical cultures from humans [8]. Likewise, we did not assess isolates for the presence of additional rarely reported mechanisms of staphylococcal oxacillin resistance, such as penicillin binding protein modifications other than PBP2a or *blaZ* β-lactamase hyperproduction, both of which have principally been identified in *S. aureus* rather than SOSA [20]. Given that *mecA*-mediated resistance accounts for the majority of oxacillin resistance among staphylococci, we believe that the omission of testing for other potential resistance mechanisms is unlikely to have affected the conclusions of our study.

Finally, we did not undertake strain typing as part of this work, and so cannot exclude the possibility that some isolates from this single-institution study were clonal; however, the inclusion of isolates from multiple different SOSA species improves generalizability. Data from multicenter studies with whole-genome sequencing–based typing analyses would permit a more robust assessment of our approach.

Of potential interest to laboratories considering integration of PBP2a testing into their SOSA AST algorithms is the existence of a lateral flow format PBP2a assay (Clearview PBP2a SA Culture Colony Test, Abbott Diagnostics, Inc., Scarborough, ME, USA) that requires less hands-on time to perform than the Oxoid PBP2' Latex Agglutination Test that we utilized in this study. To date, the Abbott assay has only been FDA cleared for use with *S. aureus* isolates, while the Oxoid test is cleared for use with both *S. aureus* and coagulase-negative staphylococci, but excellent performance of the Abbott assay with SOSA has been reported [8–11]. If the manufacturer were to seek and gain FDA clearance for use of this lateral flow test with SOSA, it would likely facilitate broader implementation of PBP2a testing, thus improving the accuracy of characterization of β-lactam susceptibility in clinical microbiology laboratories. In the interim, interested laboratories might consider validation of the test for off-label use.

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