Comparison of Tests To Detect Oxacillin Resistance in *Staphylococcus intermedius*, *Staphylococcus schleiferi*, and *Staphylococcus aureus* Isolates from Canine Hosts

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Multiple tests were compared to the reference standard PBP2a latex agglutination test for detection of *mecA*-mediated oxacillin resistance in canine staphylococci. Cefoxitin disk diffusion, using breakpoints for human isolates of coagulase-negative *Staphylococcus* spp., had low sensitivity for detection of oxacillin resistance in members of the *Staphylococcus intermedius* group.

Oxacillin-resistant *Staphylococcus* spp. are being increasingly recovered from canine clinical specimens (2, 5, 14, 16, 17, 21, 23). Interpretation of oxacillin resistance in coagulase-positive *Staphylococcus* pathogens of the *Staphylococcus intermedius*-S. schleiferi-S. delphini-S. hyicus clade (13) has been an uncertainty for veterinary diagnosticians, because guidelines are predicated on data derived from human clinical studies with *S. aureus* and coagulase-negative *Staphylococcus* spp. (CNS) (18, 19). Laboratory tests for human isolates are selected for best correlations with *mecA*-mediated resistance (3, 7, 9, 27). Detection of PBP2a antigen by latex agglutination or of the *mecA* gene by PCR is considered a definitive laboratory test for oxacillin resistance of *Staphylococcus* spp. (19, 20). The *mecA* gene has been detected by PCR in isolates of *S. intermedius*, *S. schleiferi*, and *S. aureus* from canine hosts (11, 15). Also, an *S. sciuri*-specific homolog of *mecA*, as well as a *mecA* sequence identical to that found in oxacillin-resistant *S. aureus* strains from human hosts, has been reported for *S. sciuri* isolates from humans and dogs (25). Recently, cefoxitin disk susceptibility testing was recommended as an equivalent or improved replacement for oxacillin susceptibility testing of *S. aureus* and most CNS from humans (26).

We compared several tests for oxacillin and cefoxitin susceptibility to the standard reference PBP2a latex agglutination test for predicting *mecA*-mediated oxacillin resistance in canine *Staphylococcus* isolates. Isolates were from canine clinical specimens submitted to the University of Tennessee Veterinary Teaching Hospital Clinical Bacteriology Laboratory. Initially, 60 isolates, collected from 1996 to 2005, were tested (Table 1). An additional 98 isolates that represented all target *Staphylococcus* species obtained from 1 October to 31 December 2005 were tested (Table 2). Isolates were from multiple body sites, but only one isolate of each unique oxacillin susceptibility profile was included per patient.

Procedures to isolate and identify *S. aureus*, *S. intermedius*, and *S. schleiferi* were as previously described (1, 5, 17). A PBP2a latex agglutination test kit (penicillin-binding protein [PBP2] latex agglutination test; Oxoid Ltd., Basingstoke, Hampshire, England) was applied, following oxacillin induction, in accordance with the manufacturer’s instructions. Disk diffusion susceptibility testing was performed as recommended by the CLSI (19). Commercially prepared Mueller-Hinton agar plates without NaCl supplementation (Remel, Lenexa, Kans.) were used. Disk diffusion test samples were incubated at 35°C for 24 h and examined by incident light to detect heteroresistence. Oxacillin breakpoints were those recommended for bacterial isolates from animals (19). Cefoxitin breakpoints for predicting oxacillin resistance were those recommended for human *Staphylococcus* isolates (20). Breakpoints for human CNS isolates were applied to *S. intermedius* and *S. schleiferi*. Oxacillin Etests using Mueller-Hinton agar plates with 2% NaCl supplementation (Remel) were performed according to the manufacturer’s instructions (Etest; AB Biodisk, Solna, Sweden). Etests were incubated at 35°C for 48 h. Quality control strains, *S. aureus* (ATCC 25923 and ATCC 43300) and *Escherichia coli* (ATCC 25922), were tested weekly and with each new lot of media and antimicrobial disks.

