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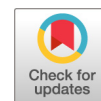
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Evaluation of Oxacillin and Cefoxitin Disk Diffusion and Microbroth Dilution Methods for Detecting *mecA*-Mediated β -Lactam Resistance in Contemporary *Staphylococcus epidermidis* Isolates

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ABSTRACT Methicillin (β -lactam) resistance in *Staphylococcus epidermidis* is mediated by the *mecA* gene, with resistance reported to be as high as 90%. The goal of this study was to evaluate oxacillin and cefoxitin disk diffusion (DD) and broth microdilution (BMD) methods for the detection of *mecA*-mediated β -lactam resistance in 100 human isolates of *S. epidermidis* (48 *mecA*-positive isolates and 52 *mecA* negative isolates). Oxacillin DD tests using the Clinical and Laboratory Standards Institute (CLSI) M100-S28 breakpoints for *S. pseudintermedius*/*S. schleiferi* accurately differentiated *mecA*-positive and -negative *S. epidermidis* isolates, with categorical agreement (CA) of 100% and no very major errors (VMEs) or major errors (MEs) identified. Likewise, oxacillin BMD and cefoxitin DD tests using the coagulase-negative *Staphylococcus* species (CoNS) breakpoints were highly reliable for detecting *mecA*-mediated β -lactam resistance in *S. epidermidis* isolates. For cefoxitin DD and BMD results interpreted using *S. aureus*/*S. lugdunensis* breakpoints, the CA was 97.6% and 96.2%, respectively. There were 4.9% VMEs for cefoxitin DD with 0% MEs, and 3.6% VMEs and 3.9% MEs for cefoxitin BMD. Oxacillin BMD using *S. aureus*/*S. lugdunensis* breakpoints yielded the highest VMEs at 17.4% and 90% CA. Our findings demonstrate that oxacillin DD tests using the CLSI M100-S28 breakpoints for *S. pseudintermedius*/*S. schleiferi* and oxacillin BMD and cefoxitin DD tests using the CoNS breakpoints reliably identified *mecA*-mediated β -lactam resistance in *S. epidermidis*. Using *mecA* PCR as the gold standard, the PBP2a SA culture colony test (Abbott Diagnostics) exhibited 100% sensitivity and specificity whereas 2 false negatives were identified using the PBP2' latex agglutination test kit (Thermo Fisher Scientific) with sensitivity and specificity of 95.8% and 100%, respectively.

KEYWORDS broth microdilution, cefoxitin, disk diffusion, *mecA*, methicillin-resistant *Staphylococcus epidermidis*, MIC, oxacillin, PBP2a, *Staphylococcus epidermidis*

Coagulase-negative *Staphylococcus* species (CoNS) represent major nosocomial pathogens commonly associated with infections related to indwelling devices and implantation of foreign materials (1). Immunocompromised patients and premature neonates are particularly vulnerable to infections caused by CoNS (1). *Staphylococcus epidermidis* is the most common CoNS recovered from clinical sources and is found ubiquitously on healthy human skin and mucous membranes (2). This species is also a common cause of infections associated with prosthetic vascular grafts, prosthetic

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TABLE 1 Summary of *Staphylococcus epidermidis* isolates collected between 2015 and 2018^a

Institution	Yr of collection	No. of isolates		Specimen source
		<i>mecA</i> negative	<i>mecA</i> positive	
CHLA	2016–2018	22	18	Arterial line, catheter, peripheral blood
BJMC	2015, 2017	10	12	Blood, CSF, joint fluid, synovial fluid
WCMC	2016–2017	20	18	Peripheral blood, catheter
Total		52	48	

^aBJMC, Barnes-Jewish Medical Center, St. Louis, MO; CHLA, Children's Hospital Los Angeles, Los Angeles, CA; CSF, cerebrospinal fluid; WCMC, New York-Presbyterian Hospital/Weill Cornell Medical Center, New York, NY.

orthopedic devices (3), and cerebrospinal fluid (CSF) shunts (4). Furthermore, *S. epidermidis* is capable of proliferating on the surfaces of indwelling devices to form biofilms which are inherently more refractory to antimicrobial therapy, resulting in frequent relapse following treatment (5).

Antimicrobial resistance is extremely common in *S. epidermidis* (1), and the species often exhibits resistance to β -lactams, rifampin, erythromycin, clindamycin, fluoroquinolones, and trimethoprim-sulfamethoxazole (6). Methicillin (β -lactam) resistance in *S. epidermidis* is mediated by the *mecA* gene, and the prevalence of methicillin-resistant *S. epidermidis* (MRSE) has been reported to be as high as 90% (7).

