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Evaluation of Surrogate Tests for the Presence of *mecA*-Mediated Methicillin Resistance in *Staphylococcus capitis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, and *Staphylococcus warneri*

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ABSTRACT Testing of staphylococci other than *Staphylococcus aureus* (SOSA) for *mecA*-mediated resistance is challenging. Isolates of *Staphylococcus capitis*, *Staphylococcus haemolyticus*, *Staphylococcus hominis*, and *Staphylococcus warneri* were evaluated by cefoxitin and oxacillin broth microdilution (BMD), disk diffusion (DD), and PBP2a immunoassay, and the results were compared to *mecA* PCR results. No phenotypic susceptibility test correlated well with PCR results across all species, although the PBP2a immunoassay yielded 100% correlation. Oxacillin BMD testing by current Clinical and Laboratory Standards Institute (CLSI) SOSA breakpoints led to 2.1% very major errors (VMEs) and 7.1% major errors (ME). Adjusting this breakpoint up by a dilution (susceptible, ≤0.5 μg/ml; resistant, ≥1.0 μg/ml) led to 2.8% VMEs and 0.3% MEs. Among species evaluated, *S. haemolyticus* had unacceptable VMEs with this new breakpoint (6.4%), as did *S. hominis* (4.0%). MEs were acceptable by this new breakpoint, ranging from 0 to 1.2%. Oxacillin DD yielded high ME rates (20.7 to 21.7%) using CLSI or European Committee on Antimicrobial Susceptibility Testing breakpoints. VMEs ranged from 0 to 5.3%. Cefoxitin BMD led to 4.9% VMEs and 1.6% MEs. Cefoxitin DD performed best when interpreted with the CLSI SOSA breakpoint, with 1.0% VMEs and 2.9% MEs. This study led CLSI to adjust the oxacillin MIC breakpoints for SOSA. Laboratories should be aware that no individual phenotypic test correlates well across all species of SOSA with *mecA* PCR results. Molecular testing for *mecA* or evaluation for PBP2a is the preferred approach.

KEYWORDS CLSI, EUCAST, PBP2a, *Staphylococcus*, breakpoint, broth microdilution, cefoxitin, disk diffusion, *mecA*, minimum inhibitory concentration

Staphylococci other than *Staphylococcus aureus* (SOSA [traditionally referred to as the coagulase-negative staphylococci, although not all are technically coagulase negative]) are normal microbiota of the human skin and mucosal surfaces and important opportunistic pathogens, associated predominantly with infection in the setting of indwelling/implanted foreign bodies and devices (1). Forty-six validly described species and 24 subspecies are members of this broad group—unified by their negative coagulase reaction (2–6). One species, *Staphylococcus schleiferi*, includes both free (tube) coagulase-negative and -positive subspecies, *S. schleiferi* subsp. *schleiferi* and *S.*

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TABLE 1 Overview of CLSI updates to staphylococcal testing recommendations to predict the presence of *mecA*

Year	Species	Recommendation(s)	Reference
1986	All staphylococci	Publication of methicillin, nafcillin, and oxacillin MIC and DD susceptibility criteria in M100, first informational supplement	26
1999	SOSA	Establishment of oxacillin MIC and DD breakpoints in M100 that are different than those for *S. aureus*	8
1999	All staphylococci	Deletion of methicillin MIC and DD susceptibility criteria—recommendation to test oxacillin alone	8
2004	*S. aureus* SOSA	Introduction of the cefoxitin disk diffusion test to predict oxacillin resistance	9
2005	*S. lugdunensis*	Inclusion of *S. lugdunensis* with *S. aureus* oxacillin and cefoxitin breakpoints	9
2006	*S. lugdunensis*	Warning that cefoxitin and not oxacillin should be used for disk diffusion of *S. lugdunensis*	9
2012	*S. aureus*	Deletion of oxacillin disk breakpoints	9
2012	SOSA	Recommendation to perform cefoxitin disk, PBP2a, or *mecA* test if oxacillin MIC of 0.5–2.0 μg/ml for species other than *S. epidermidis*	9
2014	*S. pseudintermedius*	Publication of oxacillin MIC and disk breakpoints; warning against use of cefoxitin tests for this species	10
2015	*S. schleiferi*	Publication of oxacillin MIC and disk breakpoints; warning against use of cefoxitin for this species	11
2018	*S. epidermidis*	Addition of oxacillin disk test for *S. epidermidis*, confirmation of MIC breakpoint	12
2021	SOSA	Oxacillin breakpoint updated	This work

schleiferi subsp. *coagulans*, respectively (2). Historically, identification of SOSA to the species level by morphological and biochemical testing was difficult, lacked resolution, and was often deemed unnecessary as these were commonly viewed as nonpathogenic contaminants (1). In recent years, identification of the members of the SOSA has become more commonplace, through both a better understanding of their role as opportunistic pathogens (1) and the availability of matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) in clinical laboratories (7). The ability to accurately identify SOSA to the species level has improved understanding of the clinical significance of specific species and is accompanied by a need to understand how to best evaluate these isolates for the presence of *mecA* and ultimately susceptibility to β-lactams.

Accurate detection of *mecA* (or *mecC*)-mediated resistance to the penicillinase-stable penicillins (oxacillin, nafcillin, cloxacillin, dicloxacillin, and flucloxacillin) is challenging for the staphylococci due to the heterogenous nature of *mecA* expression across this diverse group of bacteria. In 2000, the Clinical and Laboratory Standards Institute (CLSI [then the National Committee for Clinical Laboratory Standards]) first adopted SOSA oxacillin breakpoints that differed from those used for *Staphylococcus aureus*. This study was conducted using 50 isolates, weighted heavily for isolates of *Staphylococcus epidermidis* ($n = 27$). Eight isolates of *Staphylococcus hominis*, 6 of *Staphylococcus warneri*, 3 of *Staphylococcus haemolyticus*, 2 each of *Staphylococcus lugdunensis* and *Staphylococcus saprophyticus*, and a single isolate each of *Staphylococcus capitis* and *Staphylococcus simulans* were also evaluated (8). In this study, good correlation (84 to 94%) between oxacillin MIC values of $\leq$0.25 μg/ml and the absence of *mecA* was found for all isolates. Similarly, excellent correlation (99 to 100%) between oxacillin MICs of $\geq$0.5 μg/ml and the presence of *mecA* was found for *S. epidermidis*. However, poor correlation between oxacillin MICs of $\geq$0.5 μg/ml and the presence of *mecA* was shown for species other than *S. epidermidis*, ranging from 53.0 to 66.4% correlation (i.e., 33.6 to 47.0% major errors [MEs]). In 2004, CLSI recommended cefoxitin, a cephamycin, be used as a surrogate test for oxacillin as it was found that cefoxitin disk results correlated better with the presence of *mecA* for SOSA, particularly isolates with MIC values in the 0.5- to 2.0-μg/ml range (9).

