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Improved Performance of a Rapid Immunochromatographic Assay for Detection of PBP2a in Non-*Staphylococcus aureus* Staphylococcal Species

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ABSTRACT Non-*Staphylococcus aureus* staphylococcal species (non-SASS) are important pathogens in both animal and human populations. The development of β-lactam resistance in non-SASS through acquisition and expression of penicillin-binding protein 2a (PBP2a) represents a significant clinical and public health threat. Here, we evaluated the diagnostic performance of two versions of a PBP2a immunochromatographic assay with non-SASS. Our data show that the revised version of the assay, the PBP2a SA culture colony test, has superior diagnostic sensitivity compared to the previous version of the assay, the PBP2a culture colony test, 100% (95% confidence interval [CI], 93.3 to 100%) versus 67.9% (95% CI, 53.7 to 80.1%), respectively, while both assays display a specificity of 100% (95% CI, 92.5 to 100%). Therefore, the PBP2a SA culture colony test offers a rapid, accurate, and relatively inexpensive method for detecting PBP2a-mediated β-lactam resistance in clinically relevant non-SASS for the management of infections due to these organisms and for antimicrobial stewardship.

KEYWORDS beta-lactam resistance, methicillin resistance, PBP2a, *Staphylococcus*

Staphylococcus aureus is the primary pathogenic *Staphylococcus* species in human populations; however, other staphylococcal species can cause serious disease in both humans and animals (1). Several non-*S. aureus* staphylococcal species (non-SASS), e.g., *Staphylococcus epidermidis* and *Staphylococcus haemolyticus*, are notable nosocomial pathogens (1). In addition, *Staphylococcus lugdunensis* causes infectious endocarditis and skin and soft tissue infections (2, 3), while other species, especially members of the *Staphylococcus intermedius* group (notably *Staphylococcus pseudintermedius*), are significant animal pathogens (4).

Treatment of infections due to these and other non-SASS is impeded by their ability to acquire resistance to important therapeutic agents, including β-lactams (1). In staphylococci, resistance to β-lactams is predominantly conferred by the acquisition of an alternative transpeptidase, penicillin-binding protein 2a (PBP2a or PBP2′), which is encoded by the *mecA* gene and catalyzes the synthesis of the bacterial cell wall (peptidoglycan) in otherwise inhibitory concentrations of β-lactams (1, 5). Isolates that harbor PBP2a are resistant to all β-lactams, with the exception of ceftobiprole and ceftaroline (1, 5), and pose a significant clinical and public health threat.

Therefore, accurate, rapid, and inexpensive methods to detect PBP2a in staphylo-

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cocci can greatly facilitate management of infections due to these organisms, as well as antimicrobial stewardship efforts (6). Nucleic acid-based *mecA* detection methods are rapid and accurate but can be expensive and require dedicated instrumentation that prohibits their widespread adoption, especially in resource-limited settings. In contrast, rapid immunologic detection of PBP2a is relatively inexpensive, does not require specialized instrumentation, and is simple to perform in almost any laboratory setting.

The PBP2a culture colony test (Alere, Scarborough, ME), which was discontinued, was an immunochromatographic assay that employed monoclonal antibodies to detect PBP2a in *S. aureus* isolated in culture (7). The assay exhibited a sensitivity between 94.1 and 100% and a specificity between 98.8 and 100% for detection of PBP2a in *S. aureus* (6–8). In contrast, this assay demonstrated sensitivity and specificity with non-SASS of 86% and 100%, respectively (8), with the notable exceptions of *S. lugdunensis*, *Staphylococcus schleiferi*, and members of the *S. intermedius* group, where sensitivity and specificity were 100% (9).