Given the importance of accurate classification of methicillin resistance status, with the continued adoption of new technologies (e.g., matrix-assisted laser desorption ionization–time of flight mass spectrometry [MALDI-TOF MS]) by clinical laboratories that allow improved identification of staphylococci to the species level, there is a need to critically evaluate the best methods for detecting *mecA*-mediated oxacillin resistance in specific species of CoNS. The CLSI *Staphylococcus Ad Hoc* Working Group previously assessed the performance of phenotypic testing options for prediction of *mecA*-mediated oxacillin resistance in *Staphylococcus pseudintermedius* and *Staphylococcus schleiferi* (8, 9). This led to the discontinuation of cefoxitin disk diffusion (DD) testing and inclusion of oxacillin DD testing as an option for detecting *mecA*-mediated resistance in these species. Changes to the breakpoints were published in Clinical and Laboratory Standards Institute (CLSI) documents M100-S27 and M100-S28, respectively. These findings prompted further evaluation of other *Staphylococcus* species to determine whether the CLSI M100-S28 cefoxitin DD and oxacillin broth microdilution (BMD) recommendations for other species are accurate, as supporting data for current cefoxitin DD methodology were primarily derived from a small subset of *S. epidermidis* isolates (10).

Here, we present data from a study evaluating oxacillin and cefoxitin DD and BMD testing for detection of β -lactam resistance in 100 human isolates of *S. epidermidis* (48 *mecA* positive, 52 *mecA* negative).

(The findings from this study were presented to the CLSI Antimicrobial Susceptibility Testing Subcommittee in June 2018.)

MATERIALS AND METHODS

Bacterial isolates. A total of 100 human *S. epidermidis* isolates collected between 2015 and 2018 were included in this study (Table 1). Only one isolate per human subject was collected. Isolates were recovered primarily from positive-testing blood culture broths but were also obtained from cultures of other sterile sites (e.g., cerebrospinal and synovial fluids). Isolates were collected from the following 3 distinct geographical locations in the United States: New York-Presbyterian Hospital/Weill Cornell Medical Center (WCMC), New York, NY ($n = 38$); Barnes-Jewish Hospital (BJH), St. Louis, MO ($n = 22$); and Children's Hospital Los Angeles (CHLA), Los Angeles, CA ($n = 40$). Isolates were identified to the species level during routine clinical testing with either the Verigene Gram-positive blood culture test (Luminex Corporation, Austin, TX) or MALDI-TOF MS (Bruker Daltonics, Inc., Billerica, MA).

Detection of *mecA*. One hundred isolates were selected for nearly equal representation of *mecA*-positive and -negative isolates (Table 1). Isolates were evaluated for the presence of the *mecA* gene as part of routine clinical testing. At 2 sites (CHLA and WCMC), a positive blood culture broth demonstrating Gram-positive cocci upon Gram stain analysis was assayed on the Verigene Gram-positive blood culture

panel per the manufacturer's protocol. The panel has the ability to identify *S. epidermidis* and to detect the *mecA* gene. At 1 site (BJH), *S. epidermidis* isolates were first assayed for susceptibility to β -lactam antibiotics by phenotypic means following CLSI methods (M07-A10 and M02-A12), and the BJH isolates only were further interrogated for *mecA* using the Verigene Gram-positive blood culture test using a 0.5 McFarland in saline solution prepared from fresh isolates, followed by addition of 350 μ l of the suspension to the sample well of the extraction tray. This additional testing of the isolates from BJH was conducted at WCMC.

Antimicrobial susceptibility testing (AST). All isolates were shipped to 1 site (CHLA) for AST testing. Testing was conducted in 10 batches. For each batch, 10 *S. epidermidis* isolates and an associated *S. aureus* quality control (QC) isolate were tested. All isolates were stored at -80°C and subcultured twice on tryptic soy agar with 5% sheep blood agar plates (BAP) before testing.

Susceptibility to β -lactams was interrogated by DD using 1 μ g oxacillin and 30 μ g cefoxitin disks (Becton, Dickinson and Company [BD], Franklin Lakes, NJ) per CLSI recommendations (M012-A12). *Staphylococcus aureus* ATCC 25923 was utilized as QC for each batch of 10 isolates, resulting in the generation of 10 QC points. Mueller-Hinton agar (MHA) plates from Remel (Thermo Fisher Scientific, Lenexa, KS), Hardy Diagnostics (Santa Maria, CA), and BD were tested. Colonies were resuspended in 0.85% saline solution as described above and inoculated onto MHA plates within 15 min with a sterile cotton-tipped swab. Plates were allowed to dry for 3 to 5 min but for no longer than 15 min. MHA plates 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. Both the oxacillin and cefoxitin zones of inhibition were read using reflected light.

