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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. pseudintermedius* 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 ceftaroline (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 conventional phenotypic-based methods (18), resulting 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. intermedius 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, including 12 from Georgia, 24 from Iowa, 36 from Missouri, and 20 from Texas. In addition, 35 isolates sent to the Centers for Disease Control and Prevention for reference identification from state health departments across the United States were included. A single isolate 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 Microflex LT mass spectrometer (Bruker Daltonics, Billerica, MA), and the resultant spectra were queried against the Biotyper database (5,627 entries; Bruker Daltonics). Isolates identified as S. intermedius 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 subculture, 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 control, methicillin-susceptible S. aureus (S. aureus ATCC 25923), and a positive control, methicillin-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 prepared 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 mixture 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 agar 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) (immunochromatographic assay and mecA PCR negative), true positive (TP) (immunochromatographic assay and mecA PCR positive), false negative
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* group, *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* group, *S. lugdunensis*, and *S. schleiferi* isolates.

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The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

**REFERENCES**


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**TABLE 2 Diagnostic performance of the Alere PBP2a culture colony test for detecting PBP2a-mediated beta-lactam resistance in *S. intermedius* group, *S. lugdunensis*, and *S. schleiferi* clinical isolates of human and animal origin**

<table>
<thead>
<tr>
<th>Organism</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Human</td>
<td>Animal</td>
</tr>
<tr>
<td><em>S. intermedius</em> group</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>S. lugdunensis</em></td>
<td>100</td>
<td>N/A*</td>
</tr>
<tr>
<td><em>S. schleiferi</em> subsp. coagulans</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td><em>S. schleiferi</em> subsp. schleiferi</td>
<td>N/A*</td>
<td>N/A**</td>
</tr>
</tbody>
</table>

* FN (immunochromatographic assay negative and *mecA* PCR positive), or false positive (FP) (immunochromatographic assay positive and *mecA* PCR negative). Diagnostic performance was assessed using the equations for sensitivity, TP/(TP + FN), and specificity, TN/(TN + FP).

Of the 127 clinical isolates analyzed, 104 and 23 were obtained from human and animal sources, respectively, and 101 were *mecA* negative and 26 were *mecA* positive (Table 1). Compared to *mecA* PCR, the immunochromatographic assay was highly sensitive and specific (Table 2). All 101 *mecA*-negative strains tested negative and all 26 *mecA*-positive strains tested positive, irrespective of species or geographic and subject origin. Compare this to *S. aureus* (the intended test organism); an investigation of 661 *S. aureus* isolates revealed PBP2a culture colony test assay sensitivity and specificity values of 98.4% and 100%, respectively (19). Therefore, it is clear that PBP2a-negative and PBP2a-positive *S. intermedius* group, *S. lugdunensis*, and *S. schleiferi* isolates are readily differentiated using the Alere PBP2a culture colony test, and the diagnostic performance is equivalent to that observed for *S. aureus*. Nevertheless, a limitation of our study was the lack of *mecA*-positive *S. schleiferi* subsp. *coagulans* isolates (which we did not have in our possession); as such, it was not possible to determine the sensitivity of the assay for the detection of PBP2a in this subspecies.

To ensure preanalytical consistency during our evaluation, all 127 isolates were analyzed after growth on TSAB for 22 to 24 h. To understand if non-*S. aureus* isolates could be tested under clinically relevant conditions (i.e., growth on TSAB between 18 to 24 h), we assayed six isolates (three *mecA* negative and three *mecA* positive) each of *S. intermedius* group, *S. lugdunensis*, and *S. schleiferi* subsp. *coagulans* at 18 and 24 h postculture. Regardless of the species and incubation time, all *mecA*-negative isolates were PBP2a negative and all *mecA*-positive isolates were PBP2a positive at 18 and 24 h postculture, respectively. These data revealed that beta-lactam susceptibility information for non-*S. aureus* staphylococci isolates can be generated within a time frame equivalent to that of nucleic-acid-based tests and considerably faster than that of conventional phenotypic methods.

In summary, we assessed the diagnostic performance of an immunochromatographic assay for the rapid detection of PBP2a in human and animal *S. intermedius* group, *S. lugdunensis*, and *S. schleiferi* clinical isolates. To the best of our knowledge, this is the first study demonstrating the role of this assay for detecting PBP2a in clinical non-*S. aureus* human and animal isolates. The assay exhibited excellent diagnostic characteristics (Table 2) and was unaffected by species identity or subject and geographic source.