Conventional *mecA* PCR was performed using previously described primers (12). Real-time *mecA* PCR was performed with commercially described oligonucleotide primers and a *mecA* dye-quencher probe (24). Standard PCR mixtures and thermocycler profiles were employed (12, 26). DNA was extracted from a 1-ml suspension of bacteria (ca. 5 x 10^9 CFU/ml) by cell disruption with glass beads.

PBP2a detection was used as the “gold standard” to calculate the sensitivity and specificity for each test. Cutoff points for real-time PCR cycle threshold (C_T) values and cefoxitin disk diffusion inhibition zone diameters were optimized for test accuracy and specificity or sensitivity, respectively, based on data generated for construction of receiver-operator-charac-
teristic (ROC) curves. Percent agreement beyond chance was evaluated using the kappa statistic.

Results of initial comparisons of oxacillin disk diffusion, cefoxitin disk diffusion, oxacillin Etest, and conventional meC4 PCR to the PBP2a reference standard are shown in Table 1. Oxacillin disk and Etest results for S. intermedius and S. schleiferi subspecies were in 89% to 100% agreement with the PBP2a results. Cefoxitin disk results were in 0% to 46% agreement with PBP2a positive results. There was a high level of correlation between PBP2a results and those by conventional meC4 PCR (k = 0.87). The frequencies of PBP2a-positive S. intermedius, S. schleiferi subsp. coagulans, and S. aureus isolated from 1 October 2005 to 31 December 2005 were 30%, 64%, and 25%, respectively (Table 2). ROC analysis using real-time meC4 PCR C_T values to predict resistance revealed that optimum accuracy was attained at the C_T cutoff value of <29.43 for PBP2a-positive strains (sensitivity, 98%; specificity, 100%). Isolates that showed late signals with C_T values of >30 were often negative upon repeat testing. Controls lacking template DNA and extracts of other oxacillin-susceptible isolates did not produce late signals.

Overall sensitivity and specificity of the cefoxitin disk diffusion test for 150 isolates of S. intermedius and S. schleiferi were 25% and 100%, respectively. In contrast, the corresponding sensitivity and specificity of the oxacillin disk diffusion test were 97% and 98%. ROC analysis suggested that an optimal accuracy for predicting PBP2a-positive strains occurred at a cefoxitin cutoff (breakpoint) of ≤33 mm (sensitivity, 94%; specificity, 89%). It was noted previously that several meC4-positive strains of S. simulans were not detected by the cefoxitin disk diffusion test (26), nor were five meC4-positive S. epidermidis strains from a collection of 241 CNS isolates (6). In a study of 109 S. scardi isolates, 27% of which were from animals, the cefoxitin disk diffusion test had 93.5% sensitivity and 100% specificity for detecting meC4-positive strains (25).

In this study, 30% of 107 S. intermedius isolates were oxacillin resistant, as determined by PBP2a and meC4 PCR. Similarly, 59% of 37 S. schleiferi subsp. coagulans isolates were positive by both tests. Oxacillin breakpoints for S. aureus differ from those of other Staphylococcus spp. (19, 20). S. aureus, S. intermedius, and S. schleiferi have several phenotypic traits in common, and often an abbreviated number of tests are used for routine clinical identification of these species (8, 10). An error in an individual, heavily weighted test can result in misidentification and subsequent misinterpretation of susceptibility tests (22). Differentiation of these species has been further confounded by the addition of a newly proposed species, S. pseudointermedius, isolated from dogs and other animals (4).

In summary, oxacillin disk diffusion tests and Etests had high sensitivities and specificities for detecting meC4-mediated ox-

### Table 1. Comparison of tests for detection of oxacillin resistance in a collection of 60 Staphylococcus strains isolated from dogs and obtained over a 10-year period from 1996 to 2005

