Evaluation of an Immunochromatographic Assay for Rapid Detection of Penicillin-Binding Protein 2a in Human and Animal Staphylococcus intermedius Group, Staphylococcus lugdunensis, and Staphylococcus schleiferi Clinical Isolates

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The performance of a rapid penicillin-binding protein 2a (PBP2a) detection assay, the Alere PBP2a culture colony test, was evaluated for identification of PBP2a-mediated beta-lactam resistance in human and animal clinical isolates of *Staphylococcus intermedius* group, *Staphylococcus lugdunensis*, and *Staphylococcus schleiferi*. The assay was sensitive and specific, with all PBP2a-negative and PBP2a-positive strains testing negative and positive, respectively.

The genus *Staphylococcus* is currently composed of 47 species and 23 subspecies (1). Most members of the genus are resident flora; however, certain species are endowed with pathogenic traits and can cause serious disease (1). Clinically, *Staphylococcus aureus* is the most significant species worldwide (2, 3), but non-*S. aureus* species of medical and veterinary importance include members of the *Staphylococcus intermedius* group (S. pseudointermedius, S. intermedius, and S. delphini), *Staphylococcus lugdunensis*, *Staphylococcus schleiferi* subsp. coagulans, and *S. schleiferi* subsp. schleiferi (1, 4–6). These species cause a wide spectrum of diseases ranging from skin and soft tissue infections and infective endocarditis to foreign-body-related infections, and they pose a significant threat to human and animal health (1, 4–6). For instance, *S. pseudointermedius* accounts for the majority of *Staphylococcus* species isolated from canine clinical specimens and is an emerging agent of human infection (7, 8), while *S. lugdunensis* has an *S. aureus*-like proclivity for aggressive disease, notably infectious endocarditis, in humans and animals (9, 10).

Antibiotic treatment of staphylococci can be impeded by their ability to acquire resistance to multiple classes of antibiotics, especially beta-lactams (3). In staphylococci, beta-lactam resistance is typically conferred by the acquisition of an alternative penicillin-binding protein, penicillin-binding protein 2a (PBP2a or PBP2’), encoded by *mecA*, which catalyzes the synthesis of the bacterial cell wall in the presence of otherwise inhibitory concentrations of beta-lactam (3, 11). Although methicillin is no longer used clinically, staphylococcal isolates that contain *mecA*, and thus PBP2a, are called methicillin resistant, while isolates lacking *mecA* are designated methicillin susceptible (3). Presently, methicillin-resistant staphylococcal isolates are resistant to all beta-lactams, with the exception of the latest cephalosporin variants cefotaxime and ceftriaxone (12), and are of major medical and veterinary concern.

The prevalence of *mecA*-mediated beta-lactam resistance in *S. lugdunensis* is low (13); however, the number of methicillin-resistant *S. intermedius* group isolates is increasing at an alarming rate (14). Furthermore, these methicillin-resistant isolates exhibit a multidrug resistance phenotype that likely arose from indiscriminate use of antibiotics in the animal population (14, 15). Therefore, by limiting treatment options and threatening the conservation of antibiotic efficacy in animals, multidrug-resistant *S. intermedius* group isolates are a threat to animal health. Similarly, they are a danger to human wellbeing through transmission to humans and present a reservoir of antibiotic resistance. Further complicating the issue is the poor sensitivity of some phenotypic assays, in particular cefoxitin disk diffusion, to detect *mecA*-mediated resistance in *S. intermedius* group and *S. schleiferi* isolates (16). Thus, rapid, accurate, and inexpensive methods to differentiate methicillin-susceptible and methicillin-resistant *S. intermedius* group, *S. lugdunensis*, and *S. schleiferi* isolates will greatly facilitate the management of infections due to these species in human and animal populations. Herein, we assess the diagnostic performance of a rapid immunochromatographic assay, the Alere PBP2a culture colony test, to detect PBP2a in human and animal *S. intermedius* group, *S. lugdunensis*, and *S. schleiferi* clinical isolates grown in culture. The assay, indicated for the detection of PBP2a in *S. aureus* (17), is facile and differentiates methicillin-susceptible and methicillin-resistant isolates in approximately 5 min, which is considerably faster than existing methods.

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faster than conventional phenotypic-based methods (18), result-
ing in the opportunity for rapid administration of appropriate
antistaphylococcal antibiotics (19).

The collection of strains analyzed in this study is tabulated in
Table 1. It was composed of 127 clinical isolates (101 mecA negative
and 26 mecA positive) obtained from humans (104 isolates; 95
mecA negative and 9 mecA positive) and animals (23 isolates; 6
mecA negative and 17 mecA positive) and included 37 S. interme-
di us group, 67 S. lugdunensis, 12 S. schleiferi subsp. coagulans, and
11 S. schleiferi subsp. schleiferi isolates. Organisms were obtained
from four geographically distinct sites in the United States, includ-
ing 12 from Georgia, 24 from Iowa, 36 from Missouri, and 20 from
Texas. In addition, 35 isolates sent to the Centers for Disease Con-
tral and Prevention for reference identification from state health
departments across the United States were included. A single iso-
late was obtained per subject.

