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Evaluation of Oxacillin and Cefoxitin Disk Diffusion and MIC Breakpoints Established by the Clinical and Laboratory Standards Institute for Detection of *mecA*-Mediated Oxacillin Resistance in *Staphylococcus schleiferi*

H. K. Huse,[a] S. A. Miller,[a] S. Chandrasekaran,[a] J. A. Hindler,[a] S. D. Lawhon,[b] D. A. Bemis,[c] L. F. Westblade,[d] R. M. Humphries,[a]* on behalf of the *Staphylococcus Ad Hoc* Working Group of the CLSI Antimicrobial Susceptibility Testing Subcommittee

[a]UCLA David Geffen School of Medicine, Los Angeles, California, USA
[b]Texas A&M University College of Veterinary Medicine, College Station, Texas, USA
[c]University of Tennessee College of Veterinary Medicine, Knoxville, Tennessee, USA
[d]Weill Cornell Medicine, New York, New York, USA

ABSTRACT *Staphylococcus schleiferi* is a beta-hemolytic, coagulase-variable colonizer of small animals that can cause opportunistic infections in humans. In veterinary isolates, the rate of *mecA*-mediated oxacillin resistance is significant, with reported resistance rates of >39%. The goal of this study was to evaluate oxacillin and cefoxitin disk diffusion (DD) and MIC breakpoints for detection of *mecA*-mediated oxacillin resistance in 52 human and 38 veterinary isolates of *S. schleiferi*. Isolates were tested on multiple brands of commercial media and according to Clinical and Laboratory Standards Institute (CLSI) methods. Zone diameters and MIC values were interpreted using CLSI breakpoints (CLSI, *Performance Standards for Antimicrobial Susceptibility Testing. M100-S27*, 2017) for *Staphylococcus aureus*/*Staphylococcus lugdunensis*, coagulase-negative staphylococci (CoNS), and *Staphylococcus pseudintermedius*. Results were compared to those of *mecA* PCR. Twenty-nine of 90 (32%) isolates were *mecA* positive. Oxacillin inhibition zone sizes and MICs interpreted by *S. pseudintermedius* breakpoints reliably differentiated *mecA*-positive and *mecA*-negative isolates, with a categorical agreement (CA) of 100% and no very major errors (VMEs) or major errors (MEs) for all media. For cefoxitin DD results interpreted using *S. aureus*/*S. lugdunensis* and CoNS breakpoints, CA values were 85% and 75%, respectively, and there were 72% and 64% VMEs, respectively, and 0 MEs. For cefoxitin MICs interpreted using *S. aureus/S. lugdunensis* breakpoints, CA was 81%, and there were 60% VMEs and no MEs. Our data demonstrate that oxacillin DD or MIC testing methods using the current *S. pseudintermedius* breakpoints reliably identify *mecA*-mediated oxacillin resistance in *S. schleiferi*, while cefoxitin DD and MIC testing methods perform poorly.

KEYWORDS breakpoints, cefoxitin, *mecA*, oxacillin, PBP2a, *Staphylococcus schleiferi*

Staphylococcus schleiferi is an emerging zoonotic pathogen that colonizes the skin and mucosal surfaces of small animals (1–3). Isolates form medium to large, nonpigmented, beta-hemolytic colonies on 5% sheep blood agar (4, 5). The species is further divided into *S. schleiferi* subsp. *schleiferi* and *S. schleiferi* subsp. *coagulans*. *Staphylococcus schleiferi* subsp. *schleiferi* was first isolated from human specimens, in 1988 (4), while *S. schleiferi* subsp. *coagulans* was first isolated from dogs with otitis externa, in 1990 (5). The subspecies have high levels of DNA homology but differ in

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Address correspondence to R. M. Humphries, rhumphries@axdx.com.

* Present address: R. M. Humphries, Accelerate Diagnostics, Inc., Tucson, Arizona, USA.

several phenotypic characteristics, including clumping factor, tube coagulase, and urease production. *S. schleiferi* subsp. *schleiferi* is clumping factor positive, tube coagulase negative, and urease negative, while *S. schleiferi* subsp. *coagulans* is clumping factor negative, tube coagulase positive, and urease positive (4, 5). Matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) can reliably identify *S. schleiferi* to the species level, but further biochemical testing is needed to differentiate the two subspecies (6).

