Evaluation of Oxacillin and Cefoxitin Disk and MIC Breakpoints for Prediction of Methicillin Resistance in Human and Veterinary Isolates of Staphylococcus intermedius Group

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Staphylococcus pseudintermedius is a coagulase-positive, hemolytic species that colonizes the nares and anal mucosa of healthy dogs and cats. Human infections with S. pseudintermedius range in severity from bite wounds and rhinosinusitis to endocarditis; historically, these infections were thought to be uncommon, but new laboratory methods suggest that their true incidence is underestimated. Oxacillin and cefoxitin disk and MIC tests were evaluated for the detection of mecA- or mecC-mediated methicillin resistance in 115 human and animal isolates of the Staphylococcus intermedius group (SIG), including 111 Staphylococcus pseudintermedius and 4 Staphylococcus delphini isolates, 37 of which were mecA positive. The disk and MIC breakpoints evaluated included the Clinical and Laboratory Standards Institute (CLSI) M100-S25 Staphylococcus aureus/Staphylococcus lugdunensis oxacillin MIC breakpoints and cefoxitin disk and MIC breakpoints, the CLSI M100-S25 coagulase-negative Staphylococcus (CoNS) oxacillin MIC breakpoint and cefoxitin disk breakpoint, the CLSI VET01-S2 S. pseudintermedius oxacillin MIC and disk breakpoints, and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) S. pseudintermedius cefoxitin disk breakpoint. The oxacillin results interpreted by the VET01-S2 (disk and MIC) and M100-S25 CoNS (MIC) breakpoints agreed with the results of mecA/mecC PCR for all isolates, with the exception of one false-resistant result (1.3% of mecA/mecC PCR-negative isolates). In contrast, cefoxitin tests performed poorly, ranging from 3 to 89% false susceptibility (very major errors) and 0 to 48% false resistance (major errors). BD Phoenix, bioMérieux Vitek 2, and Beckman Coulter MicroScan commercial automated susceptibility test panel oxacillin MIC results were also evaluated and demonstrated >95% categorical agreement with mecA/mecC PCR results if interpreted by using the M100-S25 CoNS breakpoint. The Alere penicillin-binding protein 2a test accurately detected all mecA-positive isolates, although for four isolates, cefoxitin induction was required prior to testing. These data demonstrate that the cefoxitin surrogate test does not reliably detect the presence of mecA in S. pseudintermedius isolates and that laboratories should perform oxacillin disk or MIC tests of these isolates when they are encountered.


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Laboratory Standards Institute (CLSI) veterinary group revised recommendations for the detection of methicillin resistance in these isolates (22–24) and now stipulates that oxacillin disk or MIC testing be performed and interpreted by using S. pseudintermedius–specific breakpoints (Table 1). These guidelines are found in the CLSI VET01-S2 document (25).

With the close contact between humans and companion animals, zoonotic transfer of MRSP to humans is inevitable. Few, if any, clinical laboratories in the United States routinely access the CLSI VET01-S2 document and rely on the CLSI M100-S25 standard for instructions on how to perform susceptibility tests and interpret their results for human isolates. This fact, coupled with frequent misidentification of S. pseudintermedius as S. aureus, has resulted in variable S. pseudintermedius isolate antimicrobial susceptibility testing and interpretation practices across clinical laboratories. Moreover, no automated susceptibility test system is cleared by the U.S. Food and Drug Administration (FDA) for the testing of human isolates of S. pseudintermedius, and the ability of these systems to accurately detect MRSP is unknown.

The objective of this study was to evaluate cefoxitin and oxacillin disk zones and MICs, compared to mecA and mecC PCR, for a collection of 115 human and veterinary SIG isolates. Disk zones and MICs were interpreted by using the VET01-S2 oxacillin breakpoints, the European Committee on Antimicrobial Susceptibility Testing (EUCAST) cefoxitin breakpoints for S. pseudintermedius (26), and the existing S. aureus and coagulase-negative Staphylococcus (CoNS) oxacillin and cefoxitin breakpoints in M100-S25. The results of these data were presented to the CLSI Antimicrobial Susceptibility Testing Subcommittee in July 2015, which led to the addition of S. pseudintermedius–specific interpretative criteria for oxacillin disk and MIC data to the forthcoming M100-S26 document. In addition, three commercial automated susceptibility test systems used in human clinical laboratories were evaluated, along with a penicillin-binding protein 2a (PBP2a) test, for the detection of MRSP.