In 2016, CLSI formed a coagulase-negative *Staphylococcus ad hoc* working group to evaluate existing oxacillin and cefoxitin testing recommendations to determine their performance as surrogate tests for *mecA*/*C* in staphylococci other than *S. aureus* and *S. lugdunensis* (referred to herein as SOSA for simplicity). Prioritization of species evaluated include those with known or suspected testing challenges (e.g., *Staphylococcus pseudintermedius*) and those with a higher prevalence in human infections (e.g., *S. epidermidis*). The combined work has led to several changes to recommendations in the M100 standard for these isolates (Table 1), including addition of oxacillin disk breakpoints for *Staphylococcus pseudintermedius* (10), *S. schleiferi* (11), and *S. epidermidis* (12)

TABLE 2 Isolates evaluated in this study

Species (*n* isolates)	No. of isolates *mecA* positive	Countries of origin (*n* isolates)	% of isolates from blood culture
S. capitis (50)	25	Australia, Brazil (4), Canada (6), Chile (1), Italy (1), Japan (4), South Korea (1), Philippines (5), Poland (1), Sweden (1), Taiwan (3), Thailand (1), USA (21)	70
S. haemolyticus (50)	26	Australia (1), Belgium (1), Canada (10), France (1), Netherlands (1), South Africa (1), Switzerland (2), USA (33)	56
S. hominis (50)	25	Canada (12), USA (38)	84
S. warneri (48)	20	Argentina (1), Australia (3), Belgium (1), Canada (7), Colombia (1), Czech Republic (1), France (1), Germany (3), Greece (1), Israel (2), Italy (4), South Korea (1), Kuwait (1), Philippines (2), Russia (1) South Africa (1), Spain (1), Thailand (1), UK (2), USA (10), Venezuela (2)	44

and recommendation against use of cefoxitin to predict oxacillin susceptibility for *S. pseudintermedius* and *S. schleiferi* (10, 11).

The present study is a continuation of the work performed by CLSI to reevaluate SOSA breakpoints. Isolates of *S. capitis*, *S. haemolyticus*, *S. hominis*, and *S. warneri* were evaluated by reference cefoxitin and oxacillin broth microdilution (BMD) and disk diffusion to determine the best phenotypic tests to use as surrogates for the presence of *mecA*. These isolates were chosen due to their high frequency in human bloodstream infections, ranging from 3.5 to 12% for *S. capitis*, 2.8 to 36.7% for *S. hemolyticus*, 3.0 to 18% for *S. hominis*, and 2.0 to 7.8% for *S. warneri* (1).

MATERIALS AND METHODS

Bacterial isolates. A total of 198 isolates were evaluated in this study (Table 2). Isolates included *S. capitis* ($n = 50$), *S. haemolyticus* ($n = 50$), *S. hominis* ($n = 50$), and *S. warneri* ($n = 48$). Isolates were collected from multiple geographic locations between 2014 and 2018 (Table 2) and were chosen to represent a roughly even number of *mecA*-positive and -negative isolates for each species. The isolates were recovered from a variety of clinical cultures, including 126 from blood cultures (64%), 25 from sterile fluids (12.6%), 20 from skin and soft tissue (10.1%), 7 from genitourinary sources (3.5%), 6 from bone (3.0%), 3 respiratory (1.5%), 2 ocular (1.0%), and 3 from other sources (1.5%). Isolates were identified to the species level at each institution according to their standard operating procedures and confirmed at Accelerate Diagnostics, Inc. (Tucson, AZ), by MALDI-TOF MS (Bruker Daltonics, Inc., Billerica, MA) and at Washington University with Vitek MS (bioMérieux, Durham, NC).

***mecA*/*C* PCR.** All isolates were evaluated for *mecA* and *mecC* by colony PCR performed from overnight growth on tryptic soy agar with 5% sheep's blood agar plates (BAPs; Hardy Diagnostics, Santa Maria, CA) as previously described (12).

Antimicrobial susceptibility testing. Isolates were stored as described previously (10) and subcultured twice from frozen stocks on BAPs before testing. Disk diffusion (DD) and BMD were performed as described by CLSI (13, 14). DD was evaluated on Mueller-Hinton agar (MHA) plates obtained from 3 vendors: Remel (Lenexa, KS), Hardy Diagnostics, and BD (Sparks, MD). Disks containing 1 μg oxacillin and 30 μg cefoxitin (BBL, BD) were used. BMD was performed by CLSI reference, frozen-form panels containing cation-adjusted Mueller-Hinton broth (CA-MHB) with cefoxitin or oxacillin. Oxacillin BMD tests were supplemented with 2% NaCl. BMD panels were made by Accelerate Diagnostics, Inc. CA-MHB samples from 3 different manufacturers (Difco [BD], BD, and Oxoid [ThermoFisher Scientific, Lenexa KS]) were evaluated on a single panel. Oxacillin and cefoxitin were tested in 2-fold dilutions at concentrations ranging from 0.016 to 32 μg/ml.

For testing, 3 to 5 isolated colonies grown overnight on a BAP at 35 to 37°C in ambient air were used to prepare a suspension equivalent to a 0.5 McFarland standard, using a nephelometer, in 0.85% saline. This suspension was used to inoculate all DD and BMD tests, in parallel (13, 14). DD tests were incubated at 35 to 37°C in ambient air, and zones of inhibition were measured at 16 to 18 h for oxacillin and 24 h for cefoxitin. BMD tests were incubated at 35 to 37°C in ambient air and read at 16 to 20 h for cefoxitin and 24 h for oxacillin. Colony counts were performed on each inoculum by subculturing the growth control well for each BMD panel. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used as quality controls (QCs) for DD and BMD, respectively, and quality control testing was performed each day of testing.

