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Comparative Evaluation of Identification Systems for Testing Methicillin-Resistant Strains of *Staphylococcus aureus*

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Several commercial systems are available to distinguish between *Staphylococcus aureus* and the coagulase-negative species of the *Micrococcaceae* family. Four latex agglutination systems (Accu-Staph, SeroSTAT, Staphaurex, and Staphylatex) and two hemagglutination systems (Hemastaph and Staphyloslide) were compared for their performance in the rapid identification of 232 isolates of staphylococci, including 114 of methicillin-resistant *S. aureus*. Accu-Staph, Staphaurex, and Staphyloslide correctly identified 100% of the methicillin-resistant *S. aureus* isolates; Hemastaph and Staphylatex, 99.1%; and SeroSTAT, 94.7%. Most reactions were easy to interpret, although 15% of the SeroSTAT reactions were weak. Autoagglutination occurred only with isolates of coagulase-negative staphylococci. False-positive reactions were rare and occurred only with systems which did not detect autoagglutination. Five of these six systems appear to be adequate for the rapid identification of *S. aureus*, including methicillin-resistant isolates.

Although many tests have been used to identify *Staphylococcus aureus*, the presence of free and bound coagulase has been the most widely used criterion. The ability of the tube coagulase test to correctly identify isolates of *S. aureus* depends on the temperature of incubation (room temperature versus 35°C), the time of incubation (4 versus 24 h), and the source and type of plasma used (2, 9, 10, 18; W. Landau and R. L. Kaplan, Clin. Microbiol. Newsl., 1980).

Essers and Radebold (5) designed a rapid test for the identification of *S. aureus* with latex particles coated with human plasma. The immunoglobulin G in the plasma reacts with protein A on the surface of the staphylococci (11), and fibrinogen reacts with clumping factor to produce visible agglutination. Flandrois and Carret (6) designed a hemagglutination procedure for the detection of clumping factor with sheep erythrocytes sensitized with fibrinogen.

The commercially available systems for the identification of *S. aureus* include four with latex agglutination (SeroSTAT, Scott Laboratories, Inc., Fiskeville, R.I.; Accu-Staph, Carr-Scarborough Microbiologicals, Inc., Decatur, Ga.; Staphylatex, American Micro Scan, Lexington, Ky.; and Staphaurex, Wellcome Diagnostics, Research Triangle Park, N.C.) and two with hemagglutination (Staphyloslide, BBL Microbiology Systems, Cockeysville, Md.; and Hemastaph, Remel, Lenexa, Kans.). These kits have been evaluated in a variety of clinical situations, and the results have been promising (3, 4, 8, 13, 15).

With the increasing frequency of methicillin-resistant *S. aureus* infections and colonization, it is imperative that these isolates be accurately distinguished from the coagulase-negative staphylococci which are often also multiply drug resistant. Methicillin-resistant *S. aureus* isolates may be a unique group of *S. aureus* in that some grow slowly, and their correct identification may be more difficult. In fact, two groups of investigators have reported failures of some of these systems or techniques to identify those *S. aureus* isolates which are methicillin resistant (1, 19). For these reasons, the available rapid agglutination systems were reevaluated with larger numbers of methicillin-resistant *S. aureus* strains.

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MATERIALS AND METHODS

Organisms. A total of 232 clinical isolates of staphylococci and 10 of diphtheroids were obtained from the Clinical Microbiology Laboratory of the East Orange Veterans Administration Medical Center. Staphylococci were identified as *S. aureus* or coagulase-negative staphylococci by colonial and microscopic morphology; catalase, coagulase, and acetoin production; and mannitol fermentation (9). The tube coagulase test was performed by inoculating one or two colonies of staphylococci into 0.5 ml of rabbit coagulase plasma containing EDTA (BBL). The tubes were incubated at 35°C and examined for the presence of clot formation at 4 and 24 h. When necessary, final identifications were made by using Staph-Ident (Analytab Products, Inc., Plainview, N.Y.). Methicillin resistance was detected with oxacillin disks (BBL) and the Bauer-Kirby disk diffusion test (14). Resistance was confirmed by microbroth dilution testing (7, 16). The tests were performed in Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) containing a final density of 5×10^5 CFU/ml. The MICs were determined at 18 to 20 h of incubation at 35°C. Resistance MICs were ≥3.1 μg/ml for nafcillin and oxacillin and ≥12.5 μg/ml for methicillin (17). Methicillin-resistant *S. aureus* isolates were obtained from various patients and were from a variety of phage types and antibiotic susceptibility patterns including 19% of strains resistant to rifampin, sulfamethoxazole-trimethoprim, or rifampin plus sulfamethoxazole-trimethoprim. The diphtheroids were identified by colonial and microscopic morphology and catalase production. Before testing, isolates were subcultured onto sheep blood agar (BBL) and incubated overnight in 5 to 8% CO_2 at 35°C. Fresh isolates were necessary to produce a strong agglutination reaction. Some strains had reduced or negative reactions by day 3.