### MATERIALS AND METHODS

**Bacterial isolates.** A total of 115 isolates were included in this study. Human isolates (n = 45) were collected from the following geographically distinct academic medical centers: the Ronald Reagan UCLA Medical Center, Los Angeles, CA (n = 12); the Emory University School of Medicine, Atlanta, GA (n = 4); Washington University, St. Louis, MO (n = 12); the Centers for Disease Control and Prevention, Atlanta, GA (n = 7); the University of Iowa Hospital and Clinics, Iowa City, IA (n = 2); and the R. M. Alden Research Laboratory, Culver City, CA (n = 8). Veterinary isolates (n = 70) were obtained from the Texas A&M School of Veterinary Medicine. Identification of isolates was performed according to the standard operating procedures of the submitting institution and confirmed at a central laboratory by a nucleic acid-based assay (9).

**Antimicrobial susceptibility testing.** Isolates were stored at −70°C in brucella broth plus 15% glycerol (BD, Sparks, MD) and subcultured twice on SBA prior to testing. Well-isolated colonies were used to prepare a suspension equivalent to a 0.5 McFarland standard. This suspension was then used to inoculate Mueller-Hinton agar (BD) for disk diffusion (DD), broth microdilution (BMD) panels and automated susceptibility cards/panels according to the CLSI (DD and BMD) or the manufacturer’s instructions. BMD was performed in cation-adjusted Mueller-Hinton broth (Difco, BD) on panels prepared at UCLA according to CLSI standards (27). Oxacillin was tested at 2-fold dilutions spanning a concentration range of 0.25 to 16 μg/mL, whereas cefoxitin was tested at a single concentration of 4 μg/mL in duplicate. For DD tests, disks containing 1 μg oxacillin or 30 μg cefoxitin (BBL, BD) were used. Both BMD and DD tests were incubated at 35°C in ambient air for 24 h, and results were examined visually by two independent readers.

Automated antimicrobial susceptibility testing was performed with the Phoenix MIC-8 panel (BD Diagnostics Systems, Franklin Lakes, NJ), the Vitek 2 AST-GP71 card (bioMerieux, Inc., Durham, NC), and the MicroScan PM29 panel (Beckman Coulter, Inc., Brea, CA) at the Children’s Hospital of Los Angeles, UCLA, and Children’s Healthcare of Atlanta, respectively, in accordance with the manufacturer’s specifications. Quality control was done by testing S. aureus ATCC 25923 (DD), S. aureus ATCC 29213 (BMD), S. aureus ATCC BAA-976, S. aureus ATCC BAA-977, and S. aureus ATCC BAA-1026 for the Vitek 2 card; S. aureus ATCC 29213, S. aureus ATCC 43300, and S. aureus BAA-977 for the MicroScan panel; and S. aureus ATCC 29213 and S. aureus BAA-977 for the Phoenix panel.

**mecA and mecC detection, SCCmec typing, and repetitive-sequence PCR.** Isolates were grown on 5% sheep blood agar (Hardy Diagnostics, Santa Maria, CA), and DNA was extracted with the BiOstic Bacteremia DNA isolation kit (Mo Bio Laboratories, Carlsbad, CA). A multiplex PCR to detect and differentiate mecA and mecC (mecALG235) was performed by using a modification of a previously described method (36). A PCR with primers mecCF (5’-GAAAAAGGCTTGAACGGCCTC-3’), mecCR (5’-GAAGATCTTCCGTTCGGAGG-3’), mecAP4 (5’-GAGCAACTTCCACAGG-3’), and mecAP7 (5’-CCACCTGATCTGTAAACG-3’) was performed with Ready-To-Go PCR beads (GE Healthcare). PCR products were visualized on a 1.5% agarose gel. Known mecA- and mecC-positive MRSA strains USA300 and 72796, respectively, were used as controls.