In small animals, *S. schleiferi* most frequently colonizes the skin, nares, ears, and rectum of dogs, where it can cause inflammatory skin disease, otitis externa, and otitis media (2, 3, 5, 7–14). Though found at a lower prevalence than that in dogs, *S. schleiferi* has also been isolated from healthy cats (1), cats with inflammatory skin disease (1), and parrots (2). Oxacillin resistance rates vary but can be high, with some studies reporting oxacillin resistance for 39 to 73% of veterinary isolates (7–9, 15, 16). Staphylococcal cassette chromosome *mec* type IV (SCC*mec* IV) has been identified in *S. schleiferi* subsp. *coagulans* (16). *S. schleiferi* has also been reported to carry SCC*mec* types I and IV (17, 18). In addition to oxacillin resistance, reduced susceptibility to clindamycin, erythromycin, and fluoroquinolones has been reported (13, 19, 20). In a small study, *S. schleiferi* isolates were negative for beta-lactamase production (21).

Though it is primarily a veterinary pathogen, *S. schleiferi* can also cause opportunistic infections in humans. Cases of endophthalmitis (22), endocarditis (23, 24), bacteremia (25), osteomyelitis (26), and wound (27), surgical site (27), and pacemaker (28) infections have been reported. *S. schleiferi* was implicated in an outbreak of surgical site infections and was originally misidentified as *Staphylococcus aureus* (29). Interestingly, while both subspecies can cause infection, *S. schleiferi* subsp. *schleiferi* is more prevalent in causing human infections (27, 29).

S. schleiferi has been reported to give false-positive results in latex agglutination tests for *S. aureus* identification, at rates of 25 to 75% (30). Use of MALDI-TOF MS will likely increase the number of *S. schleiferi* isolates identified, and the high oxacillin resistance rates in this species are concerning. Many clinical laboratories use cefoxitin disk diffusion (DD) testing for detection of oxacillin resistance in staphylococci other than *S. pseudintermedius*, as outlined by the Clinical and Laboratory Standards Institute (CLSI) M100-S27 document (31, 32). Previous data supporting the use of cefoxitin as a surrogate agent for detection of oxacillin resistance in coagulase-negative staphylococci (CoNS) were largely derived from *Staphylococcus epidermidis* isolates (33). However, the most accurate methods for detecting *mecA*-mediated oxacillin resistance in other CoNS species have yet to be determined. Some studies have shown that cefoxitin disk testing has low sensitivity for detecting oxacillin resistance in *S. schleiferi* veterinary isolates and *S. pseudintermedius* (15, 33, 34).

The goal of the present study was to evaluate oxacillin and cefoxitin DD and broth microdilution (BMD) MIC testing for detection of *mecA*-mediated oxacillin resistance in 52 human and 38 veterinary isolates of *S. schleiferi*. Oxacillin-resistant staphylococci are resistant to all beta-lactam antibiotics except for new anti-methicillin-resistant *S. aureus* (anti-MRSA) cephalosporins. We demonstrate that oxacillin DD results interpreted by the CLSI M100-S27 breakpoints for *S. pseudintermedius* reliably detect *mecA*-positive and *mecA*-negative *S. schleiferi* isolates, while cefoxitin is an unreliable surrogate agent.

(The results of this study were presented to the CLSI Antimicrobial Susceptibility Testing Subcommittee in June 2017, leading to the addition of specific breakpoints for oxacillin disk diffusion and MIC testing of *S. schleiferi* for the forthcoming 28th edition of the M100 document.)

MATERIALS AND METHODS

Specimens. A total of 90 *S. schleiferi* isolates were included in this study (Table 1). Human isolates ($n = 52$) were submitted by Becton Dickinson and Company (BD) ($n = 13$), JMI Laboratories ($n = 22$), and Weill Cornell Medicine ($n = 17$). Canine and other small animal isolates ($n = 38$) were obtained from the Texas A&M University College of Veterinary Medicine ($n = 12$), the University of Tennessee College of Veterinary Medicine ($n = 25$), and Weill Cornell Medicine ($n = 1$). Isolates were identified to the species or subspecies level at each institution, using the corresponding standard operating procedures. Sub-

TABLE 1 Summary of study isolates submitted by various institutions

Institute	No. of isolates	Source	Specimen source(s)	No. of isolates	
				S. schleiferi subsp. *schleiferi*	*S. schleiferi* subsp. *coagulans*
BD	13	Human	Unknown	13	0
JMI Laboratories	22	Human	Unknown	9	13
Weill Cornell Medicine	17	Human	Blood, catheter tip, scalp, skin, urine, unknown, wound	11	6
Texas A&M	12	Canine	Ear, lung, urine	0	12
University of Tennessee	25	Canine	Bone, ear, skin	0	25
Weill Cornell Medicine	1	Small animal	Ear	0	1
Total	90			33	57

species identifications were confirmed by urease testing at the Ronald Reagan UCLA Medical Center, Los Angeles, CA. Urease testing on urea agar slants (BD) was performed according to the manufacturer's protocol, with the slants incubated at 35°C in ambient air for 24 h.