Susceptibility to β -lactams was determined by BMD using oxacillin and cefoxitin per CLSI recommendations (M07-A10). Custom BMD panels were prepared by Thermo Fisher Scientific. Each 96-well tray consisted of six doubling dilution series such that oxacillin and cefoxitin were diluted in doubling dilutions from 32 μ g/ml to 0.015 μ g/ml using independent cation-adjusted Mueller-Hinton broth (CA-MHB) from three manufacturers: BBL (BD), Oxoid (Thermo Fisher Scientific), and Difco (Thermo Fisher Scientific). BMD plates were frozen after manufacture and stored at -80°C until use. On the day of setup, BMD trays were thawed for no longer than 60 min at room temperature in a single layer. *S. aureus* ATCC 29213 was utilized as the QC for each series of 10 experimental isolates, resulting in the generation of 10 QC points. *Staphylococcus epidermidis* colonies were resuspended in 0.85% saline solution (Hardy) using sterile cotton-tipped swabs with wooden shafts (and were adjusted to 0.5 McFarland using a Siemens turbidity meter, which translates to a reading of 0.06 to 0.10). The 0.5 McFarland suspension was subjected to vortex mixing, after which 1 ml was pipetted into tubes of 29 ml demineralized water without Tween (Thermo Fisher Scientific). This dilution was mixed by inverting the tube 3 to 5 times and was then poured into the inoculator tray of an inoculator assembly (Thermo Fisher Scientific), which was used to individually inoculate each BMD plate. A purity check was performed for each by sampling 1 μ l from the inoculator tray or growth control well of the plate and plating on a BAP. Colony counts were determined on the 5th and 10th isolate as well as the QC control of each experimental set as follows: a 10 μ l aliquot was taken from the growth control well immediately after inoculation and diluted in 5 ml of 0.85% saline solution. After mixing, a 50- μ l volume was inoculated onto the surface of a blood agar plate and spread evenly with a sterile loop, in duplicate. Plates were incubated at 35 to 37°C overnight. Following incubation, the colonies on each of the two plates were counted and averaged. The average colony count was multiplied by 10,000 to determine the total CFU count per milliliter. BMD plates were covered with the plastic covers provided with the BMD plates, stacked no more than 4 plates high, and incubated at 35 to 37°C . Cefoxitin wells were read at 16 to 20 h, and oxacillin wells were read at 24 h.

PBP2 testing. Testing for PBP2a, which is encoded by *mecA*, was conducted using two immunoassays, namely, the PBP2a SA culture colony test (Abbott Diagnostics, Lake Forest, IL), which is a lateral flow immunoassay, and the PBP2' latex agglutination test (Thermo Fisher Scientific), following manufacturer instructions. For the PBP2a SA culture colony test, instructions were followed as described for *S. aureus*, which does not require overnight oxacillin induction. For the PBP2' latex agglutination test, the assay was set up as recommended by the manufacturer following overnight oxacillin induction. For the latex agglutination test and culture colony test, the recommended negative and positive *S. aureus* controls were used, namely, ATCC 25923 (negative) and ATCC 43300 (positive), respectively.

Data analysis. MIC values for BMD experiments and DD zone diameters were read and recorded systematically at 18 h and 24 h for test and control isolates to accommodate all breakpoints. Repeat testing for *mecA*, PBP2a, and cefoxitin and oxacillin was conducted by the use of DD and BMD if the oxacillin MIC and cefoxitin DD results were discrepant with regard to *mecA* status results, and the results of the repeat testing were included for analysis. Zone diameters and MIC values were interpreted using breakpoints and analysis time points for the following organisms: (i) *S. aureus*/*S. lugdunensis*; (ii) CoNS, excluding *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*; and (iii) *S. pseudintermedius* and *S. schleiferi* (Table 2) (CLSI M100-S28). Analysis was conducted on the MIC values collected at the following time points as presented in the CLSI M100-S28 document (Table 2). For quality control (QC) strain *S. aureus* ATCC 29213, *S. aureus* MIC value breakpoints were used to evaluate oxacillin MIC values at 24 h. For *S. epidermidis* isolates, CoNS oxacillin MIC values were evaluated at 24 h and cefoxitin MIC values at 18 h. For the QC strain *S. aureus* ATCC 25923, *S. aureus* cefoxitin zone diameters were measured at 24 h. For *S. epidermidis* isolates, zone diameters were evaluated at 18 h for oxacillin and 24 h for cefoxitin.

Results were compared to those obtained using the gold standard method for determining resistance to oxacillin, which was considered here to be the presence of *mecA* as determined by molecular testing. Categorical agreement (CA), very major errors (VMEs), and major errors (MEs) were calculated as follows: for CA, $100 \times (\text{number of isolates with categorical result matches})/(\text{Total number of isolates})$

TABLE 2 CLSI M100-S28 breakpoints used at time of study^a

Organism group	Oxacillin breakpoint				Cefoxitin breakpoint			
	Zone diam (mm) ^b		MIC value (μg/ml) ^c		Zone diam (mm) ^d		MIC value (μg/ml) ^e	
	S	R	S	R	S	R	S	R
<i>S. aureus</i> / <i>S. lugdunensis</i>			≤2	≥4	≥22	≤21	≤4	≥8
CoNS (except <i>S. lugdunensis</i> , <i>S. pseudintermedius</i> , <i>S. schleiferi</i>)			≤0.25	≥0.5	≥25	≤24		
<i>S. pseudintermedius</i> / <i>S. schleiferi</i>	≥18	≤17	≤0.25	≥0.5				

^aCoNS, coagulase-negative *Staphylococcus* species; R, resistant; S, susceptible.

^bThe organisms of the *S. pseudintermedius*/*S. schleiferi* group were incubated for 16 to 20 h prior to reading.

^cThe organisms of all three groups were incubated for 24 h prior to reading.

^dThe organisms of the *S. aureus*/*S. lugdunensis* group and the CoNS (except *S. lugdunensis*, *S. pseudintermedius*, *S. schleiferi*) group were incubated for 24 h prior to reading.