In 2015, a revised version of the assay was released in the United States, the PBP2a SA culture colony test (Alere, Scarborough, ME), which utilizes recombinant monoclonal antibody fragments to detect PBP2a in *S. aureus* isolated in culture. The sensitivity and specificity of the assay for *S. aureus* are reported to be >98% (10). In addition, a study examining atypical *S. aureus* isolates demonstrated a sensitivity of ≥90% and a specificity of 100% (11). However, limited data are available for non-SASS isolates, with the exception of recent work evaluating 54 *S. schleiferi* isolates, where the PBP2a SA culture colony test had a sensitivity and specificity of 100% (12). Therefore, we sought to determine the performance of the PBP2a SA culture colony test using a collection of 100 non-SASS isolates composed of 10 species obtained from animal and human subjects collected from four geographically distinct regions in the United States: Georgia (two distinct sites), Iowa, Missouri, and Texas.

MATERIALS AND METHODS

Bacterial isolates. Bacterial isolates were identified to the species level using matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Daltonics, Inc., Billerica, MA) (13). One hundred clinical isolates were chosen based on isolate availability and included species commonly recovered from human clinical specimens (1). While the majority of isolates were coagulase-negative species, *S. pseudintermedius* and *S. schleiferi* have coagulase activity and were also included in this study because they represent important animal- and human-associated pathogens (4, 14, 15). The non-SASS clinical isolates were recovered from adult and pediatric populations (with the exception of two *S. pseudintermedius* and two *S. schleiferi* isolates obtained from animals) across four geographically distinct regions of the United States and included five separate centers, as follows: site 1, Children's Healthcare of Atlanta (Atlanta, GA; $n = 35$); site 2, Emory University School of Medicine (Atlanta, GA; $n = 26$); site 3, University of Iowa Carver College of Medicine (Iowa City, IA; $n = 16$); site 4, Texas A&M College of Veterinary Medicine (College Station, TX; $n = 4$), and site 5, Washington University in St. Louis School of Medicine (St. Louis, MO; $n = 19$).

Multiplex *mecA* PCR. The *mecA* PCR was performed as previously described (9) by multiple operators at one location (Children's Healthcare of Atlanta). Briefly, a multiplex PCR was used to detect the *mecA* gene, together with an internal control (a portion of the 16S rRNA gene). Bacterial extracts were resuspended in 50 μl of nuclease-free water and incubated at 100°C for 10 min. After removal of cellular debris by centrifugation, PCRs were set up with a final reaction volume of 20 μl, consisting of 2 μl bacterial extracts (~50 to 200 ng DNA), 0.5 μM primers, and 1× AmpliTaq Gold Fast PCR master mix (Applied Biosystems, Foster City, CA). Samples were subjected to the following cycling conditions: 95°C for 10 min; 35 cycles of 96°C for 3 s, 52°C for 3 s, and 68°C for 5 s; and 72°C for 10 sec. Agarose gel electrophoresis was used to visualize the PCR products. On each day of testing, positive (*S. aureus* ATCC 43300) and negative (*S. aureus* ATCC 25923) controls were performed.

Cefoxitin and oxacillin disk diffusion. Cefoxitin disk (30 μg; Becton, Dickinson and Company [BD], Franklin Lakes, NJ) diffusion was performed for all *Staphylococcus* species (except *S. pseudintermedius* and *S. schleiferi*) at one location (Children's Healthcare of Atlanta) by a single operator, while oxacillin disk (1 μg; BD) diffusion was performed for *S. pseudintermedius* and *S. schleiferi* at another site (NewYork-Presbyterian Hospital–Weill Cornell Medical Center, New York, NY) by a single operator, and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) recommendations (16).

Immunochromatographic detection of PBP2a. The PBP2a culture colony test permits detection of PBP2a in *S. aureus* isolates recovered in culture using immunochromatography. This technique is dependent upon anti-PBP2a monoclonal antibodies applied to a nitrocellulose membrane (7). If PBP2a is detected, the isolate is assumed to be methicillin (β-lactam)-resistant *S. aureus*. Conversely, if PBP2a is not detected, the isolate is classified as methicillin (β-lactam)-susceptible *S. aureus*. The PBP2a SA culture