***mecA* PCR.** Fifty-four isolates with known *mecA* genotypes were submitted. For the remaining isolates, colony PCR was performed on 18- to 24-h isolates grown on 5% sheep blood agar plates (SBAP). The following primers were used to amplify a 533-bp product from the *mecA* gene: 5′-AAAATCGATGGTAA AGGTTGGC-3′ and 5′-AGTTCTGCAGTACCGGATTTGC-3′ (35). A pipette tip was used to transfer a pinpoint amount of an isolated colony to a 25-μl PCR mixture containing 12.5 μl AmpliTaq Gold 360 master mix (Applied Biosystems, Thermo Fisher Scientific, Waltham, MA), 0.5 μM (each) *mecA* primers, and 11.25 μl water. Reaction mixtures were incubated at 95°C for 10 min, followed by 35 cycles of 95°C for 15 s, 55°C for 30 s, and 72°C for 30 s. The final extension was performed at 72°C for 7 min. As a positive control, colony PCR was performed on *S. aureus* ATCC 43300, a *mecA*-positive strain, and 1 μl purified genomic DNA from the same strain. PCR products were visualized on a 4% precast agarose gel (Lonza, Basel, Switzerland).

Antimicrobial susceptibility testing (AST). Isolates were stored as described previously (34) and subcultured twice on 5% SBAP before testing. DD and BMD tests were performed as described by the CLSI (31, 36). DD was evaluated on Mueller-Hinton agar (MHA) plates obtained from 3 vendors: Remel (Lenexa, KS), Hardy Diagnostics (Santa Maria, CA), and BD. Disks containing 1 μg oxacillin and 30 μg cefoxitin (BBL, BD) were used. BMD was performed by the CLSI reference method, using frozen-form panels containing cation-adjusted Mueller-Hinton broth (CA-MHB) with cefoxitin (unsupplemented) or oxacillin supplemented with 2% NaCl. BMD panels were made by Thermo Fisher. MIC tests using CA-MHB from 3 different manufacturers (Difco, BD, and Oxoid) were evaluated on a single panel. Oxacillin and cefoxitin were tested in 2-fold dilutions at concentrations ranging from 0.015 μg/ml to 32 μg/ml. Isolated colonies grown overnight on SBAP at 35 to 37°C in ambient air were resuspended in 0.85% saline to obtain a 0.5 McFarland standard. The suspensions were used to inoculate all DD and MIC plates per CLSI recommendations (31, 36). DD test plates were incubated at 35°C in ambient air, and zones of inhibition were measured at 16 to 18 h for oxacillin and 24 h for cefoxitin. During preliminary studies, zones of inhibition were read with both transmitted and reflected light, and there was no difference noted between the two methods. Therefore, for the final study, both oxacillin and cefoxitin zones of inhibition were read using reflected light. BMD test plates were incubated at 35°C in ambient air and read at 16 to 20 h for cefoxitin and 24 h for oxacillin. *S. aureus* ATCC 25923 and *S. aureus* ATCC 29213 were used as quality control strains for DD and BMD tests, respectively.

Data analysis. Zone diameters and MIC values were interpreted using breakpoints for the following organisms, obtained from the CLSI M100-S27 document: (i) *S. aureus*/*S. lugdunensis*; (ii) CoNS, excluding *S. lugdunensis* and *S. pseudintermedius*; and/or (iii) *S. pseudintermedius* (Table 2) (32). Results were

TABLE 2 Breakpoints used for prediction of *S. schleiferi mecA*-mediated oxacillin resistance in this study[a]

Organism	Oxacillin breakpoint				Cefoxitin breakpoint			
	DD inhibition zone (mm)		MIC (μg/ml)		DD inhibition zone (mm)		MIC (μg/ml)	
	S	R	S	R	S	R	S	R
S. aureus/*S. lugdunensis*	NA	NA	≤2	≥4	≥22	≤21	≤4	≥8
Coagulase-negative staphylococci, except *S. lugdunensis* and *S. pseudintermedius*	NA	NA	≤0.25	≥0.5	≥25	≤24	NA	NA
S. pseudintermedius[b]	≥18	≤17	≤0.25	≥0.5	NA	NA	NA	NA

[a]From the CLSI M100-S27 document (32). NA, not applicable; S, susceptible; R, resistant.