^eThe organisms of the *S. aureus*/*S. lugdunensis* group were incubated for 16 to 20 h prior to reading.

tested)); for VME rate, $100 \times ([\text{number of VMEs}]/[\text{total number of resistant isolates by reference method}])$; for ME rate, $100 \times ([\text{number of MEs}]/[\text{total number of susceptible isolates by reference method}])$. Statistical calculations of 95% confidence intervals (CI) were performed using the Web-based MedCalc statistical software.

RESULTS

Performance of cefoxitin DD. Results from cefoxitin DD testing are summarized in Table 3 and Fig. 1A. The zones of inhibition showed a clear divide between *mecA*-positive and -negative isolates across the 3 brands of media tested. This demarcation coincides with the existing M100-S28 CoNS (excluding *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*) breakpoint (Table 2) with 100% CA and no MEs or VMEs, indicating that this breakpoint is appropriate for prediction of *mecA* status for *S. epidermidis* by the use of cefoxitin DD. Upon application of the M100-S28 *S. aureus*/*S. lugdunensis* breakpoints for cefoxitin DD testing, the CA ranged from 96% to 99% but the VMEs were 8.3% (4/48), 4.2% (2/48), and 2.1% (1/48) for the BD, Remel, and Hardy media, respectively. The size distributions of zones of inhibition were similar across manufacturers (see Table S1 in the supplemental material).

Performance of cefoxitin BMD. Cefoxitin BMD results are summarized in Table 3 and Fig. 1B. Three isolates (SEP052 [*mecA* positive; CHLA]; SEP072 [*mecA* negative; CHLA] and SEP099 [*mecA* positive; BJH]) could not be assayed by BMD using media from all 3 manufacturers due to absence of growth. All 3 isolates exhibited appropriate growth on the associated purity plates. Cefoxitin BMD is not an approved method for determining susceptibility to β -lactams for CoNS, and the resultant MIC value distributions for *S. epidermidis* shows that the MIC values for *mecA*-positive and -negative isolates overlap between 4 to 8 $\mu\text{g/ml}$ (Fig. 1B), which coincides with the M100-S28 *S. aureus*/*S. lugdunensis* breakpoint for cefoxitin MIC (Table 2), thus supporting the absence of cefoxitin BMD breakpoints for CoNS. Interpreting the cefoxitin MIC values with the M100-S28 *S. aureus*/*S. lugdunensis* breakpoint, all CA-MHB media performed similarly, with CA ranging from 95.9% to 96.9%. The distributions of MIC values were similar across manufacturers (Table S2). The VME rates were 2.2% (1/46) for Difco and 4.3% (2/46) for both BBL and Oxoid. The ME rate was 3.9% (2/51) for all 3 brands of CA-MHB.

Performance of oxacillin DD. Results from testing oxacillin DD are summarized in Table 4 and Fig. 1C. One isolate (SEP099 [*mecA* positive; BJH]) failed to grow sufficiently in the 16-to-18-h time range and was not included in the subsequent analysis; however, it had sufficient growth at 24 h and was resistant to oxacillin and cefoxitin. At the time of the study, oxacillin DD was recommended by CLSI as a method to detect *mecA*-mediated β -lactam resistance in *S. pseudintermedius*/*S. schleiferi* only (M100-S28). Zones of inhibition read between 16 and 18 h after inoculation in this study showed a clear demarcation between *mecA*-positive and -negative isolates, with a distribution along the M100-S28 *S. pseudintermedius*/*S. schleiferi* oxacillin DD breakpoint (Fig. 1C). The

TABLE 3 Performance of cefoxitin disk diffusion and broth microdilution for detection of *mecA*-mediated β -lactam resistance in *S. epidermidis*^a

Test and species (breakpoints [μ g/ml]; read time)	% positive isolates (no. of positive isolates/total no. of isolates tested)											
	Summary ^b			BD medium			Remel medium			Hardy medium		
Cefoxitin disk diffusion	CA	ME	VME	CA	ME	VME	CA	ME	VME	CA	ME	VME
CoNS ($S \geq 25$, $R \leq 24$ h)	100 (300/300)	0 (0/156)	0 (0/144)	100 (100/100)	0 (0/52)	0 (0/48)	100 (100/100)	0 (0/52)	0 (0/48)	100 (100/100)	0 (0/52)	0 (0/48)
<i>S. aureus</i> / <i>S. lugdunensis</i> ($S \geq 22$; $R \leq 21$; 24 h)	97.7 (293/300)	0 (0/156)	4.9 (7/144)	96 (96/100)	0 (0/52)	8.3 (4/48)	98 (98/100)	0 (0/52)	4.2 (2/48)	99 (99/100)	0 (0/52)	2.1 (1/48)
Cefoxitin broth microdilution	Summary ^b			Difco CA-MHB			BBL CA-MHB			Oxoid CA-MHB		
	CA	ME	VME	CA	ME	VME	CA	ME	VME	CA	ME	VME
<i>S. aureus</i> / <i>S. lugdunensis</i> ($S \leq 4$, $R \geq 8$; 16–20 h)	96.2 (280/291) ^c	3.9 (6/153)	3.6 (5/138)	96.9 (94/97)	3.9 (2/51)	2.2 (1/46)	95.9 (93/97)	3.9 (2/51)	4.3 (2/46)	95.9 (93/97)	3.9 (2/51)	4.3 (2/46)

^aBMD, broth microdilution; CA, categorical agreement; CA-MHB, cation-adjusted Mueller-Hinton broth; CoNS, coagulase-negative *Staphylococcus* species; DD, disk diffusion; VME, very major error; ME, major error; R, resistant; S, susceptible.