PBP2a testing. PBP2a testing was performed using the Abbott PBP2a SA Culture Colony test (Abbott Diagnostics, Inc., Scarborough, ME), according to the package insert instructions for testing *S. aureus* and from the same plate used to make inocula for BMD and DD. In advance, it was agreed that isolates that displayed a different PBP2a result versus *mecA* PCR result would be repeated in duplicate and that using growth from around a cefoxitin disk and growth from a BAP would be performed, although no isolates required induction. *S. aureus* ATCC 43300 and *S. aureus* ATCC 25923 were used as positive and negative controls, respectively.

Data analysis. Zone diameters and MIC values were interpreted using the following breakpoints in the CLSI M100, 29th edition, document (Table 3): *S. aureus*/*S. lugdunensis* cefoxitin disk diffusion (FOX DD

TABLE 3 Breakpoints evaluated in this study

Breakpoint descripton[a]	Breakpoint value for: Susceptible	Resistant	Abbreviation
Oxacillin MIC, *Staphylococcus* other than *S. aureus* and *S. lugdunensis*	≤0.25 μg/ml	≥0.5 μg/ml	OX MIC SOSA
Oxacillin MIC, *S. aureus*/S. *lugdunensis*	≤2 μg/ml	≥4 μg/ml	OX MIC SAU
Oxacillin zone diameter, *S. schleiferi*, *S. pseudintermedius*, *S. epidermidis*	≥18 mm	≤17 mm	OX DD SOSA
Oxacillin zone diameter, EUCAST, *S. pseudintermedis*	≥20 mm	≤19 mm	OX DD EUCAST
Cefoxitin MIC, *S. aureus*/*S. lugdunensis*	≤4 μg/ml	≥8 μg/ml	FOX MIC SAU
Cefoxitin zone diameter, *S. aureus*/*S. lugdunensis*	≥22 mm	≤21 mm	FOX DD SAU
Cefoxitin zone diameter, *Staphylococcus* other than *S. aureus*, *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*	≥25 mm	≤24 mm	FOX DD SOSA
New, oxacillin MIC breakpoint for SOSA (this study)	≤0.5 μg/ml	≥1 μg/ml	New OX MIC SOSA

[a]All breakpoints are CLSI, unless otherwise noted.

SAU), *S. aureus*/*S. lugdunensis* cefoxitin MIC (FOX MIC SAU), *S. aureus*/*S. lugdunensis* oxacillin MIC (OX MIC SAU), SOSA excluding *S. lugdunensis* and *S. pseudintermedius* cefoxitin disk (FOX DD SOSA), SOSA oxacillin MIC (OX MIC SOSA), and *S. pseudintermedius*/*S. schleiferi*/*S. epidermidis* oxacillin disk (OX DD SOSA) (15). Additionally, results were evaluated with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 2019 oxacillin disk *S. pseudintermedius* breakpoint (16). Results were compared to *mecA* (and *mecC*, although all isolates were negative) PCR as the "gold standard" for oxacillin resistance. Categorical agreement (CA), very major errors (VMEs), and major errors (MEs) were calculated as previously described (17). VMEs were for isolates that were *mecA* positive but were oxacillin or cefoxitin susceptible. MEs were defined as those isolates that were *mecA* negative but were oxacillin or cefoxitin resistant. Disk-to-MIC correlates were evaluated using dBETs software (https://dbets.shinyapps.io/dBETS/). As each isolate was evaluated on three brands of MHA, data were pooled such that each isolate yielded $n = 3$ results.

RESULTS

Ninety-six isolates were positive for *mecA*, and 102 were negative by PCR (Table 2). No isolates were positive for *mecC*. The breakdown by species included *S. capitis* (25 *mecA* positive, 25 *mecA* negative), *S. haemolyticus* (26 *mecA* positive, 24 *mecA* negative), *S. hominis* (25 *mecA* positive, 25 *mecA* negative), and *S. warneri* (20 *mecA* positive, 28 *mecA* negative). PBP2a testing correlated 100% with the *mecA* PCR results, and no induction was required for any isolate.

***S. capitis*.** Clear separation of the *mecA*-positive and -negative isolates was observed by both oxacillin and cefoxitin BMDs (see Fig. S1A and S2A in the supplemental material). Most oxacillin MIC values for isolates with *mecA* were >32 μg/ml (89.3%). Only Sigma CA-MHB yielded oxacillin MICs of <32 μg/ml, with 2 isolates with a MIC of 4 μg/ml, 1 at 8 μg/ml and 1 at 16 μg/ml (Fig. S1A). Both the OX MIC SOSA and OX MIC SAU breakpoints yielded 100% CA (Table 4). Most cefoxitin MIC values for isolates with *mecA* were >32 μg/ml (85.3%; Fig. S2A), whereas *mecA*-negative isolates in general had a cefoxitin MIC of 2 or 4 μg/ml. Overall, no statistical difference was noted between brands of media with regard to VME or ME rates.

DD performed with a 1-μg oxacillin disk similarly split the *mecA*-positive and -negative isolates (see Fig. S3A in the supplemental material). Using the OX DD SOSA