Slide agglutination tests. Slide agglutination tests were performed by the instructions of the manufacturers. *S. aureus* and *S. epidermidis* control organisms were included

TABLE 1. Comparison of systems for the identification of *S. aureus*[a]

System	Sensitivity (%)	Specificity (%)	Predictive value (%)	
			Positive	Negative
Accu-Staph	100.0	98.1	98.3	100.0
Hemastaph	99.1	100.0	100.0	99.0
SeroSTAT	94.7	100.0	100.0	94.3
Staphaurex	100.0	99.1	99.1	100.0
Staphylatex	99.1	100.0	100.0	99.0
Staphyloslide	100.0	100.0	100.0	100.0

[a] Values are based on 114 isolates of methicillin-resistant *S. aureus* and 106 of coagulase-negative staphylococci.

each day with the test isolates for each system. Additional controls were performed when indicated by the manufacturer.

The Accu-Staph test was performed by placing 1 drop of Accu-Staph reagent (pink plasma-coated latex particles) on a provided reusable glass test slide. A wooden applicator stick was used to transfer a visible amount of growth onto the slide next to the reagent. The bacteria were then mixed into the reagent to obtain a homogeneous suspension, and the slide was tilted back and forth for up to 45 s. The test was considered positive when agglutination was present and negative when agglutination was absent.

The SeroSTAT latex agglutination test was performed by placing 1 drop of supplied 0.85% saline on a glass slide. No fewer than five isolated colonies were mixed with the saline to produce a smooth suspension. SeroSTAT reagent (plasma-coated latex particles) (1 drop) was added and mixed. The slide was rocked by hand for up to 45 s and read for the presence of agglutination.

The Staphaurex test was performed by dispensing 1 drop of the latex reagent onto the provided card. The flat end of a wooden applicator stick was used to transfer and to mix the equivalent of six colonies of bacteria with the latex reagent. The card was rotated by hand for approximately 20 s. A positive test was indicated by clumping of the latex particles with clearing of the milky background, whereas a negative test was indicated by no agglutination.

The Staphylatex test was performed by placing 1 drop of the supplied 0.85% saline onto a provided agglutination card. Three or four colonies were suspended in the saline with a wooden applicator stick, and 1 drop of reagent (plasma-coated latex particles) was added and mixed. The card was rocked for 45 s, and the presence or absence of agglutination was recorded.

The Hemastaph hemagglutination test was performed with reagent cells (formalinized sheep erythrocytes sensitized with human fibrinogen) and control cells (sheep erythrocytes not sensitized with human fibrinogen). Three to five colonies of the test organism were inoculated onto two different surface areas of the provided card. A total of 1 drop of each cell suspension (reagent and control) was then added to the two inoculated surfaces and mixed for 15 s. The slide was rocked for an additional 30 s. Presence of agglutination of the reagent cells with no agglutination of the control cells was considered a positive test, whereas no agglutination of both cell suspensions was interpreted as a negative test. A noninterpretable test was one in which agglutination was observed in the control suspension regardless of the agglutination pattern in the reagent cell suspension.

The Staphyloslide test was performed with the reagent cells (azide-preserved sheep erythrocytes sensitized with fibrinogen) and control cells (unsensitized sheep erythrocytes). A total of 1 drop of each cell suspension was dispensed onto two separate areas of a clean glass slide. Two to five colonies of growth were mixed with each reagent. The slide was rocked for 15 s and read immediately for the presence of agglutination with results of positive, negative, or noninterpretable as described above.

RESULTS

The results were comparable for five of the six systems in this evaluation of 114 isolates of methicillin-resistant *S. aureus* and 106 of the coagulase-negative staphylococci. The sensitivities ranged from 94.7 to 100% and the specificities from 98.1 to 100% (Table 1). In the present study, the SeroSTAT system had the lowest sensitivity for correctly identifying isolates of *S. aureus*, with 94.7%, while three of the systems (Accu-Staph, Staphaurex, and Staphyloslide) had sensitivities of 100%. The predictive values of a positive and negative test for each system are also listed.

The discrepancies in identification among the systems are given in Table 2. There were eight false-negative results involving three systems. The majority of the false-negative results (six of eight) occurred with the SeroSTAT system. One isolate gave false-negative results with three systems. Similarly, the majority of the noninterpretable results also occurred with the SeroSTAT system. All of the noninterpretable results were with the coagulase-negative staphylococci. Excluding those isolates of coagulase-negative staphylococci which autoagglutinated, all strains were correctly identified with the exception of three false-positive results which involved two systems.