^bData include compiled numbers from all 3 manufacturers.

^cThree isolates (two *mecA* positive, one *mecA* negative) did not grow sufficiently using any liquid media.

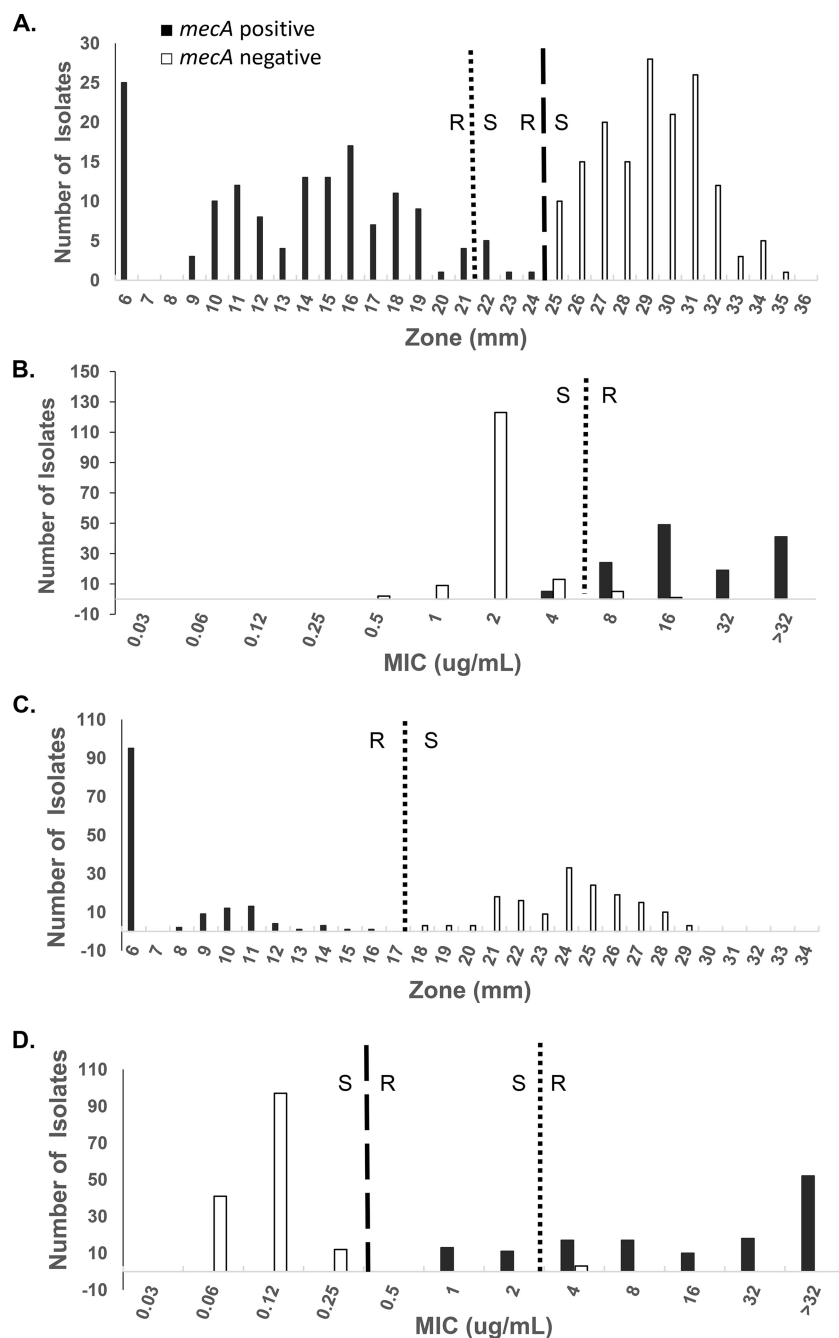


FIG 1 Distribution of cefoxitin and oxacillin growth inhibition zones and MIC values as determined by DD and BMD testing compiled for media from all three manufacturers. Breakpoints are derived from the CLSI M100-S28 document. (A) Cefoxitin DD (BD) (Hardy and Remel media; $n = 300$). The dotted line represents the breakpoint for *S. aureus* and *S. lugdunensis*; the dashed line represents the breakpoint for CoNS except *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*. (B) Cefoxitin BMD (Difco, BD, and Oxoid media; $n = 291$). The dotted line represents the breakpoint for *S. aureus* and *S. lugdunensis*. (C) Oxacillin DD (BD, Hardy and Remel media; $n = 297$). The dotted line represents the breakpoint for *S. pseudintermedius* and *S. schleiferi*. (D) Oxacillin BMD (Difco, BD, and Oxoid media; $n = 291$). The dotted line represents the breakpoint for *S. aureus* and *S. lugdunensis*; the dashed line represents the breakpoint for CoNS except *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*.