<table>
<thead>
<tr>
<th>PBP2a result</th>
<th>Staphylococcus species (no. of isolates)</th>
<th>Oxacillin disk diffusion</th>
<th>Cefoxitin disk diffusion</th>
<th>Oxacillin Etest</th>
<th>Conventional meC4 PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>S. intermedius (9)</td>
<td>9 (100)</td>
<td>4 (44)</td>
<td>9 (100)</td>
<td>9 (100)</td>
</tr>
<tr>
<td></td>
<td>S. schleiferi subsp. coagulans (13)</td>
<td>13 (100)</td>
<td>6 (46)</td>
<td>13 (100)</td>
<td>12 (92)</td>
</tr>
<tr>
<td></td>
<td>S. schleiferi subsp. schleiferi (5)</td>
<td>5 (100)</td>
<td>0 (0)</td>
<td>5 (100)</td>
<td>5 (100)</td>
</tr>
<tr>
<td></td>
<td>S. aureus (4)</td>
<td>4 (100)</td>
<td>4 (100)</td>
<td>4 (100)</td>
<td>4 (100)</td>
</tr>
<tr>
<td></td>
<td>All tested (31)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>S. intermedius (18)</td>
<td>16 (89)</td>
<td>18 (100)</td>
<td>16 (89)</td>
<td>16 (89)</td>
</tr>
<tr>
<td></td>
<td>S. schleiferi subsp. coagulans (10)</td>
<td>10 (100)</td>
<td>10 (100)</td>
<td>9 (90)</td>
<td>9 (90)</td>
</tr>
<tr>
<td></td>
<td>S. schleiferi subsp. schleiferi (1)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td></td>
<td>S. aureus (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>All tested (29)</td>
<td></td>
<td></td>
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</tbody>
</table>

*For PBP2a-positive S. aureus strains, results in the intermediate or resistant category were considered correct.

### Table 2. Frequency of oxacillin resistance in 98 Staphylococcus isolates (from canine hosts) collected from 1 October to 31 December 2005

<table>
<thead>
<tr>
<th>PBP2a result</th>
<th>Staphylococcus species (no. of isolates)</th>
<th>Oxacillin disk diffusion</th>
<th>Cefoxitin disk diffusion</th>
<th>Real-time meC4 PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>S. intermedius (24)</td>
<td>22 (92)</td>
<td>4 (17)</td>
<td>23 (96)</td>
</tr>
<tr>
<td></td>
<td>S. schleiferi subsp. coagulans (9)</td>
<td>9 (100)</td>
<td>1 (11)</td>
<td>9 (100)</td>
</tr>
<tr>
<td></td>
<td>S. aureus (1)</td>
<td>1 (100)*</td>
<td>1 (100)</td>
<td>1 (100)</td>
</tr>
<tr>
<td></td>
<td>All tested (34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>S. intermedius (56)</td>
<td>56 (100)</td>
<td>56 (100)</td>
<td>56 (100)</td>
</tr>
<tr>
<td></td>
<td>S. schleiferi subsp. coagulans (5)</td>
<td>5 (100)</td>
<td>5 (100)</td>
<td>5 (100)</td>
</tr>
<tr>
<td></td>
<td>S. aureus (3)</td>
<td>3 (100)</td>
<td>3 (100)</td>
<td>3 (100)</td>
</tr>
<tr>
<td></td>
<td>All tested (64)</td>
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<td></td>
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</tbody>
</table>

*a No S. schleiferi subsp. schleiferi organisms were identified in phase 2 of the study.

b For PBP2a-positive strains, a real-time PCR C_T value of <30 was considered correct.

c For PBP2a-positive S. aureus strains, a result in the intermediate or resistant category was considered correct.
acillin resistance among the tested isolates of *S. intermedius* and *S. schleiferi* from canine hosts. Cefoxitin disk diffusion tests with *S. intermedius* and *S. schleiferi* resulted in an unacceptable level of very major susceptibility errors (resistant isolates called susceptible). With further study, the cefoxitin zone diameter breakpoint might be suitably modified for use with the *S. intermedius* group. All tests used in this study appeared satisfactory for the detection of oxacillin resistance in the few encountered isolates of *S. aureus* from canine hosts. Veterinary laboratories that have been unaccustomed to seeing substantial numbers of oxacillin-resistant staphylococci as pathogens in clinical samples should ascertain that their routine method used to screen for oxacillin resistance correlates well with reference standards for the *Staphylococcus* spp. being tested. Multiple testing should be performed, as recommended by CLSI, to confirm the presence of PBP2a or meca in isolates with borderline susceptibility test results. Some screening tests, if not well standardized for the species being tested, may not prompt questioning of strains that give a false appearance of susceptibility.

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**REFERENCES**