The identity of each strain was confirmed to the species level
using matrix-assisted laser desorption ionization–time of flight
mass spectrometry (20). Mass spectra were obtained with a Mi-
croflex LT mass spectrometer (Bruker Daltonics, Billerica, MA),
and the resultant spectra were queried against the Biotyper data-
base (5,627 entries; Bruker Daltonics). Isolates identified as S. in-
termidus or S. pseudointermedius were reported as S. intermedius
group (no S. delphini isolates were included in the collection). To
confirm S. schleiferi to the subspecies-level, urea hydrolysis and
free (tube) coagulase (S. schleiferi subsp. coagulans, positive; S.
schleiferi subsp. schleiferi, negative) were assayed using urea agar
(Thermo Fisher Scientific, Waltham, MA) and rabbit coagulase
plasma (Thermo Fisher Scientific).

Prior to testing, organisms were passaged twice on tryptic soy
agar with 5% sheep blood (TSAB) (Thermo Fisher Scientific) and
incubated at 35°C in 5% to 10% carbon dioxide. After the first
passage, cultures were incubated between 18 and 24 h before sub-
culture, while after the second passage, cultures were incubated
between 22 and 24 h before analysis. Testing was performed per
the manufacturer’s instructions (17) and without knowledge of
the mecA PCR result. On all days of testing, both a negative con-
trols and a positive control, methicillin-susceptible S. aureus
(Staphylococcus aureus ATCC 25923), and a positive control, me-
thicillin-resistant S. aureus (S. aureus ATCC 43300), were analyzed in conjunction with test isolates.

The reference method for detecting mecA (PBP2a)-mediated
beta-lactam resistance was mecA PCR. Fragments of the mecA
gene and an internal control were detected using a multiplex PCR
assay (21). A bacterial extract containing genomic DNA was pre-
pared by resuspending colonies in 50 μl of nuclease-free water
(BioExpress, Kaysville, UT) and by heating at 100°C for 10 min.
Cellular debris was removed by centrifugation for 5 min at 21,130×
g. Each PCR mixture contained 2 μl of the bacterial extract (between
50 and 200 ng of DNA), primers at a final concentration of 0.5 μM,
and 10 μl of 2× AmpliTaq Gold Fast PCR master mix (Applied
Biosystems, Foster City, CA) in a final volume of 20 μl. The mix-
ture was cycled as follows: 10 min at 95°C, 35 cycles of 3 s at 96°C,
3 s at 52°C, and 5 s at 72°C, followed by 10 s at 72°C. The resultant
PCR products were analyzed using agarose gel electrophoresis.
Again, negative (S. aureus ATCC 25923) and positive (S. aureus
ATCC 43300) controls were analyzed in conjunction with test
isolates on all days of analysis. Immunochromatographic assay
results were defined as true negative (TN) (immunochromato-
graphic assay and mecA PCR negative), true positive (TP) (immu-
nochromatographic assay and mecA PCR positive), false negative

<table>
<thead>
<tr>
<th>Geographic origin of isolates (no.)</th>
<th>Human isolates</th>
<th>Animal isolates</th>
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<tbody>
<tr>
<td>Geometric origin of isolates (no.)</td>
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<td>mecA positive</td>
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<td>Michigan (11)</td>
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<tr>
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<tr>
<td>Total (127)</td>
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</table>

<table>
<thead>
<tr>
<th>Human isolates</th>
<th>Animal isolates</th>
</tr>
</thead>
<tbody>
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<td>mecA positive</td>
<td>mecA negative</td>
</tr>
<tr>
<td>16</td>
<td>2</td>
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<tr>
<td>7</td>
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</tr>
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<td>21</td>
<td>2</td>
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<td>104</td>
<td>0</td>
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</table>

TABLE 1. S. lugdunensis, and S. schleiferi clinical isolates of human and animal origin included in the study.
Recently, a new version of the Alere PBP2a assay, the PBP2a SA culture colony test (22), was released. The diagnostic performances of the PBP2a culture colony test and the PBP2a SA culture colony test for S. aureus grown on TSAB (the intended test organism for the two systems and the same medium employed in this study) are equivalent; PBP2a culture colony test sensitivity and specificity was >98% and PBP2a SA culture colony test sensitivity and specificity was >99%. Consequently, the PBP2a SA culture colony test should accurately differentiate PBP2a-negative and PBP2a-positive S. intermedius, S. lugdunensis, and S. schleiferi isolates. Therefore, we believe the Alere PBP2a culture colony test (and the PBP2a SA culture colony test) will benefit medical and veterinary clinical microbiologists and infectious disease specialists by affording rapid, accurate, and inexpensive detection of PBP2a in S. intermedius, S. lugdunensis, and S. schleiferi isolates.

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