majority (35/48, 72.9%) of *mecA*-positive isolates grew all the way to the edge of the oxacillin disk (i.e., a zone size of 6 mm). Identical results were obtained for all three brands of media with 100% CA and no VMEs or MEs, and the size distributions of zones of inhibition were similar across manufacturers (Table S3). This indicates that for *S.*

TABLE 4 Performance of oxacillin disk diffusion and broth microdilution for detection of *mecA*-mediated β -lactam resistance in *S. epidermidis*^a

Test and species (breakpoints [μ g/ml]; read time)	% positive isolates (no. of positive isolates/total no. of isolates tested)											
	Summary ^b			BD medium			Remel medium			Hardy medium		
Oxacillin disk diffusion	CA	ME	VME	CA	ME	VME	CA	ME	VME	CA	ME	VME
<i>S. pseudintermedius</i> / <i>S. schleiferi</i> ($S \geq 18$, $R \leq 17$; 16–18 h)	100 (297/297) ^c	0 (0/156)	0 (0/144)	100 (99/99)	0 (0/52)	0 (0/47)	100 (99/99)	0 (0/52)	0 (0/47)	100 (99/99)	0 (0/52)	0 (0/47)
Oxacillin broth microdilution	Summary ^b			Difco CA-MHB			BBL CA-MHB			Oxoid CA-MHB		
	CA	ME	VME	CA	ME	VME	CA	ME	VME	CA	ME	VME
<i>S. pseudintermedius</i> / <i>S. schleiferi</i> + CoNS ($S \leq 0.25$, $R \geq 0.5$; 24 h)	99.0 (288/291) ^d	2.0 (3/153)	0 (0/138)	99.0 (96/97)	2.0 (1/51)	0 (0/46)	99.0 (96/97)	2.0 (1/51)	0 (0/46)	99.0 (96/97)	2.0 (1/51)	0 (0/46)
<i>S. aureus</i> / <i>S. lugdunensis</i> ($S \leq 2$, $R \geq 4$; 24 h)	90.7 (264/291) ^d	2.0 (3/153)	17.4 (24/138)	93.9 (92/98)	2.0 (1/51)	10.9 (5/46)	93.9 (91/97)	2.0 (1/51)	10.9 (5/46)	84.7 (82/97)	2.0 (1/51)	30.4 (14/46)

^aCA, categorical agreement; CA-MHB, cation-adjusted Mueller-Hinton broth; CoNS, coagulase-negative *Staphylococcus* species; VME, very major error; ME, major error.

^bData include compiled numbers from all 3 plates.

^cOne isolate exhibited growth too faint to allow zone reading.

^dThree isolates (two *mecA* positive, one *mecA* negative) did not grow sufficiently using Difco and BBL CA-MHB.

epidermidis, oxacillin DD is appropriate to predict *mecA* status using the M100-S28 *S. pseudintermedius*/*S. schleiferi* breakpoints (Table 2).

Performance of oxacillin BMD. Results from tests of the oxacillin MIC are summarized in Table 4 and Fig. 1D. Again, as with cefoxitin BMD testing, the same three isolates (SEP052, SEP072, and SEP099) could not be assayed by BMD across all CA-MHB media because they yielded no growth, despite growing on a BAP purity plate. MIC value distributions revealed a clear separation between *mecA*-positive and -negative isolates at between 0.25 and 0.5 $\mu\text{g/ml}$ (Fig. 1D). This is in agreement with the M100-S28 CoNS breakpoints for oxacillin. Using CoNS breakpoints for oxacillin BMD yielded 99% CA, 2% (1/51) MEs, and 0% (0/46) VMEs for all 3 CA-MHB brands. Interpreted with the M100-S28 *S. aureus*/*S. lugdunensis* breakpoints, however, CA dropped to 84.7% overall for Oxoid and 93.9% overall for Difco and BBL. The MEs were consistent at 1/51 (2%) for all 3 CA-MHB brands, but the VMEs dramatically increased to 14/46 (30.4%) for Oxoid and 5/46 (10.9%) for Difco and BBL. The distributions of MIC values were similar across manufacturers (Table S4). Taken together, these data suggest that oxacillin BMD continues to be an appropriate method for detecting *mecA*-mediated β -lactam resistance using the M100-S28 CoNS breakpoints (Table 2).

Performance of PBP2a immunoassays. PBP2a testing was performed on all isolates using two immunoassays. The PBP2a SA culture colony test is approved by the U.S. Food and Drug Administration (FDA) for detection of PBP2a in *S. aureus* only, but it showed 100% agreement (100% sensitivity and specificity) with *mecA* as the gold standard using the default manufacturer instructions. The PBP2' latex agglutination test kit is indicated by the FDA for both *S. aureus* and CoNS, with a recommendation to utilize overnight oxacillin induction for the latter. Using *mecA* as a gold standard, 2 *mecA*-positive isolates were miscategorized, resulting in overall sensitivity and specificity of 95.8% and 100%, respectively.