A multiplex PCR to detect and differentiate SCCmec types I to V was also performed on all isolates by a method described previously (28). To

### Table 1: Breakpoints evaluated for prediction of S. pseudintermedius methicillin resistance

<table>
<thead>
<tr>
<th>Breakpoint standard</th>
<th>Oxacillin breakpoint</th>
<th>Cefoxitin breakpoint</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Disk (mm)</td>
<td>MIC (μg/ml)</td>
</tr>
<tr>
<td></td>
<td>S*</td>
<td>R†</td>
</tr>
<tr>
<td>CLSI M100-S25 S. aureus/S. lugdunensis</td>
<td>NA*</td>
<td>NA</td>
</tr>
<tr>
<td>CLSI M100-S25 CoNS</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>CLSI VET01-S2 S. pseudintermedius</td>
<td>≥18</td>
<td>≥17</td>
</tr>
<tr>
<td>EUCAST S. pseudintermedius</td>
<td>NA</td>
<td>NA</td>
</tr>
</tbody>
</table>

* S, susceptible.  
† R, resistant.  
* NA, not applicable.
analyze the phylogenetic relatedness of the isolates used in this study, repetitive-sequence PCR (rep-PCR) was performed as previously described (29, 30). The banding patterns obtained on a virtual gel were compared to determine the similarity index. Isolates with a similarity index of ≥95% were considered to be identical. For analysis, isolates that clustered together were given similar letter designations (A to F) to indicate similarity.

**PBPA2a test.** The Alere PBPA2a Culture Colony Test (Alere Inc., Scarborough, ME) was used to test colonies grown for 24 h according to the manufacturer’s instructions for *S. aureus*. All 115 isolates were tested for noninduced and induced PBPA2a expression. Colonies from the same plate used to prepare inoculums for cefoxitin and oxacillin DD, as well as BMD susceptibility testing, were used for the noninduced PBPA2a test. Bacteria from the edge of the cefoxitin zone of growth inhibition were harvested and used for the induced PBPA2a tests. Quality control was done with the *S. aureus* ATCC 43300 (positive control) and ATCC 25923 (negative control) strains for each new lot of PBPA2a tests.

**Data analysis.** MIC and disk results were interpreted by using the following breakpoints: (i) CLSI VET01-S2 *S. pseudintermedius* oxacillin MIC and disk, (ii) CLSI M100-S25 *S. aureus/S. lugdunensis* oxacillin MIC, (iii) CLSI M100-S25 *S. aureus/S. lugdunensis* cefoxitin MIC and disk, (iv) CLSI M100-S25 CoNS oxacillin MIC, (v) CLSI M100-S25 CoNS cefoxitin MIC and disk, and (vi) EUCAST S. *pseudintermedius* cefoxitin disk (Table 1). Categorical agreement (CA), major errors (MEs), and very MEs (VMEs) were calculated as described by Clark et al. (31). CA was defined by using the results of the mecA/mecC PCR. MEs were defined as findings that isolates were resistant to oxacillin or cefoxitin but negative for mecA and mecC by PCR. The VME rate was calculated by using the number of the mecA/mecC PCR. MEs were defined as findings that isolates were susceptible to oxacillin or cefoxitin but positive for mecA or mecC by PCR. The VME rate was calculated by using the number of the mecA/mecC PCR and mecC by PCR as the denominator. VMEs were defined as findings that isolates were susceptible to oxacillin or cefoxitin but positive for mecA or mecC by PCR. The VME rate was calculated by using the number of the mecA/mecC PCR and mecC by PCR as the denominator.

**Discrepant-result resolution.** Isolates with an ME or VME result compared to the gold standard (mecA/mecC PCR) were retested by subculturing a new culture from the frozen stock and testing it by both the method that generated the error and the reference method in parallel.

### RESULTS

#### Molecular studies.

One hundred fifteen SIG isolates were included in this study, which were further identified as 111 *S. pseudintermedius* and 4 *S. delphini* isolates. Forty-five *S. pseudintermedius* isolates were from human, 56 were from canine, 7 were from feline, and 2 were from avian specimens, and 1 was from a porcine specimen (Table 2). The *S. delphini* isolates were from avian (*n* = 1) and equine (*n* = 3) specimens. mecA was detected in 37/115 (32.2%) isolates, all *S. pseudintermedius*, including 4/45 (8.9%) human and 33/64 (51.6%) veterinary isolates. mecC was not detected in any isolate in this study.