[b]In the 28th edition of the CLSI M100 document, the guidance for *S. pseudintermedius* will also apply to *S. schleiferi*.

compared to the results of *mecA* PCR, which was considered the gold standard for oxacillin resistance. Categorical agreement (CA), very major errors (VMEs), and major errors (MEs) were calculated as previously described (37). CA was determined using *mecA* PCR results as the reference to define isolates as resistant or susceptible. VMEs were counted as identifications of isolates that were *mecA* positive but oxacillin or cefoxitin susceptible. MEs were defined as identifications of isolates that were *mecA* negative but oxacillin or cefoxitin resistant.

Discrepancy analysis. For any isolates with oxacillin results that were discordant with *mecA* PCR results, testing was repeated by both AST and PCR. If the error resolved, it was excluded as an error.

PBP2a testing. Penicillin binding protein 2a (PBP2a) testing was performed on 54 *S. schleiferi* isolates by use of Alere PBP2a SA culture colony test kits (Alere Inc., Scarborough, ME) and Oxoid PBP2′ latex agglutination test kits (Thermo Fisher Scientific, Waltham, MA). Only 54 isolates were tested due to a limited number of available testing kits. Isolates were chosen based on *mecA* PCR results to give an almost even distribution of *mecA*-positive and *mecA*-negative isolates (28 and 26, respectively). Forty-three isolates were *S. schleiferi* subsp. *coagulans*, and 11 were *S. schleiferi* subsp. *schleiferi*. Thirty-eight isolates were from animals (all *S. schleiferi* subsp. *coagulans*), and 16 were from humans (5 *S. schleiferi* subsp. *coagulans* isolates and 11 *S. schleiferi* subsp. *schleiferi* isolates). Colonies from SBAP used for DD and BMD inoculum preparation were tested for noninduced PBP2a expression according to the manufacturers' instructions for *S. aureus*. *S. aureus* ATCC 43300 and *S. aureus* 25923 were used as positive and negative controls, respectively.

RESULTS

Isolates. Ninety isolates were tested in this study (Table 1). Fifty-two (58%) were isolated from human specimens (blood, wounds, urine, ears, catheters, skin, and the scalp). Thirty-eight (42%) isolates were urine, bone, lung, pyoderma (of the ear), or skin isolates from canines or other small animals. Thirty-three (37%) isolates were identified as *S. schleiferi* subsp. *schleiferi*, all of which were isolated from human specimens. Fifty-seven (63%) isolates were identified as *S. schleiferi* subsp. *coagulans*; 19 (33%) of these were isolated from human specimens, and 38 (67%) were isolated from animals.

***mecA* PCR.** Twenty-nine (32%) isolates were *mecA* positive. All of the *mecA*-positive isolates were *S. schleiferi* subsp. *coagulans*, and four of these were isolated from human specimens. Sixty-one (68%) isolates were *mecA* negative. Of these, 33 (54%) were *S. schleiferi* subsp. *schleiferi* and 28 (46%) were *S. schleiferi* subsp. *coagulans*.

Cefoxitin DD and BMD testing. Results from the cefoxitin DD and BMD tests are summarized in Table 3 and Fig. 1. For cefoxitin tests, neither the zones of inhibition nor the MICs showed clear divisions between *mecA*-positive and *mecA*-negative isolates for any medium (either MHA or CA-MHB) brand tested (Fig. 1; see Fig. S1 and S2 in the supplemental material). For DD testing, 17 isolates showed only faint growth on Remel MHA medium (Fig. 2), and thus zones of inhibition could not be measured. Therefore, a total of 253 data points were collected for all media (Fig. 1A).