TABLE 4 Overview of phenotypic test results

Breakpoint	No. (%) of results[a] *S. capitis* VMEs	*S. capitis* MEs	*S. haemolyticus* VMEs	*S. haemolyticus* MEs	*S. hominis* VMEs	*S. hominis* MEs	*S. warneri* VMEs	*S. warneri* MEs	Overall VMEs	Overall MEs
OX MIC SOSA	0/75 (0)	0/75 (0)	**3/78 (3.8)**	1/75 (1.4)	**3/75 (4.0)**	**5/75 (6.7)**	0/59 (0)	**16/84 (19.0)**	**6/287 (2.1)**	**22/309 (7.1)**
OX MIC SAU	0/75 (0)	0/75 (0)	**15/78 (19.2)**	0/75 (0)	**3/75 (4.0)**	0/75 (0)	0/59 (0)	0/84 (0)	**18/287 (6.2)**	0/309 (0)
OX DD SOSA	0/75 (0)	0/75 (0)	**5/78 (6.4)**	0/75 (0)	**4/75 (5.3)**	0/75 (0)	0/59 (0)	**67/84 (79.8)**	**9/287 (3.1)**	**67/309 (21.7)**
OX DD EUCAST	0/75	**3/75 (4)**	**2/78 (2.6)**	0/75 (0)	**4/75 (5.3)**	2/75 (2.7)	**1/59 (1.7)**	**59/84 (70.2)**	**7/287 (2.4)**	**64/309 (20.7)**
FOX MIC SAU	0/75 (0)	0/75 (0)	**6/78 (7.7)**	0/72 (0)	**6/75 (8.0)**	**5/75 (6.7)**	**2/59 (3.4)**	0/84 (0)	**14/287 (4.9)**	5/309 (1.6)
FOX DD SOSA	0/75 (0)	0/75 (0)	**3/78 (3.8)**	0/72 (0)	0/75 (0)	**9/75 (12)**	0/59 (0)	0/84 (0)	3/287 (1.0)	9/309 (2.9)
FOX DD SAU	0/75 (0)	0/75 (0)	**4/78 (5.1)**	0/72 (0)	**3/75 (4.0)**	0/75 (0)	**1/59 (1.7)**	0/84 (0)	**8/287 (2.8)**	0/309 (0)
New OX MIC SOSA	0/75 (0)	0/75 (0)	**5/78 (6.4)**	0/72 (0)	**3/75 (4.0)**	0/75 (0)	0/59 (0)	1/84 (1.2)	**8/287 (2.8)**	1/309 (0.3)

[a]Boldface values indicate errors above acceptance limits.

breakpoint, 100% CA was seen (Table 4). Using the OX DD EUCAST breakpoint, 3/75 results represented MEs (4%; Table 4). These errors were for a single isolate with a zone of inhibition of 18 mm on all three brands of MHA (Fig. S3A). DD performed with the 30-μg cefoxitin disk yielded 100% CA using both the FOX DD SOSA and the FOX DD SAU breakpoints (Table 4).

***S. haemolyticus*.** No test provided good differentiation of *mecA*-positive from -negative isolates of *S. haemolyticus* (see Fig. S1B to S4B in the supplemental material). While over half of the oxacillin MIC values for isolates with *mecA* were >32 μg/ml (60.3%), 19.2% of isolates with *mecA* yielded oxacillin MICs of 0.25 to 1 μg/ml. Isolate WU-09, which harbored *mecA*, had an oxacillin MIC value of 0.25 μg/ml by all three manufacturers of media (Table 4), yielding 3.8% VMEs by the OX MIC SOSA breakpoint. Both *mecA* PCR and oxacillin tests were repeated for this isolate and yielded the same results. Additionally, a 1.4% ME rate was observed by this breakpoint (Table 4). The OX MIC SAU breakpoint yielded 19.2% VMEs and 0% MEs (Table 4). Cefoxitin MIC values ranged from $\leq$0.016 to 4 μg/ml for *mecA*-negative isolates and 0.25 to >32 μg/ml for *mecA*-positive isolates, with substantial overlap in MICs between *mecA*-positive and -negative isolates (Fig. S2B). The VME rate was 7.7% by the FOX MIC SAU breakpoint, with no MEs (Table 4).

DD performed with 1-μg oxacillin disks resulted in zones of growth inhibition ranging from 20 to 26 mm for *mecA*-negative isolates and 6 to 20 mm for *mecA*-positive isolates (Fig. S3). Using the OX DD SOSA breakpoint, 6.4% VMEs and 0% MEs were observed (Table 4). Using the OX EUCAST breakpoint, 2.6% VMEs were seen and there were no MEs. DD performed with the 30-μg cefoxitin disk yielded zones of 26 to $\geq$30 mm and 6 to 28 mm for *mecA*-negative and -positive isolates, respectively (Fig. S4B). Again, only VMEs were observed, at 5.1% by the FOX DD SAU and 3.8% by the FOX DD SOSA breakpoints (Table 4).

***S. hominis*.** The *mecA*-negative isolates yielded oxacillin MICs of 0.03 to 0.5 μg/ml, and *mecA*-positive isolates had MICs of 0.125 to $>32\mu$g/ml (Fig. S1C). Three VMEs were observed by both the OX MIC SOSA and OX MIC SAU breakpoints (Table 4), all with a MIC of 0.125 μg/ml for the same isolate (CHLA Shom 14). The only MEs were for oxacillin BMD results interpreted by the OX MIC SOSA breakpoint (Table 4). Cefoxitin MICs ranged from 0.25 to 8 μg/ml for *mecA*-negative isolates and 4 to >32 μg/ml for *mecA*-positive isolates. The VME rate was 8.0% by the FOX MIC SAU breakpoint, with 6.7% MEs (Table 4).

Oxacillin DD resulted in zones of growth inhibition ranging from 19 to 26 mm for *mecA*-negative isolates and 6 to 22 mm for *mecA*-positive isolates (Fig. S3C). Cefoxitin DD results for *mecA*-positive isolates ranged broadly from 6 to 23 mm and from 22 to $\geq$30 mm for *mecA*-negative isolates (Fig. S4C). Using the OX DD SOSA breakpoint, 5.3% VMEs and 0% MEs were seen (Table 4). Using the OX DD EUCAST breakpoint, 5.3% VMEs and 2.6% MEs were seen (Table 4). The FOX DD SOSA breakpoint yielded 0% VMEs and 12% MEs, whereas the FOX DD SAU breakpoint yielded 4% VMEs and 0% MEs (Table 4).