SCCmec typing was performed for all isolates with a multiplex PCR assay that detects and differentiates SCCmec types I to V. As shown in Table 2, types III (*n* = 9), IV (*n* = 10 isolates), and V (*n* = 8 isolates) were identified and found only in mecA-positive isolates (*i.e.*, no mecA dropout events were detected). Among the human isolates, only the traditional community-associated SCCmec types IV and V were identified (Table 2), whereas the veterinary isolates included types III, IV, and V, as well as seven isolates that were not typeable by this SCCmec assay. The isolates clustered into six groups by rep-PCR, including 22 in group A (10 human, 12 veterinary), 1 in group B (veterinary), 2 in group C (veterinary), 4 in group D (2 human, 2 veterinary), 2 in group E (1 human, 1 veterinary), and 84 in group F (32 human and 52 veterinary). There was no clear division by rep-PCR of isolates from human, avian, equine, canine, or feline specimens or by the geographic locations at which the isolates were recovered.

#### Cefoxitin and oxacillin DD and BMD.

A clear division between oxacillin MICs was found between mecA-positive and -negative isolates—MICs were ≥0.5 μg/ml for all mecA-positive isolates and ≥0.25 μg/ml for all mecA-negative isolates but one (Fig. 1). Similarly, oxacillin zones of ≤17 mm were observed for all mecA-positive isolates, and all mecA-negative isolates but one had oxacillin zones of ≥18 mm (Fig. 2). The outlier isolate was the same for both disk and MIC tests, for which the measured oxacillin zone was 13 mm and the MIC was 1 μg/ml. These results, along with the mecA and mecC PCR results, were confirmed in duplicate for this isolate. In contrast to the oxacillin test results, no clear division in cefoxitin zones was observed between mecA-positive and -negative isolates (Fig. 3) and only 4/37 (10.8%) mecA-positive isolates grew at 4 μg/ml cefoxitin (data not shown).

Categorical interpretation of the disk and MIC results, compared to mecA PCR assay results, was done by using six breakpoints, and the results are presented in Table 3. With the cefoxitin CLSI M100-S25 *S. aureus/S. lugdunensis* breakpoints, 28/37 mecA-positive isolates were interpreted as susceptible by disk (*i.e.*, 75.7% VMEs) and 33/37 were interpreted as susceptible by MIC (89.1% VMEs, Table 3). Among the mecA-positive isolates for which

<table>
<thead>
<tr>
<th>Institute (state of isolate origin)</th>
<th>No. of isolates</th>
<th>Source(s)</th>
<th>Specimen source(s)</th>
<th>No. of mecA-positive isolates</th>
<th>SCCmec type (no. of isolates)</th>
<th>Rep-PCR clonal lineage(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCLA (CA)</td>
<td>12</td>
<td>Human</td>
<td>Blood, ethmoid sinus drainage, wound, ear, skin, abscess</td>
<td>3</td>
<td>IV (2), V (1)</td>
<td>A, D, F</td>
</tr>
<tr>
<td>R. M. Alden Research Laboratory (CA)</td>
<td>8</td>
<td>Human</td>
<td>Blood, dog bites, cat bites, wounds</td>
<td>0</td>
<td>None detected</td>
<td>F</td>
</tr>
<tr>
<td>Washington University (MO)</td>
<td>12</td>
<td>Human</td>
<td>Wounds</td>
<td>1</td>
<td>V (1)</td>
<td>F</td>
</tr>
<tr>
<td>Emory University School of Medicine (GA)</td>
<td>4</td>
<td>Human</td>
<td>Wounds</td>
<td>0</td>
<td>Not detected</td>
<td>F</td>
</tr>
<tr>
<td>University of Iowa (IA)</td>
<td>2</td>
<td>Human</td>
<td>Unknown</td>
<td>0</td>
<td>Not detected</td>
<td>A, F</td>
</tr>
<tr>
<td>CDC†</td>
<td>7</td>
<td>Human</td>
<td>Unknown</td>
<td>0</td>
<td>Not detected</td>
<td>A, E, F</td>
</tr>
<tr>
<td>Texas A&amp;M*</td>
<td>70</td>
<td>Canine, feline, equine, porcine</td>
<td>Pyoderma</td>
<td>33</td>
<td>III (9), IV (8), V (9)</td>
<td>A, B, C, D, E, F</td>
</tr>
</tbody>
</table>

† The state from which the isolate was acquired was unavailable.