DD and MIC results were interpreted using the cefoxitin breakpoints listed in Table 2. On applying the CLSI M100-S27 *S. aureus*/*S. lugdunensis* breakpoints for DD testing using cefoxitin, the CA values for BD, Hardy, and Remel media were 78%, 76%, and 71%, respectively. For cefoxitin, there were 20/29 (69%), 22/29 (76%), and 21/29 (72%) VMEs for the BD, Hardy, and Remel media, respectively. There were no MEs because all *mecA*-negative isolates were susceptible by DD testing on all media tested, with zone sizes ranging from 29 to 45 mm (Fig. 1). For BMD, all brands performed similarly with cefoxitin. The CA values and numbers of VMEs for BD, Difco, and Remel CA-MHB were 81% and 12/29 (41%), 81% and 12/29 (41%), and 80% and 11/29 (38%), respectively. There were no MEs for any of the CA-MHB medium brands tested. On applying the CoNS breakpoints for DD testing using cefoxitin, the CA value and percentage of VMEs were 81% and 59% for BD medium, 77% and 72% for Hardy medium, and 75% and 62% for Remel medium (Table 3). There were no MEs with application of the CoNS breakpoints.

Overall, for cefoxitin DD results interpreted using *S. aureus*/*S. lugdunensis* and CoNS breakpoints, CA was 85% and 75%, respectively, and there were 63/87 (72%) and 56/87 (64%) VMEs, respectively. Cefoxitin MICs interpreted using *S. aureus/S. lugdunensis* breakpoints yielded an overall CA of 81% and 52/87 (60%) VMEs. There were no MEs for either DD or MIC results interpreted using any breakpoints.

Oxacillin DD and BMD testing. Results from the oxacillin DD and BMD tests are summarized in Table 4 and Fig. 1. In contrast to the cefoxitin results, there was a clear

TABLE 3 Performances of cefoxitin DD and BMD testing for detection of *mecA*-mediated oxacillin resistance in *S. schleiferi*

Breakpoints	Summary			BD medium			Hardy medium			Remel medium			Difco CA-MHB			BD CA-MHB			Oxoid CA-MHB		
	CA (%)	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)		CA (%)[a]	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)	
		VMEs	MEs		VMEs	MEs		VMEs	MEs		VMEs	MEs[a]		VMEs	MEs		VMEs	MEs		VMEs	MEs
CLSI M100-S27 DD breakpoints																					
S. aureus/S. lugdunensis	85	63/87 (72)	0/166 (0)	78	20/29 (69)	0/61 (0)	76	22/29 (76)	0/61 (0)	71	21/29 (72)	0/44 (0)									
CoNS staphylococci (except for *S. lugdunensis* and *S. pseudintermedius*)	75	56/87 (64)	0/166 (0)	81	17/29 (59)	0/61 (0)	77	21/29 (72)	0/61 (0)	75	18/29 (62)	0/44 (0)									
CLSI M100-S27 MIC breakpoints																					
S. aureus/S. lugdunensis	81	52/87 (60)	0/183 (0)										81	12/29 (41)	0/61 (0)	81	12/29 (41)	0/61 (0)	80	11/29 (38)	0/61 (0)

[a]We were unable to read zones for 17 *mecA*-negative isolates due to poor growth; these were not included in the denominator.