***S. warneri*.** Isolates without *mecA* yielded oxacillin MICs of 0.125 to 1 μg/ml, and isolates with *mecA* had MICs of 4 to $>32\mu$g/ml (Fig. S1D). Only MEs were observed, at 19% by the OX MIC SOSA breakpoint but 0% by the OX MIC SAU breakpoint (Table 4). Cefoxitin MICs ranged from 1 to 4 μg/ml for *mecA*-negative isolates and 4 to >32 μg/ml for *mecA*-positive isolates (Fig. S2D). The VME rate was 3.4% by the FOX MIC SAU breakpoint, with no MEs (Table 4). The two VMEs were for two different isolates, WCM1249671 and SW-02, on two different manufacturers' media, suggesting random errors. However, 27% of results for isolates with *mecA* were at a MIC value of 8 μg/ml at the FOX MIC SAU breakpoint.

DD performed with a 1-μg oxacillin disk resulted in zones of growth inhibition ranging from 14 to 22 mm for *mecA*-negative isolates and 6 to 15 mm for *mecA*-positive isolates. Most results (94.9%) from *mecA*-positive isolates generated a 6-mm zone of inhibition (i.e., no zone). In contrast, 29.8% of *mecA*-negative isolates yielded a zone of inhibition of 19 mm, with a normal distribution of zones for all isolates ranging from 14

to 22 mm. Cefoxitin DD results for *mecA*-positive isolates ranged broadly from 6 to 22 mm, and the majority of *mecA*-negative isolates had zones of inhibition of 29 mm (38%) or ≥30 mm (47.6%) (Fig. S4D). Using the OX DD SOSA breakpoint, 0% VMEs and 79.8% MEs were seen (Table 4). Using the OX EUCAST breakpoint, 0% VMEs and 70.2% MEs were seen. CA improved with the cefoxitin disk, with 100% CA for the FOX DD SOSA breakpoint and only 1 VME (1.7%) by the FX DD SAU breakpoint.

Evaluation of results for all species. No single MIC breakpoint performed well across the species of SOSA evaluated (Table 4). When evaluated against CLSI criteria of <3% MEs and <1.5% VMEs (18), only the FOX DD SOSA breakpoint had an acceptable VME rate, whereas the OX MIC SAU, FOX MIC SAU, FOX DD SOSA, and FOX DD SAU breakpoints had acceptable ME rates. While some error rates were near acceptance limits for an individual species, each method had at least one species for which performance was particularly poor: OX MIC SOSA with 19% MEs with *S. warneri*, OX MIC SAU with 19% VMEs for *S. haemolyticus*, OX DD SOSA with 80% MEs for *S. warneri*, FOX MIC SAU with 8% VMEs for *S. hominis*, FOX DD SOSA with 12% MEs for *S. hominis*, and FOX DD SAU with 5.1% VMEs for *S. haemolyticus* (Table 4). For each species, however, one or more methods could be considered acceptable (Table 4).

Currently, the CLSI recommends confirmation of oxacillin MICs in the 0.5- to 2.0-μg/ml range (resistant by the OX MIC SOSA breakpoints) by PBP2a or *mecA* detection methods. Historically, use of cefoxitin DD was also considered acceptable. We evaluated isolates with oxacillin VMEs or MEs for which the MIC values that generated the errors were in this range to determine if cefoxitin DD could resolve the error. For *S. hominis*, all 5 MEs were noted at a MIC of 0.5 μg/ml, and all cefoxitin disk zones for these isolates yielded zones of 22 to 23 mm (resistant by FOX DD SOSA but susceptible by FOX DD SAU). For *S. warneri* isolates with MEs, oxacillin MIC values were 0.5 μg/ml ($n = 15$) and 1 μg/ml ($n = 1$). For the 7 isolates that encompassed these 15 MEs, all disk results obtained were susceptible by the FOX SOSA DD breakpoint. Finally, 3 VMEs and 1 ME were due to *S. haemolyticus* isolates with oxacillin MIC values in the 0.5- to 2.0-μg/ml range. None of the 3 VMEs were resolved by cefoxitin DD, but the 1 ME was. As such, the strategy historically proposed by CLSI performed well for resolving errors for *S. warneri*, but not the other species evaluated herein.

New proposed breakpoint for oxacillin MIC and evaluation of disk correlates. The majority (21/22 [95.5%]) of OX SOSA MIC MEs in this study occurred due to an oxacillin MIC of 0.5 μg/ml (Fig. 1). VMEs for the OX SOSA MIC breakpoint were due to oxacillin MICs of 0.125 μg/ml ($n = 3$) and 0.25 μg/ml ($n = 3$) (Fig. 1). Adjusting the OX SOSA susceptible MIC breakpoint from ≤0.25 μg/ml to ≤0.5 μg/ml resulted in an overall reduction in MEs from 7.2% to 0.3% and an increase in VMEs from 2.1% to 2.8%, due to two additional VMEs for the same *S. haemolyticus* isolate (Fig. 1 and Table 4). The two species at this new breakpoint with unacceptable error rates were *S. hominis*, with 4.0% VMEs, and *S. haemolyticus*, with 6.4% VMEs. All three VMEs for *S. hominis* were for an isolate with an oxacillin MIC of 0.125 μg/ml by all three CA-MHB brands evaluated. This isolate also yielded 1 VME by FOX MIC SAU (not shown). For *S. haemolyticus*, two isolates yielded VMEs—one of these isolates yielded a VME by all breakpoints evaluated in this study but gave a positive PBP2a result (data not shown).

In order to determine if changing the oxacillin breakpoint would impact other species, data from prior studies were reevaluated, including evaluation of *S. epidermidis* ($n = 100$ isolates tested across three brands of media [note some isolates did not grow on some media, yielding 291 results]), *S. pseudintermedius* ($n = 111$ isolates), and *S. schleiferi* ($n = 90$ isolates). This resulted in 0% VMEs and only 3% MEs (Table 5). The ME was for an isolate of *S. epidermidis* with an oxacillin MIC value of 4 μg/ml but which was *mecA* negative. The overall MIC distribution is shown in Fig. 1. Combined, this new breakpoint resulted in 1.6% VMEs (8/490 *mecA*-positive results) and 0.9% MEs (5/562 *mecA*-negative results). In contrast, the 2019 CLSI OX MIC SOSA breakpoint yielded 1.2% VMEs (6/490) and 4.5% MEs (25/562). The biggest change was for *S. warneri*, where the ME rate went from 21.3% to 1.3% with this change to the breakpoint.