### Table 2 Summary of study isolates

<table>
<thead>
<tr>
<th>Institute (state of isolate origin)</th>
<th>No. of isolates</th>
<th>Source(s)</th>
<th>Specimen source(s)</th>
<th>No. of mecA-positive isolates</th>
<th>SCCmec type (no. of isolates)</th>
<th>Rep-PCR clonal lineage(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UCLA (CA)</td>
<td>12</td>
<td>Human</td>
<td>Blood, ethmoid sinus drainage, wound, ear, skin, abscess</td>
<td>3</td>
<td>IV (2), V (1)</td>
<td>A, D, F</td>
</tr>
<tr>
<td>R. M. Alden Research Laboratory (CA)</td>
<td>8</td>
<td>Human</td>
<td>Blood, dog bites, cat bites, wounds</td>
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<td>None detected</td>
<td>F</td>
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<tr>
<td>Washington University (MO)</td>
<td>12</td>
<td>Human</td>
<td>Wounds</td>
<td>1</td>
<td>V (1)</td>
<td>F</td>
</tr>
<tr>
<td>Emory University School of Medicine (GA)</td>
<td>4</td>
<td>Human</td>
<td>Wounds</td>
<td>0</td>
<td>Not detected</td>
<td>A, D, E, F</td>
</tr>
<tr>
<td>University of Iowa (IA)</td>
<td>2</td>
<td>Human</td>
<td>Unknown</td>
<td>0</td>
<td>Not detected</td>
<td>A, F</td>
</tr>
<tr>
<td>CDC†</td>
<td>7</td>
<td>Human</td>
<td>Unknown</td>
<td>0</td>
<td>Not detected</td>
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</tr>
<tr>
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<td>70</td>
<td>Canine, feline, equine, porcine</td>
<td>Pyoderma</td>
<td>33</td>
<td>III (9), IV (8), V (9)</td>
<td>A, B, C, D, E, F</td>
</tr>
</tbody>
</table>

† The state from which the isolate was acquired was unavailable.
VMEs were observed, ceftoxitin zones ranged from 22 to 38 mm (Fig. 3) and MICs were ≤4 μg/ml. All mecA-negative isolates were susceptible to ceftoxitin by both disk and MIC tests, yielding 0% MEs (Table 3). When the data were interpreted by the CLSI M100-S25 S. aureus/S. lugdunensis oxacillin MIC breakpoint, 8/37 mecA-positive isolates were susceptible to oxacillin by MIC (21.6% VMEs). All eight VMEs were obtained with isolates with measured oxacillin MICs of 1 to 2 μg/ml, just below or at the susceptibility
breakpoint of 52 μg/mL (Fig. 1). In contrast, 78/78 mecA-negative isolates were susceptible to oxacillin (i.e., 0% MEs) (Table 3). Repeat testing confirmed all VMEs, and no trend in SCCmec type or rep-PCR pattern was observed for isolates that yielded VMEs for either oxacillin or cefoxitin (data not shown).

Many laboratories routinely test both oxacillin and cefoxitin MICs for S. aureus and report an isolate as oxacillin resistant if it is resistant to either oxacillin or cefoxitin (data not shown). Thus, it is possible that VMEs observed for oxacillin and cefoxitin MICs individually are due to the combined strategy. Using the M100-S25 breakpoints, MICs indicating susceptibility to both oxacillin and cefoxitin were observed for 7 (18.9%) of 37 mecA-positive isolates. Disk and MIC data interpreted by CLSI breakpoints for CoNS MEs were observed for 11/37 mecA-positive isolates interpreted as susceptible by cefoxitin disk (29.7% VMEs) and 0/78 mecA-negative isolates interpreted as resistant (0% MEs, Table 3).

The M100-S25 CoNS breakpoints for oxacillin and cefoxitin are the same as those for S. pseudintermedius. Oxacillin MICs were interpreted as indicating resistance for 8 (21.6%) of 37 mecA-positive isolates and 0/78 mecA-negative isolates (Table 3).