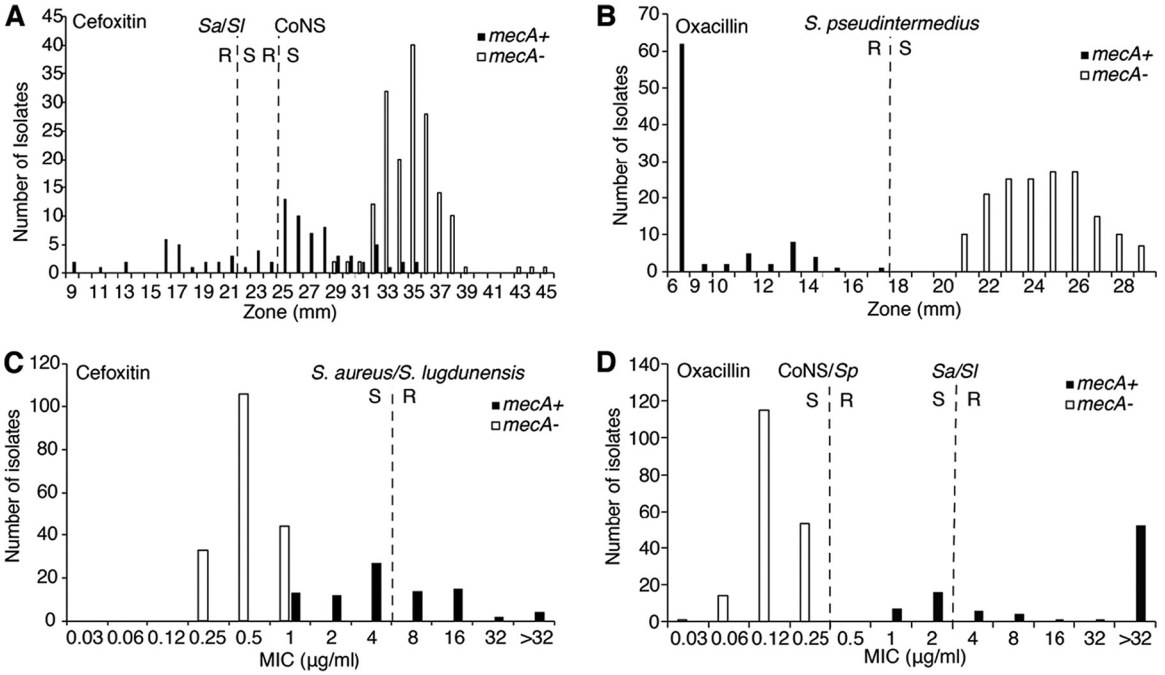

FIG 1 Distributions of cefoxitin and oxacillin growth inhibition zone diameters and MICs as determined by DD and BMD testing on all media tested. M100-S27 DD and MIC breakpoints are shown for *S. aureus*/*S. lugdunensis* (*Sa*/*Sl*), coagulase-negative staphylococci (excluding *S. lugdunensis* and *S. pseudintermedius*) (CoNS), and *S. pseudintermedius*. R, resistant; S, susceptible. (A) Cefoxitin DD testing (for BD, Hardy, and Remel media, $n = 253$). (B) Oxacillin DD testing (for BD, Hardy, and Remel media, $n = 253$). (C) Cefoxitin MICs (for Difco, BD, and Oxoid media, $n = 270$). (D) Oxacillin MICs (for Difco, BD, and Oxoid media, $n = 270$).

division between *mecA*-positive and *mecA*-negative isolates for both zones of inhibition and MICs for all medium brands tested (Fig. 1; Fig. S3 and S4). On applying the *S. pseudintermedius* breakpoints for DD testing, CA was 100% for all medium brands. There were no VMEs or MEs. On Remel medium, 16 isolates did not grow well enough for reading of the oxacillin DD zones, so the denominator (*n*) was 45.

For oxacillin BMD, CA values with the *S. aureus*/*S. lugdunensis* breakpoints were 98%, 92%, and 89% for Difco, BD, and Oxoid CA-MHB, respectively. The numbers of VMEs for Difco, BD, and Oxoid CA-MHB were 6/29 (21%), 7/29 (24%), and 10/29 (35%), respectively. There were no MEs for the three medium brands tested. The oxacillin MIC breakpoints for CoNS and *S. pseudintermedius* are the same. CA was 100%, and there were no VMEs or MEs for the three medium brands.

Overall, for oxacillin DD results interpreted using the *S. pseudintermedius* breakpoints, overall CA was 100%, and there were no VMEs or MEs. Oxacillin MICs interpreted using the

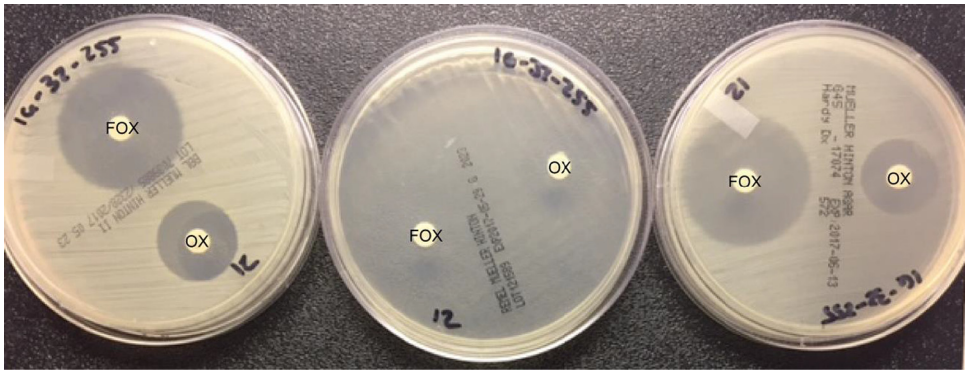

FIG 2 Growth of a single strain of *S. schleiferi* on BD (left), Remel (middle), and Hardy (right) MHA plates with oxacillin (OX) and cefoxitin (FOX) disks.