A total of 12 isolates of methicillin-susceptible *S. aureus* were also tested, and all systems identified them correctly (data not shown). However, when 10 randomly collected strains of diphtheroids were tested, 2 gave false-positive results with both SeroSTAT and Staphylatex, and 1 with Accu-Staph. Noninterpretable results were obtained with one isolate each for Hemastaph and Staphyloslide, confirming the need to perform Gram stains before reporting the results of these systems.

DISCUSSION

With the increasing number of serious infections caused by the staphylococci and with the advent of a new hospital reimbursement system (diagnosis related groups), the laboratory is obligated to correctly identify microorganisms rapidly to provide for efficient patient management. *S. aureus* has been traditionally identified by its production of coagulase. The slide coagulase test has been the most rapid method for identification; however, 10 to 15% of the strains yield negative results. Because of this low sensitivity, a negative slide coagulase test requires additional procedures such as a thermostable nuclease (12, 20) or a tube coagulase

TABLE 2. Discrepancies and noninterpretable results with the rapid systems for the identification of *S. aureus*

System	No. false-positive	No. false-negative	No. noninterpretable[a]
Accu-Staph	2	0	0
Hemastaph	0	1	2
SeroSTAT	0	6	6
Staphaurex	1	0	0
Staphylatex	0	1	4
Staphyloslide	0	0	2

[a] All coagulase-negative staphylococci.

test. However, these additional tests require not only additional reagent costs and technologist time, but also prolong the time necessary for final organism identification. A more rapid test was needed to identify *S. aureus* with a sensitivity equal to that of the tube coagulase test.

The several commercial agglutination systems distinguish *S. aureus* from other members of the *Micrococcaceae* on the basis of detectable protein A on the surface of *S. aureus* and clumping factor (5, 11). However, earlier reports (1, 19) have noted that some agglutination systems produced a larger number of false-negative results for isolates of methicillin-resistant *S. aureus*. In the present study, six agglutination systems were evaluated with 114 isolates of methicillin-resistant *S. aureus*. Five of the six systems had sensitivities of greater than 99%, a sensitivity similar to that expected for the standard tube coagulase test (2, 8). Three of these systems have been previously evaluated with a limited number of methicillin-resistant *S. aureus* isolates with similar results (1). The SeroSTAT had a sensitivity of only 94.7%, comparable with the overall percentage reported by Jungkind et al. (8) but lower than that reported by others (2, 3, 13, 15). Data for the actual number of methicillin-resistant *S. aureus* isolates in those reports are often lacking.

As with the slide coagulase test, autoagglutination may occur with some of the agglutination systems. The hemagglutination systems provide a separate negative control with nonsensitized sheep erythrocytes to detect autoagglutination. The two latex systems (SeroSTAT and Staphylatex) in which microorganisms are suspended in saline before the addition of the latex reagent also allow for such observations. In the present study, autoagglutination was found only with coagulase-negative staphylococci and some isolates of diphtheroids. SeroSTAT had 6 of the 14 noninterpretable results; Staphylatex had 4. Each of the two hemagglutination systems had two noninterpretable results.

Although the time allowed for the agglutination reaction varied with the systems, most positive reactions were visible within 15 s. The hemagglutination tests were more involved procedures to perform because both the reagent and control cells were tested with each isolate. Three of the kits (Hemastaph, Staphaurex, and Staphylatex) provided disposable cards which helped decrease the time necessary to perform the test and often provided for better observation of the agglutination reactions. The agglutination patterns with the hemagglutination kits were easy to interpret, although care must be taken to initially resuspend the erythrocytes. The Staphaurex test had the easiest of the latex agglutination patterns to read. The pink color of the Accu-Staph pattern made observation of clumping easy, as was the case in reading of the Staphylatex pattern. With the SeroSTAT test, about 15% of the positive reactions were considered weak and were the most difficult to interpret. During the evaluation, it was found that cultures must be fresh (preferably overnight cultures), since the strength of the agglutination reaction decreased with the increased age of the isolate. Deviations from strictly fresh isolates may have accounted for the low detection rate for methicillin-resistant *S. aureus* isolates noted in some published reports.

Care must be taken to test only the gram-positive cocci which are catalase positive, since diphtheroids may give false-positive reactions. Of the 10 diphtheroid isolates tested, 2 each gave false-positive agglutination in the SeroSTAT and Staphylatex kits and 1 in the Accu-Staph. These data would indicate that all isolates which are nonhemolytic must have a Gram stain performed to determine morphology and confirm identification.

In summary, Accu-Staph, Hemastaph, Staphaurex, Staphylatex, and Staphyloslide provided rapid and accurate identifications of *S. aureus* comparable to that of the tube coagulase test regardless of methicillin susceptibility. These systems should be considered for routine use, especially in those laboratories in which rapid identifications can be translated into large cost savings under the diagnosis related group system.

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