% MEs determined by PBP2a uninduced, NA

 Positive 96.5 4 (10.8) 0 (0.0)

 Positive 100 0 (0.0) 0 (0.0)

 a NA, not applicable.
TABLE 4 Performance of commercial automated systems when testing 115 isolates of S. pseudintermedius for oxacillin susceptibilitya

<table>
<thead>
<tr>
<th>System/panel (conc range [µg/ml])</th>
<th>CLSI M100-S25 S. aureus/S. lugdunensis oxacillin breakpoint (R, ≥4 µg/ml)</th>
<th>CLSI M100-S25 CoNS oxacillin breakpoint (R, ≥0.5 µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CA (%)</td>
<td>No. (%) of VMEs</td>
</tr>
<tr>
<td>BD Phoenix PMIC-8 (0.25, 0.5, 1, 2)</td>
<td>90.4</td>
<td>11 (29.7)</td>
</tr>
<tr>
<td>bioMérieux Vittek AST-GP71 (0.5, 1, 2)</td>
<td>93.0</td>
<td>8 (21.6)</td>
</tr>
<tr>
<td>Beckman Coulter MicroScan Pos MIC 29 (0.25, 0.5, 1, 2)</td>
<td>95.7</td>
<td>5 (13.5)</td>
</tr>
</tbody>
</table>

Oxacillin MICs were interpreted by using the CLSI M100-S25 S. aureus/S. lugdunensis or CoNS breakpoints; the reference is a meca PCR result. Results obtained with all three systems are currently for research use only.

a R, resistance.

One isolate had growth failure (repeated twice) in the AST-GP71 panel.

37/37 meca-positive isolates by these breakpoints (0% VMEs) and susceptibility for 77/78 meca-negative isolates (i.e., 1.3% MEs) (Table 3 and Fig. 1). The VET01-S2 document also includes oxacillin disk breakpoints for S. pseudintermedius, whereas oxacillin disk testing is no longer recommended for human isolates of staphylococci. Oxacillin disk test results, interpreted by the VET01-S2 breakpoint, indicated resistance for 37/37 meca isolates (0% VMEs) and susceptibility for 77/78 meca-negative isolates (1.3% MEs). This ME was observed for the same meca-negative isolate that was oxacillin resistant by MIC (Fig. 2).

EUCAST cefoxitin disk breakpoints for S. pseudintermedius (Table 1) were evaluated. One of 37 meca-positive isolates was interpreted as susceptible (2.7% VMEs). This isolate had a measured cefoxitin zone of 38 mm, which was reproduced twice. In contrast, 48/78 meca-negative isolates were resistant to cefoxitin by the EUCAST disk breakpoint, a 61.5% ME rate.

Performance comparison of automated susceptibility test systems. The performance of three commercial, automated susceptibility test systems, the BD Phoenix, bioMérieux Vittek 2, and Beckman Coulter MicroScan, was assessed. The test panels, MIC ranges, and performance compared to meca PCR are summarized in Table 4. Notably, 0/37 meca-positive isolates had a cefoxitin MIC of >4 µg/ml—which represents a 100% cefoxitin VME rate for all three antimicrobial susceptibility test systems (not shown). Given this poor performance, only oxacillin MICs were evaluated, and they were interpreted manually by using the CLSI M100-S25 S. aureus/S. lugdunensis and M100-S25 CoNS/VET01-S2 S. pseudintermedius oxacillin MIC breakpoints. When interpreted by the S. aureus/S. lugdunensis oxacillin breakpoint, 11 (29.7%), 8 (21.6%), and 5 (13.5%) VMEs were obtained with the Phoenix, Vittek 2, and MicroScan systems, respectively. No MEs were observed. When evaluated by the M100-S25 CoNS/VET01-S2 S. pseudintermedius breakpoint, 4 (10.8%), 1 (2.7%), and 0 (0.0%) VMEs were obtained with the Phoenix, Vittek 2, and MicroScan systems, respectively. When these isolates were retested, the VME obtained with the Vittek 2 system was resolved (i.e., the MIC of 0.5 µg/ml was ≤0.25 µg/ml on retesting) and all of the VMEs obtained with the Phoenix system were reproduced. Two of the Phoenix VMEs were obtained with isolates that also had lower MICs in the other commercial test systems. One, a canine isolate, had an oxacillin MIC of 0.5 µg/ml in the Vittek 2 system and 1 µg/ml in the MicroScan system, and the other, a porcine isolate, had oxacillin MICs of 0.5 and 1 µg/ml in those systems, respectively. Notably, all four isolates had oxacillin MICs of >16 µg/ml by the BMD method. One ME (1.3%) was observed when the M100-S25 CoNS/VET01-S2 S. pseudintermedius breakpoint was used in all three systems, for the same canine isolate that yielded a ME by the BMD and DD methods. One growth failure was observed, for a meca-positive isolate, in the Vittek 2 system. This was confirmed by repeat testing. The overall CA levels when using the M100-S25 CoNS/VET01-S2 oxacillin S. pseudintermedius breakpoint were 95.7, 98.3, and 99.1%, for the Phoenix, Vittek 2, and MicroScan systems, respectively.

