

Efficacies of Rapid Agglutination Tests for Identification of Methicillin-Resistant Staphylococcal Strains as *Staphylococcus aureus*

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Four commercially available rapid agglutination tests for the identification of *Staphylococcus aureus* were compared with the tube coagulase test for the identification of 300 methicillin-resistant isolates of staphylococci. Isolates tested included 207 methicillin-resistant *S. aureus* and 93 coagulase-negative staphylococci, collected from five medical centers. Strain variability was documented by phage typing and antimicrobial susceptibility patterns. Results of rapid identification tests ranged between 82 and 86% sensitivity, significantly poorer than the 98% sensitivity which the tube coagulase test provided.

The standard test for differentiating *Staphylococcus aureus* from coagulase-negative staphylococci is the tube coagulase test. With a sensitivity of 95 to 98%, it takes 4 to 24 h for completion (10, 16). The slide coagulase test takes less than 1 min but has a 10 to 15% false-negative rate (10). Recently marketed rapid agglutination tests offer comparable rapidity, with results in less than 1 to 2 min. Initial studies reported accuracy similar to that of the tube coagulase test (8, 9, 14). However, later reports documented false-negative tests (*S. aureus* identified as coagulase-negative staphylococci) among methicillin-resistant *S. aureus* strains (MRSA), with false-negative rates of up to 20 to 25% (1, 5, 15). Discrepancies between these investigations may have been due to small numbers of test organisms, variable numbers of MRSA, and other interhospital strain differences. In one study with isolates from multiple hospitals, more false-negative tests were recorded (15). Because of concern about lower sensitivity among methicillin-resistant strains, we attempted to assess the tests using a large, varied population of methicillin-resistant staphylococci, including many MRSA, to overcome possible strain bias.

The study used 300 methicillin-resistant staphylococcal isolates from Wilford Hall U.S. Air Force Medical Center, Audie L. Murphy Memorial Veterans Hospital, University of Texas Health Science Center—San Antonio, Brook Army Hospital Burn Center, and University of Colorado Medical Center. All isolates were subcultured onto 5% sheep blood agar (Remel, Lenexa, Kans.), incubated for 18 to 24 h at 35°C for fresh growth before testing, and confirmed by characteristic colony morphology, Gram stain, and catalase reaction. Accustaph (Curtin Matheson, Houston, Tex.), Bacto (Difco Laboratories, Detroit, Mich.), Staphaurex (Wellcome Diagnostics, Dartford, England), and Staphyloslide (BBL Microbiology Systems, Cockeysville, Md.) were compared with the tube coagulase test for the identification of *S. aureus*. The rapid tests were independently performed

by two investigators; the results of the tube coagulase and biochemical tests were not available at the time of rapid agglutination testing. All discrepancies were resolved with thermanuclease agar (Remel) (3), mannitol salt and DNase agar (Remel), and biochemical identification with Staph-Ident strips (Analytab Products, Plainview, N.Y.).

Methicillin resistance was confirmed by growth on oxacillin salt agar (Remel) containing 6 mg of oxacillin per liter and incubated at 35°C for 24 h. Antimicrobial susceptibility testing was repeated by the standardized disk diffusion method of Bauer et al. (4, 12) with oxacillin, vancomycin, teicoplanin, clindamycin, erythromycin, trimethoprim-sulfamethoxazole, rifampin, gentamicin, minocycline, ampicillin-clavulanic acid, imipenem-cilastatin, and ciprofloxacin disks (BBL) on Mueller-Hinton agar (Remel). Plates were incubated at 35°C and read at 24 h. Phage typing was done at the U.S. Air Force School of Aerospace Medicine, Epidemiology Division, Laboratory Services Branch, Brooks Air Force Base, by the technique described by Blouse et al. (6). Strain variability was established by sorting isolates simultaneously for various combinations of antimicrobial susceptibility patterns and phage types.

There were 207 MRSA and 93 methicillin-resistant coagulase-negative staphylococci identified. In this series, the rapid agglutination tests were less accurate than the tube coagulase test. The tube coagulase test gave two false-negative results, compared with 35 for Accustaph, 29 for Bacto and Staphaurex, and 37 for Staphyloslide. There were no false-positive tests recorded. The results are shown in Table 1. Of 207 MRSA confirmed by oxacillin salt agar plates, 21 appeared to be susceptible by disk diffusion, confirming earlier reports of lower sensitivity by disk diffusion (2). Other susceptibilities ranged from 30% for erythromycin to 100% for vancomycin. Rates of susceptibility were not appreciably different for strains yielding false-negative tests with rapid agglutination methods. Among the 207 clinical isolates of MRSA, 74 distinct strains were identified. Many isolates were nontypeable; further diversity among these strains could be documented only by other methods. Among the 41 MRSA isolates yielding false-negative tests, 19 distinct strains were identified. There was a large differ-

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TABLE 1. Results of the rapid tests for the identification of MRSA^a

Test	Sens. (%)	NPV (%)
Tube coagulase	99	98
Accustaph	83	73
Bacto	86	76
Staphaurex	86	76
Staphyloslide	82	72

^a Sens., Sensitivity; NPV, negative predictive value. All results for specificity and positive predictive value were 100%.

ence in the number of organisms from each institution yielding false-negative tests. Only 2 of 67 MRSA isolates from the University of Colorado Medical Center and 2 of 37 from Wilford Hall U.S. Air Force Medical Center yielded false-negative tests, as opposed to 9 of 12 MRSA from Brook Army Hospital Burn Center and 17 of 73 from University of Texas Health Science Center—San Antonio. Table 2 shows the number and proportion of organisms and distinct strains yielding false-negative tests, organized by institution.