DISCUSSION

This study evaluated oxacillin and cefoxitin DD and BMD testing for the detection of *mecA*-mediated β -lactam resistance in 100 contemporary human *S. epidermidis* isolates obtained from three geographical locations in the United States between 2015 and 2018. Nearly equal distributions of *mecA*-positive and -negative isolates were tested to ensure even distributions of resistant and susceptible isolates. Data generated from three MHA manufacturers (BD, Remel, and Hardy) and three CA-MHB manufacturers (Difco, BD, and Oxoid) demonstrated that cefoxitin DD using CLSI M100-S28 CoNS breakpoints and oxacillin DD using CLSI M100-S28 *S. pseudintermedius*/*S. schleiferi* breakpoints accurately predicted the presence and absence of *mecA* in *S. epidermidis* with 100% CA. Oxacillin BMD using CoNS breakpoints also yielded results that were highly accurate (with only one false-positive isolate), resulting in a ME rate of 2% and CA of 99%. While the zone of inhibition that was observed using Remel MHA was fainter than that observed using the other two brands, as previously described (8), the zone sizes were still readable and no notable differences in performance between all three brands were identified.

A 2005 study of 310 CoNS isolates that included an undisclosed number of *S. epidermidis* isolates supported the use of cefoxitin DD to predict the presence of *mecA* in CoNS (10). Although there were four *mecA*-positive isolates of *S. epidermidis* that were falsely classified as susceptible by cefoxitin DD, the errors were observed to be related to media lot number or media brand rather than species-specific issues. An important aspect of our study is the use of 100 contemporary and geographically diverse isolates of *S. epidermidis* to confirm the reliability of cefoxitin DD in detecting *mecA*-mediated β -lactam resistance in *S. epidermidis* when CoNS breakpoints are used. In contrast, cefoxitin DD failed to detect the presence of *mecA* in *S. pseudintermedius* and *S. schleiferi* by the use of the M100-S27 breakpoints for CoNS, prompting the removal of cefoxitin DD as an option for testing of *S. pseudintermedius* and *S. schleiferi* isolates in the M100-S26 and M100-S28 documents, respectively (8, 9). This highlights

the need to critically evaluate other *Staphylococcus* species to determine the most appropriate testing modalities.

As demonstrated here, oxacillin DD testing using breakpoints of ≥ 18 mm (susceptible) and ≤ 17 mm (resistant) is reliable for predicting *mecA*-mediated β -lactam resistance in *S. epidermidis*, which is consistent with previous studies on *S. pseudintermedius* (9) and *S. schleiferi* (8). Likewise, our data support the use of oxacillin BMD with the CoNS (including *S. pseudintermedius*/*S. schleiferi*) breakpoints to accurately detect *mecA*-mediated resistance in *S. epidermidis*. There are currently no ceftiofur MIC breakpoints for non-*S. aureus*/*S. lugdunensis* species, and our data are consistent with those from other studies that reported poor predictive value using *S. aureus*/*S. lugdunensis* ceftiofur MIC breakpoints. Those studies reported very high VME rates of 89.2% and 60% for *S. pseudintermedius* and *S. schleiferi* by the use of *S. aureus*/*S. lugdunensis* ceftiofur MIC breakpoints, respectively, but no MEs were identified (8, 9). Although our finding of 3.6% VMEs for *S. epidermidis* is significantly lower than the rates reported from the aforementioned studies, we also reported false-positive results for 6 isolates (ME rate of 3.9%).

The PBP2a SA culture colony test, which is approved by the FDA for PBP2a detection in *S. aureus*, was 100% sensitive and 100% specific for *S. epidermidis* isolates by the use of the default manufacturer instructions (i.e., without oxacillin induction). These findings are consistent with other studies that reported 100% sensitivity and specificity for other staphylococcal species, including 50 *S. epidermidis* (11) and 54 *S. schleiferi* (8) isolates. Although the PBP2' latex agglutination test kit is FDA approved for both *S. aureus* and CoNS (with oxacillin induction), two false negatives were identified, resulting in overall sensitivity and specificity of 95.8% and 100%, respectively. However, previous studies reported sensitivity and specificity of 100% in testing *S. epidermidis* isolates by the use of the PBP2' latex agglutination test kit after oxacillin induction (12, 13). We initially conducted a pilot testing without induction, but the results were difficult to interpret, and all 100 *S. epidermidis* isolates were subsequently tested for PBP2a using the PBP2' latex agglutination test kit with oxacillin induction. Likewise, a previous study concluded that induction is necessary for successful detection of oxacillin resistance in CoNS since testing without induction detected only 57.6% of *mecA*-positive isolates (13).