PBP2a test. The Alere PBP2a test, when performed with colonies from the edge of the cefoxitin zone, was 100% sensitive and 100% specific, compared to the meca PCR result. In contrast, if it was performed uninduced, following the package insert instructions for S. aureus, four meca-positive isolates were repeatedly negative for PBP2a (n = 3 replicates), yielding a sensitivity of 89.2% and a specificity of 100%. No correlation was found between isolates that required induction and oxacillin MIC or cefoxitin disk zones (data not shown).

DISCUSSION

Clinical laboratories almost universally rely on the use of the surrogate agent cefoxitin to detect meca-mediated beta-lactam resistance when testing human isolates of staphylococci (27). The cefoxitin disk test has been shown in several studies to be the most reliable predictor of the presence of meca in both S. aureus and CoNS. However, the present study demonstrates that this test falls short for SIG isolates of both human and veterinary origins. Regardless of the breakpoint employed, cefoxitin VME rates compared to meca PCR were unacceptably high and ranged from 3 to 89% (Table 3). On the low end of this range was the EUCAST cefoxitin disk test, with 3% VMEs observed among the 37 meca-positive isolates tested. However, we obtained a very high ME rate (62.8%) when using this breakpoint, further demonstrating the limitation of this surrogate agent for SIG. Indeed, we were unable to find a clear breakpoint in cefoxitin zones that could differentiate meca-positive from meca-negative isolates (Fig. 3). No correlation between SCCmec type and/or rep-PCR clonal type and performance of cefoxitin tests was identified, suggesting that our findings were not related to a specific lineage of this organism group. It should be noted that we incubated all tests for 24 h, which is longer than the recommended 16- to 20-h incubation endorsed by EUCAST (26)—we cannot exclude the possibility that additional 4 h of incubation contributed to some MEs. Nonetheless, it is clear that neither the cefoxitin nor the oxacillin test will adequately detect meca-mediated resistance in all species of Staphylococcus—both VMEs and MEs (32) have been documented. In particular, the cefoxitin disk test performs poorly with S. saprophyticus (32) and S. simulans (33) when results are inter-
ory, only 6.7 or 43.3% were mecA-positive (88/206 isolates, 230 mecA positive), they found 0.9% VMEs and 0.6% MEs. Similarly, when Schissler and colleagues evaluated a collection of 30 mecA-positive veterinary isolates of *S. pseudintermedius*, only 6.7 or 43.3% were resistant when the cefoxitin disk test was used and interpreted according to the CLSI M100-S25 *S. aureus*/*S. lugdunensis* or CoNS breakpoint, respectively. These findings, which correlated with our study of human isolates, confirm that cefoxitin is not predictive of mecA-mediated resistance in *S. pseudintermedius* isolates, regardless of the isolate source. In response to the data presented here, the CLSI Antimicrobial Susceptibility Testing Subcommittee voted in June 2015 to add *S. pseudintermedius*-specific oxacillin MIC and disk breakpoints to the forthcoming M100-S26 document. These new breakpoints for human isolates of *S. pseudintermedius* are the same as those published in the VET01-S2 document and will help raise awareness regarding the use of oxacillin tests to detect mecA-mediated resistance in *S. pseudintermedius* among clinical laboratories, in particular as cefoxitin testing has been recommended and oxacillin testing has been discouraged by the CLSI for other species of staphylococci. However, the use of these new oxacillin breakpoints, when they are published, is contingent on the laboratory’s accurate identification of *S. pseudintermedius* when it is isolated from diagnostic specimens. Accurate identification may be a challenge to laboratories that do not have MALDI-TOF MS technology for bacterial identification and impossible for laboratories that rely solely on a coagulase or *S. aureus* latex test for the identification of staphylococci, as both tests yield positive reactions with SIG isolates. We have found that commercial systems will identify isolates of *S. pseudintermedius* and *S. intermedius* (L. Westblade, unpublished data). Thus, if these isolates are encountered, laboratories should perform oxacillin MIC or disk tests on them to predict the presence of mecA.