Assuming that previous investigators followed the instructions of the manufacturer, discrepancies between earlier studies of rapid agglutination tests should be limited to interpretation of reactions and bacterial strain differences. In the current study, two investigators completed the rapid tests independently. Organisms from five different medical centers were collected to provide better strain variability. Strain variability was documented by phage typing and differing antimicrobial susceptibility patterns. An unacceptably high number of MRSA were misidentified as coagulase-negative staphylococci, which could result in inadequate treatment and isolation of patients with MRSA and contribute to patient morbidity and mortality as well as to the spread of this troublesome pathogen (7).

Reasons for the poor results are unclear. The tube coagulase test detects free and bound coagulase, a relatively species-specific product of *S. aureus*, by activation of fibrinogen in the plasma to form a clot. Between 2 and 5% of *S. aureus* strains are not detected by the tube coagulase test because of inadequate amount or poor stability of the enzyme or timing of observation (10, 16). Slide coagulase tests detect only bound coagulase, by clumping of a bacterial suspension in plasma; 10 to 15% of isolates do not test

TABLE 2. Numbers of MRSA isolates yielding false-negative tests (FNT), grouped by institution

Institution ^a	Isolates			Strains	
	Total ^b	No. of FNT ^c	% FNT ^d	Total ^e	No. of FNT ^f
UCMC	67	2	3	30	2
WHMC	44	3	7	23	3
ALMMVH	27	8	30	12	6
UTHSC	58	19	33	27	9
BABC	11	9	82	7	3

^a Institutions include the University of Colorado Medical Center (UCMC), Wilford Hall U.S. Air Force Medical Center (WHMC), Audie L. Murphy Memorial Veterans Hospital (ALMMVH), University of Texas Health Science Center—San Antonio (UTHSC), and Brook Army Burn Center (BABC).

^b Number of isolates from each institution.

^c Number of isolates yielding false-negative tests.

^d Percentage of isolates from each institution yielding false-negative tests.

^e Number of distinct strains isolated from each institution. Some strains were found in more than one institution.

^f Number of distinct strains yielding false-negative tests.

positive, presumably because they have only free (unbound) coagulase (9, 10). The rapid agglutination tests detect bound coagulase by agglutination of *S. aureus* latex particles or fixed erythrocytes coated with plasma or fibrinogen and immunoglobulin G. The detection of protein A, another relatively species-specific protein, is intended to make up the difference in sensitivity between the tube and slide tests. Coating the latex particles or blood cells with serum containing immunoglobulin allows binding of the protein A to the Fc portion of the immunoglobulin and the resulting agglutination (9). Despite theoretical considerations, detection of MRSA was inadequate with these systems.

One reason for the decreased accuracy could be the protein A molecule itself. Most *S. aureus* strains produce a bound protein A molecule which would be detected in a slide test. However, some MRSA may produce only a secreted, or free, protein A (11). Free protein A is detected in many protein A assays but would not contribute to clumping of latex particles in a rapid agglutination test. Furthermore, unlike penicillin resistance, which is plasmid mediated, methicillin resistance is due to altered penicillin-binding proteins and is coded in chromosomal DNA (C. Thornsberry, Antimicrob. Newsl. 1:43-47, 1984). Because MRSA have a different genome than methicillin-susceptible *S. aureus* strains, other staphylococcal proteins and their modes of elaboration may also vary between the two groups of organisms. A study of virulence and metabolic products of *S. aureus* showed differences in the amounts of catalase produced by methicillin-susceptible *S. aureus* and MRSA (13). A difference in the amount or type (free or bound) of coagulase produced, or in a nonspecific factor contributing to clumping in staphylococci, as well as the difference in protein A noted above, may contribute to a lower sensitivity of rapid agglutination tests among MRSA.

Interestingly, certain hospitals had a higher proportion of isolates and strains testing falsely negative. At Brook Army Hospital Burn Center, the high rate of false-negative results was due to the fact that a single strain made up the majority of the isolates. Of the 27 distinct strains isolated at University of Texas Health Science Center—San Antonio, 11 yielded false-negative tests. On the other hand, only 2 of 30 strains isolated at University of Colorado Medical Center and 3 of 23 strains isolated at Wilford Hall U.S. Air Force Medical Center yielded false-negative tests. This may explain why the tests have performed well at some institutions and less well at others.

The rapid latex agglutination tests did not perform as well as the tube coagulase test among MRSA, identifying approximately 20% of MRSA as methicillin-resistant coagulase-negative staphylococci. Interpretation of test results was performed by two individuals to eliminate interpretation variation. Strain variability was documented. Thus, if a high proportion of *S. aureus* isolates are resistant to methicillin, rapid agglutination procedures may misidentify a significant number of the isolates. The use of these kits should be determined by the incidence of MRSA isolated, the ability of the kit to identify MRSA at the user institution, and the necessity of providing same-day identification of *S. aureus*.

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