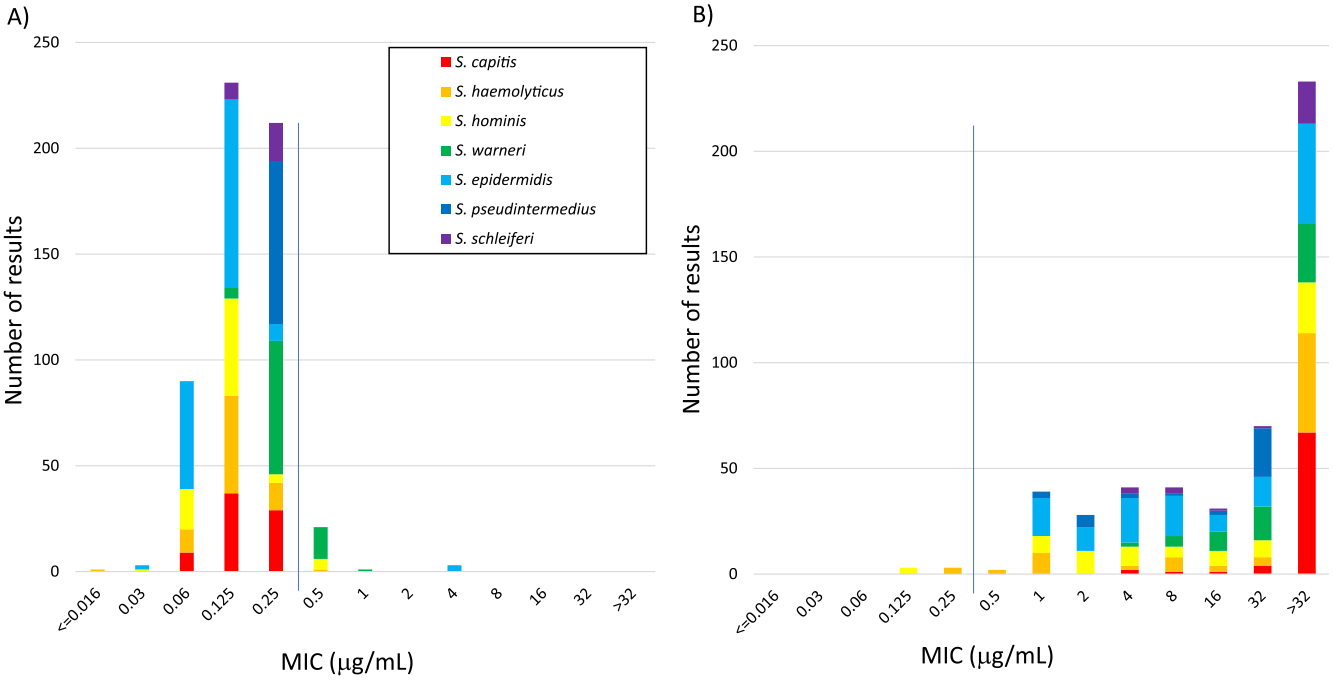

FIG 1 Oxacillin MIC distribution for staphylococci evaluated in this and prior studies (Table 5). Oxacillin MICs were determined in CA-MHB plus 2% NaCl. *mecA*-negative isolates are presented in panel A, and *mecA*-positive isolates are presented in panel B. The lower end of the MIC distribution for *S. pseudintermedius* was 0.25 μg/ml, and the upper end was 32 μg/ml. All other species were tested across the entire oxacillin concentration range shown. The vertical blue line represents the 2020 CLSI oxacillin breakpoint.

Disk diffusion breakpoints are traditionally set by correlating zones of growth inhibition to MICs, and this was attempted to see if an improved disk breakpoint could be established with the new oxacillin MIC breakpoint. Disk correlates were evaluated using the dBETS program, which indicated for cefoxitin a susceptible disk breakpoint of ≥25 mm was preferred, with a rate of 0.2% VMEs and 2.4% MEs. This is the same disk diffusion breakpoint currently endorsed by CLSI. Similarly, a susceptible breakpoint of ≥18 mm was calculated for the oxacillin disk, which was associated with a 0.5% VME rate and a 3.0% ME rate compared to the oxacillin MIC. This too is the same disk breakpoint currently endorsed by CLSI. Manual evaluation of the disk results compared to *mecA* PCR (as opposed to the oxacillin MIC, which is used by dBETs) demonstrated overlap between cefoxitin zones for *mecA*-positive and *mecA*-negative isolates of *S. haemolyticus* (at 27 and 28 mm; Fig. 2) and *S. hominis* (at 22 and 23 mm; Fig. 2). Overlap of oxacillin zones for *mecA*-positive and *mecA*-negative

TABLE 5 Comparison of *mecA* results with oxacillin MIC values, using the M100 S30 and M100 S31 breakpoints, for isolates in the present and prior studies

Species	No. of results for:		No. (%) of results for:				Reference
			Old breakpoint (M100 S30)		New breakpoint (M100 S31)		
	mecA-negative isolates	*mecA*-positive isolates	VMEs at ≤0.25 μg/ml	MEs at ≥ 0.5 μg/ml	VMEs at ≤0.5 μg/ml	MEs at ≥1.0 μg/ml	
S. capitis	75	75	0	0	0	0	This work
S. epidermidis	153	138	0	3 (2.0)	0	3 (2.0)	12
S. haemolyticus	72	78	3 (3.8)	1 (1.4)	5 (6.4)	1 (1.4)	This work
S. hominis	84	59	3 (5.0)	5 (6.0)	3 (5.0)	0	This work
S. pseudintermedius	77	37	0	0	0	0	10
S. schleiferi	26	28	0	0	0	0	11
S. warneri	75	75	0	16 (21.3)	0	1 (1.3)	This work
Overall	562	490	6 (1.2)	25 (4.4)	8 (1.6)	5 (0.89)	This work