TABLE 4 Performances of oxacillin DD and BMD testing for detection of *mecA*-mediated oxacillin resistance in *S. schleiferi*

Breakpoints	Summary			BD medium			Hardy medium			Remel medium			Difco CA-MHB			BD CA-MHB			Oxoid CA-MHB		
	CA (%)[a]	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)		CA (%)[a]	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)		CA (%)	No. of errors/no. of isolates (%)	
		VMEs	MEs[a]		VMEs	MEs		VMEs	MEs		VMEs	MEs[a]		VMEs	MEs		VMEs	MEs		VMEs	MEs
CLSI M100-S27 DD breakpoints																					
S. pseudintermedius	100	0/87 (0)	0/167 (0)	100	0/29 (0)	0/61 (0)	100	0/29 (0)	0/61 (0)	100	0/29 (0)	0/45 (0)									
CLSI M100-S27 MIC breakpoints																					
S. aureus/S. lugdunensis	91	23/87 (26)	0/183 (0)										98	6/29 (21)	0/61 (0)	92	7/29 (24)	0/61 (0)	89	10/29 (35)	0/61 (0)
Coagulase-negative staphylococci (except for *S. lugdunensis* and *S. pseudintermedius*)	100	0/87 (0)	0/183 (0)										100	0/29 (0)	0/61 (0)	100	0/29 (0)	0/61 (0)	100	0/29 (0)	0/61 (0)
S. pseudintermedius[b]	100	0/87 (0)	0/183 (0)										100	0/29 (0)	0/61 (0)	100	0/29 (0)	0/61 (0)	100	0/29 (0)	0/61 (0)

[a]We were unable to read zones for 16 *mecA*-negative isolates; these were not included in the denominator.

[b]In the 28th edition of the CLSI M100 document, the guidance for *S. pseudintermedius* will also apply to *S. schleiferi*.

S. aureus/*S. lugdunensis* breakpoints yielded an overall CA of 91%, and there were 23/87 (26%) isolates with VMEs and no MEs. Overall CA for oxacillin MICs interpreted using CoNS and *S. pseudintermedius* breakpoints was 100%, with no VMEs or MEs.

Discrepant analysis. One isolate tested *mecA* PCR negative but was PBP2a positive and oxacillin resistant by DD and BMD testing. When all tests were repeated, the *mecA* PCR was positive, so this error was excluded from our analyses.

PBP2a testing. Compared to *mecA* PCR as the gold standard, PBP2a results showed 100% CA for all 54 *S. schleiferi* isolates tested, using both Alere PBP2a SA culture colony test kits and Oxoid PBP2′ latex agglutination test kits.

DISCUSSION

The widespread adoption of MALDI-TOF MS for bacterial identification has allowed clinical laboratories to better identify staphylococci to the species level (6, 38). Rapid adoption of this technology, combined with an increasing immunocompromised population and close contact between humans and companion animals, has increased the number and diversity of clinically significant CoNS isolates identified (39). This increase leads to the issue of determining which methods are best for detecting *mecA*-mediated oxacillin resistance in CoNS, which has not been critically evaluated for many species.

Here we present oxacillin and cefoxitin DD and BMD data for detection of *mecA*-mediated oxacillin resistance in 90 human and veterinary isolates of *S. schleiferi*. PCR for *mecA* was used to define oxacillin resistance. The correlation between oxacillin resistance and *mecA* detection has previously been reported to be 93 to 95% (12, 16). In previous studies, the mechanism of oxacillin resistance in *mecA*-negative isolates was not determined (12, 16).

While a similar study has been performed on veterinary isolates (15), our study included 52 human isolates, MIC testing, MHA manufactured by BD, Hardy, and Remel, and CA-MHB manufactured by Difco, BD, and Oxoid. The 3 brands of CA-MHB performed similarly for both oxacillin and cefoxitin MIC testing (Tables 3 and 4). Some isolates did not grow satisfactorily on Remel MHA to produce a readable zone of inhibition (Fig. 2). However, for those isolates that grew well, major differences in performance between brands were not noted (Fig. 1; Tables 3 and 4). Nonetheless, laboratories should be cognizant of medium-to-medium variability for commercial MHAs.