In summary, our data demonstrate that oxacillin DD testing using the CLSI M100-S28 breakpoints for *S. pseudintermedius*/*S. schleiferi* can reliably differentiate between *mecA*-positive and -negative *S. epidermidis* isolates. The results of testing using the breakpoints for oxacillin BMD and ceftiofur DD for CoNS, other than *S. lugdunensis*, *S. pseudintermedius*, and *S. schleiferi*, also showed high accuracy. Ceftiofur BMD testing should not be used to predict *mecA*-mediated β -lactam resistance in *S. epidermidis* due to unacceptable false-positive and false-negative results. The findings from this study were presented to the CLSI Antimicrobial Susceptibility Testing Subcommittee in June 2018, leading to the addition of specific breakpoints for oxacillin and ceftiofur DD and oxacillin BMD testing for *S. epidermidis* in the 29th edition of the M100 document. The CLSI *Staphylococcus Ad Hoc* Working Group will continue to systematically evaluate the performance of current recommended phenotypic testing options for predicting *mecA*-mediated β -lactam resistance in other *Staphylococcus* species.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/JCM.00961-19>.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

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J.D.B., S.N.N., and L.F.W. designed the study. C.-A.D.B., J.D.B., S.N.N., and L.F.W. analyzed data and wrote the manuscript. S.N.N., K.C., and M.A.W. collected and curated relevant *S. epidermidis* isolates.

REFERENCES

1. Becker K, Heilmann C, Peters G. 2014. Coagulase-negative staphylococci. Clin Microbiol Rev 27:870–926. <https://doi.org/10.1128/CMR.00109-13>.
2. Widerstrom M, Wistrom J, Sjostedt A, Monsen T. 2012. Coagulase-negative staphylococci: update on the molecular epidemiology and clinical presentation, with a focus on *Staphylococcus epidermidis* and *Staphylococcus saprophyticus*. Eur J Clin Microbiol Infect Dis 31:7–20. <https://doi.org/10.1007/s10096-011-1270-6>.
3. Hellmark B, Unemo M, Nilsdotter-Augustinsson A, Söderquist B. 2009. Antibiotic susceptibility among *Staphylococcus epidermidis* isolated from prosthetic joint infections with special focus on rifampicin and variability of the *rpoB* gene. Clin Microbiol Infect 15:238–244. <https://doi.org/10.1111/j.1469-0691.2008.02663.x>.
4. Conen A, Walti LN, Merlo A, Fluckiger U, Battegay M, Trampuz A. 2008. Characteristics and treatment outcome of cerebrospinal fluid shunt-associated infections in adults: a retrospective analysis over an 11-year period. Clin Infect Dis 47:73–82. <https://doi.org/10.1086/588298>.
5. Raad I. 1998. Intravascular-catheter-related infections. Lancet 351: 893–898. [https://doi.org/10.1016/S0140-6736\(97\)10006-X](https://doi.org/10.1016/S0140-6736(97)10006-X).
6. Littorin C, Hellmark B, Nilsdotter-Augustinsson Å, Söderquist B. 2017. In vitro activity of tedizolid and linezolid against *Staphylococcus epidermidis* isolated from prosthetic joint infections. Eur J Clin Microbiol Infect Dis 36:1549–1552. <https://doi.org/10.1007/s10096-017-2966-z>.
7. Krediet TG, Jones ME, Janssen K, Gerards LJ, Fleer A. 2001. Prevalence of molecular types and *mecA* gene carriage of coagulase-negative Staphylococci in a neonatal intensive care unit: relation to nosocomial septicemia. J Clin Microbiol 39:3376–3378. <https://doi.org/10.1128/JCM.39.9.3376-3378.2001>.
8. Huse HK, Miller SA, Chandrasekaran S, Hindler JA, Lawhon SD, Bemis DA, Westblade LF, Humphries RM. 24 January 2018, posting date. 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*. J Clin Microbiol <https://doi.org/10.1128/JCM.01653-17>.
9. Wu MT, Burnham CA, Westblade LF, Dien Bard J, Lawhon SD, Wallace MA, Stanley T, Burd E, Hindler J, Humphries RM. 2016. Evaluation of oxacillin and cefoxitin disk and MIC breakpoints for prediction of methicillin resistance in human and veterinary isolates of *Staphylococcus intermedius* group. J Clin Microbiol 54:535–542. <https://doi.org/10.1128/JCM.02864-15>.
10. Swenson JM, Tenover FC; Cefoxitin Disk Study Group. 2005. Results of disk diffusion testing with cefoxitin correlate with presence of *mecA* in *Staphylococcus* spp. J Clin Microbiol 43:3818–3823. <https://doi.org/10.1128/JCM.43.8.3818-3823.2005>.
11. Canver MC, Gonzalez MD, Ford BA, Arnold AR, Lawhon SD, Burnham CA, Jenkins SG, Burd EM, Westblade LF. 28 March 2019, posting date. Improved performance of a rapid immunochromatographic assay for detection of PBP2a in non-*Staphylococcus aureus* staphylococcal species. J Clin Microbiol <https://doi.org/10.1128/JCM.01417-18>.
12. Horstkotte MA, Knobloch JK, Rohde H, Mack D. 2001. Rapid detection of methicillin resistance in coagulase-negative staphylococci by a penicillin-binding protein 2a-specific latex agglutination test. J Clin Microbiol 39:3700–3702. <https://doi.org/10.1128/JCM.39.10.3700-3702.2001>.
13. Hussain Z, Stoakes L, Garrow S, Longo S, Fitzgerald V, Lannigan R. 2000. Rapid detection of *mecA*-positive and *mecA*-negative coagulase-negative staphylococci by an anti-penicillin binding protein 2a slide latex agglutination test. J Clin Microbiol 38:2051–2054.