TABLE 1 PBP2a culture colony test and PBP2a SA culture colony test results

Staphylococcus species (*n*)	No. of *mecA*-negative isolates	No. of negative PBP2a culture colony tests	No. of negative PBP2a SA culture colony tests	No. of *mecA*-positive isolates	No. of positive PBP2a culture colony tests	No. of positive PBP2a SA culture colony tests
S. capitis (8)	7	7	7	1	1	1
S. caprae (3)	2	2	2	1	1	1
S. epidermidis (50)	14	27	14	36	23	36
S. haemolyticus (3)	2	3	2	1	0	1
S. hominis (12)	8	11	8	4	1	4
S. lugdunensis (9)	5	5	5	4	4	4
S. pseudintermedius (6)	3	3	3	3	3	3
S. schleiferi (6)	3	3	3	3	3	3
S. simulans (1)	1	1	1	0	0	0
S. warneri (2)	2	2	2	0	0	0
All species (100)	47	64	47	53	36	53

colony test is a revised version of the PBP2a culture colony test that employs recombinant anti-PBP2a monoclonal antibody fragments bound to a nitrocellulose membrane (10). To determine the accuracy of both assays compared to that of *mecA* PCR results, testing was performed according to the manufacturer's instructions without oxacillin or cefoxitin induction. All testing of the PBP2a culture colony test was performed at one site (Children's Healthcare of Atlanta), while testing of the PBP2a SA culture colony test was performed at another site (NewYork-Presbyterian Hospital–Weill Cornell Medical Center). The testing was performed by a single operator. It was not possible to test the collection of isolates simultaneously with both versions of the assay. To ensure consistency with the timing of testing, all isolates were tested with each test version after 22 to 24 h of incubation (which is within the 18- to 24-h time frame in which testing is typically performed) on tryptic soy agar with 5% sheep blood at 35°C in 5 to 10% carbon dioxide. On each day of testing, positive (*S. aureus* ATCC 43300) and negative (*S. aureus* ATCC 25923) controls were performed. The reproducibility (precision) of the PBP2a SA culture colony test for non-SASS was determined using two representative isolates of *S. epidermidis*, one *mecA* negative and the other *mecA* positive, at a single site (NewYork-Presbyterian Hospital–Weill Cornell Medical Center). Each isolate was tested in triplicate on three different days by three different operators using three different lot numbers of kits to assess the intra- and interassay reproducibility of the test. On each day of reproducibility testing, positive (*S. aureus* ATCC 43300) and negative (*S. aureus* ATCC 25923) controls were performed.

Data analysis. A true positive (TP) was defined as a positive immunochromatographic assay in the setting of a positive *mecA* PCR. A true negative (TN) was defined as a negative immunochromatographic assay in the setting of a negative *mecA* PCR. A false positive (FP) was defined as a positive immunochromatographic assay in the setting of a negative *mecA* PCR. A false negative (FN) was defined as a negative immunochromatographic assay in the setting of a positive *mecA* PCR. Sensitivity and specificity were calculated using the following equations: $[TP/(TP + FN)] \times 100$ or $[TN/(TN + FP)] \times 100$, respectively. Confidence intervals (CIs) were calculated using MedCalc version 15.1, available at https://www.medcalc.org (MedCalc Software, Ostend, Belgium). All other data analysis was performed using Microsoft Excel software.

RESULTS

The *mecA* status of each isolate was determined using an in-house PCR assay (9), which identified 47 *mecA*-negative and 53 *mecA*-positive isolates (Table 1). The results of the *mecA* PCR were accepted as the gold standard. Cefoxitin and oxacillin disk diffusion results were concordant with *mecA* PCR results for 100% of the isolates tested.

Upon testing the collection of 100 isolates using the PBP2a culture colony test, the sensitivity and specificity were determined to be 67.9% (95% CI, 53.7 to 80.1%) and 100% (95% CI, 92.5 to 100%), respectively. Notably, there were 17 false-negative results when compared to *mecA* PCR, consisting of 13 *S. epidermidis* isolates (13/36 *mecA*-positive *S. epidermidis* isolates; 36.1%), three *S. hominis* isolates (3/4 *mecA*-positive *S. hominis* isolates; 75%), and one *S. haemolyticus* isolate (1/1 *mecA*-positive *S. haemolyticus* isolate; 100%). The 17 isolates that exhibited false-negative results were from four of the five sites, as follows: six from site 1 (6/17 *mecA*-positive isolates; 35.3%), four from site 2 (4/11 of *mecA*-positive isolates; 36.4%), one from site 3 (1/9 *mecA*-positive isolates; 11.1%), and six from site 5 (6/12 *mecA*-positive isolates, 50.0%). While the 13 *S. epidermidis* false-negative results were for isolates obtained from sites 1, 2, 3, and 5, the three *S. hominis* isolates that yielded false-negative results were from site 2 (however, site 2 was the only site that provided *mecA*-positive *S. hominis* isolates; $n = 4$), and the