Nonetheless, the inclusion of oxacillin disk breakpoints in M100-S26 is particularly helpful to laboratories in the United States, as no commercial antimicrobial susceptibility test systems are cleared by the FDA for *S. pseudintermedius*. While *S. pseudintermedius* is the only *Staphylococcus* species for which the oxacillin disk test would be used, many laboratories use oxacillin DD as the surrogate test for penicillin susceptibility in isolates of *Streptococcus pneumoniae*. These laboratories would thus already have the disk on hand and quality controlled. As an alternative, the commercial antimicrobial susceptibility test systems performed well; oxacillin MICs interpreted by M100-S25 CoNS breakpoints from all three instruments demonstrated a >95% CA. These systems appear to be able to detect mecA-mediated resistance if the CoNS breakpoints are used, with the exception of the BD Phoenix system, where a 10.8% VME rate was noted. Importantly, 0 of the 37 mecA-positive isolates had a positive cefoxitin screening result in these systems, reinforcing the poor performance of this surrogate agent for SIG and the potential negative implication of misidentification of SIG as *S. aureus*. Furthermore, it is worth noting that we encountered some difficulty extracting oxacillin results from the commercial platforms. For example, the Phoenix system required the operator to annotate “unspecified” for an organism’s identification in order to retrieve oxacillin MIC results, and Vitek 2 required similar manipulation of the platform’s software and/or setting to obtain results. Laboratories should be aware that while the results of the present study are encouraging, they are reported for research use only and would require laboratory verification prior to use in determining patient care. Therefore, the oxacillin disk test is an attractive option.

The Alere PBP2a Culture Colony test demonstrated 100% agreement with the mecA PCR results, if colonies from the edge of the cefoxitin zone were used for testing. Cefoxitin, rather than oxacillin, was used as the inducer for mecA expression in our study on the basis of literature about *S. aureus* that demonstrates that cefoxitin is the superior inducer of mecA expression in this species (35). It is perhaps fortuitous that we chose this approach, since it allows laboratories that do not stock an oxacillin disk to perform a PBP2a test for *S. pseudintermedius* with colonies from the edge of the cefoxitin disk (which is stocked by nearly all laboratories in the United States) zone of inhibition.

Throughout this study, we encountered one isolate, of canine origin, that yielded a consistently elevated oxacillin MIC and small oxacillin zones but was mecA and mecC negative by PCR. This isolate had an oxacillin zone diameter of 12 mm, a cefoxitin zone diameter of 27 mm, and an oxacillin MIC of >16 μg/ml but a cefoxitin MIC of ≤4 μg/ml. The oxacillin MICs were similarly high (1 to >2 μg/ml) by all three commercial systems. The mechanism of oxacillin resistance in this isolate remains to be determined, but it may be due to either hyperexpression of blaZ or other altered penicillin-binding proteins not detected by our molecular analysis-based methods. While these alternative mechanisms of low-level oxacillin resistance have been documented in *S. aureus*, they have not, to our knowledge, been confirmed in isolates of SIG.

In summary, our data demonstrate that the VET01-S2 oxacillin disk and MIC breakpoints are appropriate for use with *S. pseudintermedius* isolates obtained from human clinical specimens. Laboratories should attempt to identify coagulase-positive staphylococci that appear atypical in morphology (double zone of hemolysis, white in color) by MALDI-TOF MS or nucleic acid methods to ensure the appropriate determination and interpretation of oxacillin MICs and DD zones.

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