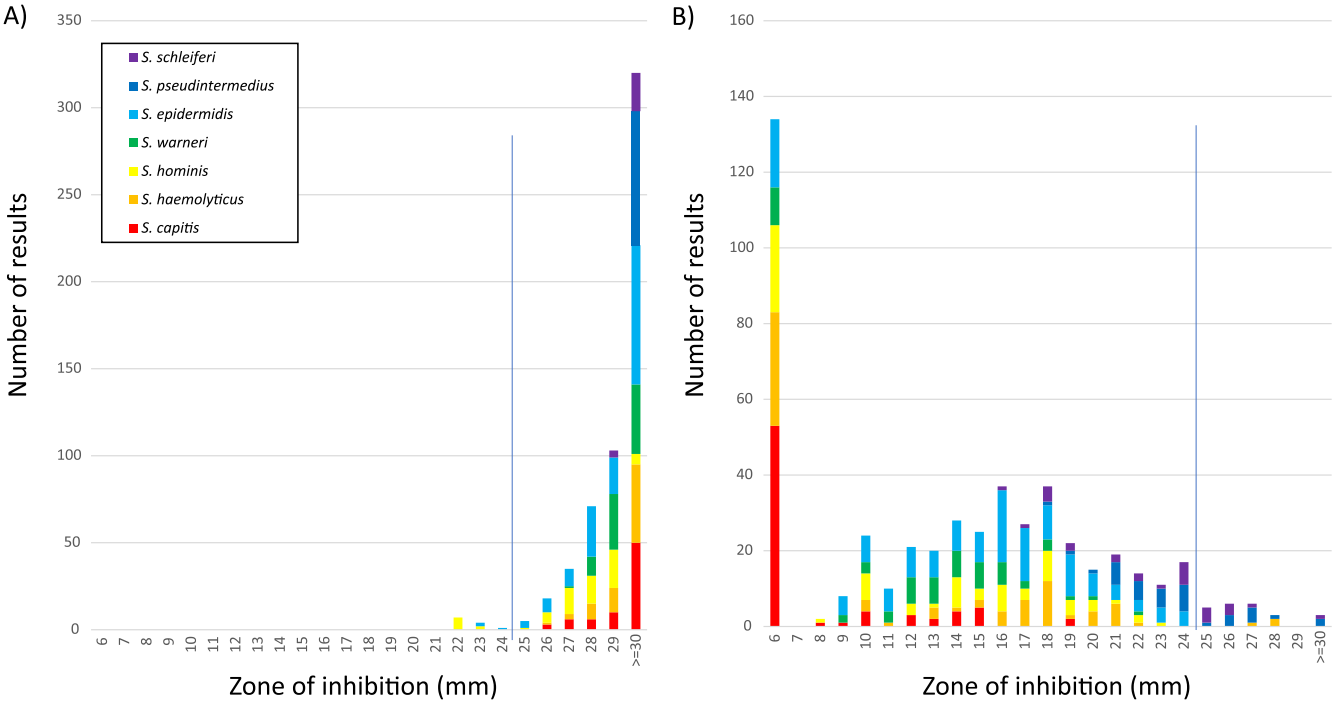

FIG 2 Cefoxitin DD distribution for staphylococci evaluated in this and prior studies (Table 5). *mecA*-negative isolates are presented in panel A, and *mecA*-positive isolates are presented in panel B. The vertical blue line represents the current CLSI FOX DD SOSA breakpoint.

isolates occurred for *S. haemolyticus* (at 20 mm), *S. warneri* (at 15 mm), and *S. hominis* (at 20 and 22 mm) (Fig. 3; Fig. S4).

Quality control results. All results were within QC ranges, as published in the CLSI M100 standard, 29th edition (15). *S. aureus* ATCC 25923 cefoxitin DD results ranged from 23 to 28 mm (mode, 25 mm). Oxacillin DDs ranged from 18 to 21 mm (mode,

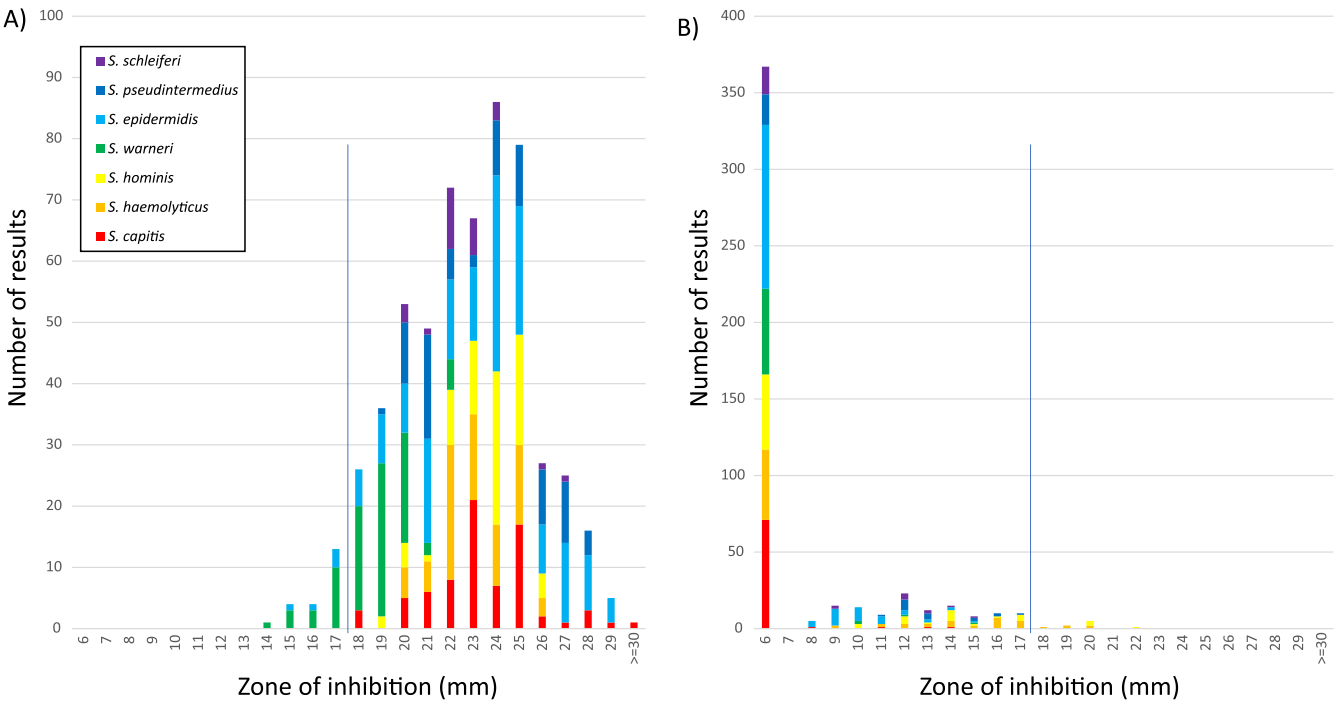

FIG 3 Oxacillin DD distribution for staphylococci evaluated in this and prior studies (Table 5). *mecA*-negative isolates are presented in panel A, and *mecA*-positive isolates are presented in panel B. The vertical blue line represents the current CLSI OX DD SOSA breakpoint.