one *S. haemolyticus* isolate with a false-negative result was from site 1 (which was the only site to provide a *mecA*-positive *S. haemolyticus* isolate). The false-negative results reported for various species and isolates from multiple centers suggest that these data more likely reflect true assay performance as opposed to a specific characteristic associated with clonal isolates. In contrast, testing of the collection of 100 isolates using the revised version of the assay, the PBP2a SA culture colony test, demonstrated 100% sensitivity (95% CI, 93.3 to 100%) and 100% specificity (95% CI, 92.5 to 100%). Data are summarized in Table 1. Finally, the intra- and interassay reproducibility of the PBP2a SA culture colony test was 100%.

DISCUSSION

Our data suggest that the PBP2a SA culture colony test has superior sensitivity for detecting PBP2a-mediated β-lactam resistance in non-SASS compared to that of the PBP2a culture colony test (100% versus 67.9%, respectively), while both assays have a specificity of 100%. These results are consistent with the known improved affinity of recombinant monoclonal antibody fragments compared to that of monoclonal antibodies, given the ability for facile antibody engineering and manipulation to improve performance (17). Recombinant antibody fragments are also advantageous because the absence of the fragment crystallizable region (Fc) domain reduces nonspecific binding, and antigen detection is often enhanced through decreased steric hindrance (17).

Interestingly, the reduced sensitivity of the PBP2a culture colony test was limited to three of the 10 species tested, *S. epidermidis*, *S. hominis*, and *S. haemolyticus*. It remains unclear if this represents a true limitation of this assay for these three species or if an insufficient number of isolates were tested for the remaining seven species. Our data demonstrated a reduced sensitivity when using the PBP2a culture colony test for non-SASS compared to that in a recent report (8). This discrepancy may be due to differences in the specific non-SASS isolates included in each study and/or due to a larger number of isolates evaluated in this current study. Nonetheless, our data show that the revised version of the assay, the PBP2a SA culture colony test, displays 100% sensitivity and specificity across 10 clinically relevant non-SASS. As such, the PBP2a SA culture colony test offers a rapid, reproducible, and inexpensive test for PBP2a detection in medically and veterinary-relevant *Staphylococcus* species.

The PBP2a SA culture colony test is only U.S. Food and Drug Administration cleared for testing *S. aureus* isolates. However, this assay allows laboratories to readily address the CLSI recommendation to test isolates of *Staphylococcus* species (other than *S. aureus*, *S. lugdunensis*, *S. epidermidis*, *S. pseudintermedius*, and *S. schleiferi*) with oxacillin MIC values between 0.5 and 2 μg/ml recovered from serious infections for the presence of *mecA* or PBP2a, because some of these strains may lack *mecA* (and ultimately PBP2a), and oxacillin MIC breakpoints may overcall resistance (16). In this situation, it is our recommendation that isolates that test *mecA* or PBP2a negative should be reported as oxacillin susceptible.

In conclusion, while molecular testing for *mecA* remains the gold standard for detecting *mecA*-mediated β-lactam resistance in staphylococci, PBP2a immunoassays, such as the PBP2a SA culture colony test, are invaluable tools for the management of infections due to *Staphylococcus* species and for antimicrobial stewardship efforts due to their rapid time to result, low cost, and ease of implementation and use in any laboratory, including those in austere settings. Through this work, we provide a framework for validating the PBP2a SA culture colony test for testing non-SASS as a laboratory-developed test, which should be of practical use to clinical laboratories.

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