For DD testing of staphylococci other than *S. pseudintermedius*, the 2017 guidance for the CLSI reference method uses cefoxitin as a surrogate agent for detecting *mecA*-mediated oxacillin resistance (31, 32). Our data show that cefoxitin DD testing does not accurately predict the presence of *mecA* in *S. schleiferi* by use of the M100-S27 breakpoints for CoNS. Although cefoxitin DD testing accurately identified *mecA*-negative isolates, VME rates were unacceptably high for *S. schleiferi* on all media. Our findings are similar to those of previous studies that have shown species-dependent results for cefoxitin disk diffusion testing of CoNS. Compared to PBP2a testing results, the overall sensitivity of cefoxitin DD testing was only 25% for 150 isolates of *S. intermedius* and *S. schleiferi* (15). In a study of 170 isolates of *mecA*-positive CoNS, cefoxitin DD testing failed to identify five *mecA*-positive isolates of *S. simulans* (33). For *S. saprophyticus*, cefoxitin DD testing was 100% sensitive but only 56% specific compared to *mecA* PCR (40). Finally, the VME rate for cefoxitin DD testing for *S. pseudintermedius* was 29.7% compared to the *mecA* PCR results (34). The findings from our study may be problematic for laboratories that identify CoNS by use of phenotypic methods alone because *S. schleiferi* can be misidentified as *S. aureus*, leading to erroneous oxacillin susceptibility results.

As a result of this study, along with the data demonstrated previously for *S. pseudintermedius* (28), the CLSI recently removed cefoxitin DD testing as an option for confirming that non-*S. epidermidis* CoNS isolates from serious infections with oxacillin MICs in the 0.5- to 2.0-μg/ml range are truly oxacillin resistant. Laboratories should confirm susceptibility for such isolates by *mecA* or PBP2a tests. At present, the Alere PBP2a SA culture colony test is FDA cleared for PBP2a testing in *S. aureus*, while the Oxoid PBP2′ latex agglutination test kit is FDA cleared for testing PBP2a in *S. aureus* and

induced CoNS. In this study, PBP2a testing was 100% sensitive and 100% specific for identifying *mecA*-positive and *mecA*-negative isolates when colonies were tested without induction. For *S. schleiferi*, the Oxoid PBP2′ latex agglutination test kit has shown 85 to 100% CA between PBP2a expression and oxacillin resistance (3, 15), while the Alere PBP2a SA culture colony test has shown 100% CA (41). Additionally, the CLSI *Staphylococcus Ad Hoc* Working Group will continue to systematically evaluate the performance of current recommended phenotypic testing options for predicting *mecA*-mediated oxacillin resistance in the genus *Staphylococcus*.

Oxacillin DD and BMD testing performed most reliably in detecting *mecA*-mediated oxacillin resistance in *S. schleiferi*. Our findings are similar to those of Bemis et al. (15), who tested 43 *S. schleiferi* canine isolates for PBP2a and correlated the results with those of oxacillin and cefoxitin DD testing. Using breakpoints for animal isolates, they found 100% CA between PBP2a testing and oxacillin DD testing for both subspecies but only 0% and 46% CA between PBP2a testing and cefoxitin DD testing for *S. schleiferi* subsp. *schleiferi* and *S. schleiferi* subsp. *coagulans*, respectively (15). Additionally, Wu et al. (34) found that oxacillin DD testing performed better than cefoxitin DD testing for detecting *mecA* in *S. pseudintermedius*, a common colonizer of dogs and cats. One limitation to our study is that all 29 *mecA*-positive isolates were *S. schleiferi* subsp. *coagulans* because we were unable to obtain *mecA*-positive *S. schleiferi* subsp. *schleiferi* isolates. There may be differences in *mecA*-positive *S. schleiferi* subsp. *schleiferi* DD and BMD testing that could not be examined in this study.

In summary, we have shown that oxacillin DD and BMD testing methods using the current *S. pseudintermedius* breakpoints accurately identify *mecA*-mediated oxacillin resistance in *S. schleiferi*. Cefoxitin DD and BMD were unreliable for identifying oxacillin resistance due to the large number of false-susceptible results observed. The results from this study were presented to the CLSI Antimicrobial Susceptibility Testing Subcommittee in June 2017, leading to specific breakpoints for oxacillin DD and MIC testing of *S. schleiferi*.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/JCM.01653-17.

SUPPLEMENTAL FILE 1, PDF file, 2.0 MB.

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