19 mm). *S. aureus* ATCC 29213 oxacillin MIC values ranged from 0.125 to 0.5 μg/ml (mode, 0.25 μg/ml) and 2 to 4 μg/ml (mode, 4 μg/ml).

DISCUSSION

Ideally, one MIC and/or one DD method would be established for all staphylococcal species evaluated, enabling a more streamlined workflow for the clinical laboratory and for device manufactures. However, each method had at least one species for which performance was particularly poor. The CLSI AST Subcommittee agreed that implementation of species-specific breakpoints for SOSA may be unrealistic for laboratories, given the complexity of testing and the fact that not all laboratories can accurately perform identification of these isolates to the species level. While routine identification of SOSA to the species level may not be indicated in all cases (e.g., likely skin microbiota contaminant in a blood culture), we believe species identification should be attempted for susceptibility testing when isolates are deemed clinically relevant, such as repeated positive blood cultures or recovery from infected prostheses.

Adoption of a new breakpoint for oxacillin for these species improved performance, both at the individual species level and generally across all isolates. Based on these data, CLSI voted to approve a revision to the oxacillin MIC breakpoint for SOSA of susceptible at $\leq$ 0.5 μg/ml and resistant at $\geq$1.0 μg/ml. While this new breakpoint was still associated with errors, it provided the best correlation between *mecA* results across the species evaluated to date. Disk-to-MIC correlates were performed in the present study, which supported existing CLSI OX DD SOSA and FOX DD SOSA breakpoints. In particular, the FOX DD SOSA performed the best among DD methods, yielding rates of 1.0% VMEs and 2.9% MEs for the isolates studied herein. However, cefoxitin tests (both MIC and DD) yield unacceptably high rates of VMEs for *S. pseudintermedius* (10) and *S. schleiferi* (11) and should not be used for these species.

The original 2005 CLSI breakpoint study similarly found challenges with phenotypic detection of *mecA*-positive and -negative SOSA isolates. In that study, the 2019 SOSA OX MIC breakpoint was associated with 6.8% VMEs and 28.4% MEs. Applying the new 2021 oxacillin CLSI breakpoint for species other than *S. aureus* and *S. lugdunensis* to the 2005 data set increased the VME rate to 9.1%, and the ME rate was reduced to 15.1%. The increase in VMEs was due to 11 errors for *S. hominis* and 9 errors for *S. epidermidis*.

This is not the first time that a susceptible breakpoint of $\leq$0.5 μg/ml has been proposed for the SOSA and oxacillin. In 1995, McDonald and colleagues recommended a breakpoint of $\leq$0.5 μg/ml for oxacillin as the preferred method to detect *mecA* presence for their collection of 38 isolates of *S. epidermidis* (19). In this study, oxacillin testing was performed in the presence of 2% NaCl, as is the current CLSI recommendation. Similarly, in 1996, Cormican and colleagues suggested an oxacillin MIC of $\leq$0.5 μg/ml correlated best with the presence or absence of *mecA* for 55 isolates of SOSA isolated from blood cultures (20). Furthermore, a susceptible breakpoint of $\leq$0.5 μg/ml is closer to the EUCAST-calculated epidemiological cutoff values for the species evaluated, which are $\leq$0.5 μg/ml for *S. epidermidis* and *S. hominis* and $\leq$1.0 μg/ml for *S. haemolyticus*, *S. capitis*, and *S. warneri* (Gunnar Kahlmeter, personal communication to R.M.H.).

There are limitations to the use of oxacillin or cefoxitin testing as a surrogate for *mecA* presence among the SOSA, even when using the new oxacillin MIC breakpoint or confirmed oxacillin or cefoxitin DD breakpoints. Every breakpoint evaluated yielded unacceptable VME rates for *S. haemolyticus*, and most did as well for *S. hominis* (Table 4). In contrast, while not FDA cleared for use with SOSA, 100% concordance was observed between the Abbott PBP2a test and *mecA* PCR. Canver and colleagues similarly demonstrated this assay to perform well for non-*S.aureus* staphylococci, including *S. capitis*, *S. caprae*, *S. epidermidis*, *S. haemolyticus*, *S. hominis*, *S. lugdunensis*, *S. pseudintermedius*, *S. schleiferi*, *S. simulans*, and *S. warneri* (21). Adaptation of this method for use with these species may be the preferred method for testing.

Limitations of the present study include use of only one brand of disk and testing performed in a single, well-controlled laboratory. Additionally, no isolates harbored

mecC, and so performance of phenotypic tests for isolates with that resistance factor was not evaluated. It should be noted that the incidence of *mecC* among SOSA is exceedingly low, with occasional reports of individual isolates of *Staphylococcus caprae* (22), *Staphylococcus xylosus* (6, 23), *Staphylococcus saprophyticus* (24, 25), *Staphylococcus stepanovicii*, *Staphylococcus edaphicus* (3), and *S. warneri* (22) harboring the gene. Several isolates of *Staphylococcus sciuri* have been reported to harbor *mecC* (22). Most laboratories perform MIC susceptibility testing using automated systems, and the breakpoints proposed herein may not correlate with *mecA* results for these systems. However, strengths of the present study include evaluation of a diverse collection of contemporary isolates from multiple geographic regions.

In conclusion, laboratories should consider performing *mecA* PCR or a PBP2a test if a penicillinase-stable penicillin, such as oxacillin, is being considered for therapy for serious infections due to SOSA.

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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