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Failure of Rapid Agglutination Methods to Detect Oxacillin-Resistant *Staphylococcus aureus*

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Although a latex agglutination test (StaphAurex) and a hemagglutination test (Staphyloslide) correctly identified all strains of *Staphylococcus aureus* that were susceptible or had intermediate susceptibility to oxacillin, 17 of 73 (23%) and 18 of 73 (25%) strains of oxacillin-resistant *S. aureus* were not identified by StaphAurex and Staphyloslide, respectively. All strains not detected were resistant to trimethoprim-sulfamethoxazole and rifampin.

Slide agglutination procedures have been developed for the rapid and reliable differentiation of *Staphylococcus aureus* from other staphylococci recovered from clinical specimens. These methods utilize sensitized sheep erythrocytes or latex particles which coagglutinate when exposed to specific cell wall components of *S. aureus*, such as clumping factor or protein A (6, 10). Sensitivities of 98 to 100% have been reported for these procedures (1, 3, 10). However, some reports have noted that *S. aureus* isolates that are resistant to semisynthetic penicillins, such as oxacillin or methicillin, may yield false-negative reactions when tested with some of these methods (1, 5, 14).

In our region, strains of *S. aureus* that possess a wide variety of antimicrobial susceptibility patterns have been recovered from clinical specimens. In addition to oxacillin-susceptible *S. aureus* and oxacillin-resistant *S. aureus*, we have encountered strains of *S. aureus*, such as those recently described (7), with intermediate resistance (oxacillin MIC, 2.0 to 4.0 μg/ml). Furthermore, strains of oxacillin-resistant *S. aureus* (MIC, >8.0 μg/ml) have shown great variation in susceptibility to other classes of antimicrobial agents.

A total of 266 recent clinical isolates of catalase-producing, gram-positive cocci were collected from the clinical microbiology laboratories of Cedars-Sinai Medical Center, Los Angeles, and the West Los Angeles Veterans Administration Medical Center, Los Angeles. In addition, 14 other strains of *S. aureus* from other institutions in Southern California were tested; these were obtained from M. Appleman, University of Southern California County Medical Center (8 isolates), and Janet Hindler, University of California at Los Angeles Medical Center Clinical Laboratories, Los Angeles (6 isolates). Each isolate was recovered from a different patient. The isolates were tested by the following methods: slide coagulase (SC) or tube coagulase (TC) or both (11), Staphyloslide (BBL Microbiology Systems, Cockeysville, Md.), and StaphAurex (Wellcome Diagnostics, Research Triangle Park, N.C.). Testing with the experimental test methods was performed as recommended by the manufacturers. If a discrepancy was noted between the results of the TC test and the experimental test systems, the identity of the organism as *S. aureus* was confirmed by mannitol utilization and the thermostable nuclease test (15).

Isolates identified as *S. aureus* were tested for susceptibility to oxacillin by standard disk diffusion methods (4), as modified by Baker et al. (2). Isolates that showed a clear zone of inhibition of ≥13 mm around a 1-μg oxacillin disk and that were not resistant to other non-beta-lactam drugs were considered to be oxacillin susceptible. Those that were intermediate or resistant to oxacillin or that were noted to be resistant to other classes of drugs were further studied for susceptibility to oxacillin by broth microdilution with cation-supplemented Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) further supplemented with 2% NaCl; inocula were prepared from overnight growth on an agar plate, and incubation was done at 35°C for 24 h (8, 12). Isolates of *S. aureus* were classified on the basis of susceptibility tests in the following way: oxacillin-susceptible *S. aureus* strains were those that had a zone of ≥13 mm in disk diffusion or MICs of <1.0 μg/ml in broth microdilution; oxacillin-intermediate *S. aureus* strains were those that had MICs of 2.0 to 4.0 μg/ml; and oxacillin-resistant *S. aureus* strains were those that had MICs of ≥8 μg/ml. Susceptibility to rifampin (MIC, <1.0 μg/ml) was determined by the same method as for oxacillin except that NaCl supplementation was omitted. Standard disk diffusion methods (4, 9) were used to determine susceptibilities to clindamycin, chloramphenicol, erythromycin, gentamicin, trimethoprim-sulfamethoxazole, and vancomycin.

Of the 280 isolates tested, 180 were identified as *S. aureus* by the SC test or TC test or both, of which 100 were oxacillin-susceptible *S. aureus*, 7 were oxacillin-intermediate *S. aureus*, and 73 were oxacillin-resistant *S. aureus*. The remaining 100 isolates were designated coagulase-negative members of the family *Micrococcaceae*.

The results of tests with StaphAurex, Staphyloslide, and SC are as follows. All oxacillin-intermediate and -susceptible *S. aureus* strains were correctly identified by the StaphAurex and Staphyloslide tests. The SC test yielded 97 of 100 (97%) and 4 of 7 (57%) positive results for those strains, respectively.

In the oxacillin-resistant *S. aureus* group, 56 of 73 (77%), 55 of 73 (75%), and 52 of 73 (71%) strains were correctly identified by the StaphAurex, Staphyloslide, and SC tests, respectively. One strain which produced latex agglutination in the StaphAurex test demonstrated delayed and equivocal hemagglutination in the Staphyloslide test and was recorded as a negative test. All strains that were misidentified by the

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TABLE 1. Results of susceptibility tests for rifampin and trimethoprim-sulfamethoxazole for 73 isolates of oxacillin-resistant *S. aureus* correlated with the results of rapid agglutination tests

Susceptibility result (no. of isolates)	No. (%) of strains with indicated rapid agglutination test result	
	Negative	Positive
Resistant to rifampin (21)	18 (86)	3 (14)
Resistant to trimethoprim-sulfamethoxazole (18)	18 (100)	0 (0)
Susceptible to rifampin (52)	0 (0)	52 (100)
Susceptible to trimethoprim-sulfamethoxazole (55)	0 (0)	55 (100)

new test systems were also misidentified by the SC test. All strains that were TC positive but negative in the latex agglutination or hemagglutination test were confirmed as *S. aureus* by demonstration of thermostable nuclease activity and mannitol utilization.

The StaphAurex test correctly identified 100% of the coagulase-negative strains. In the Staphyloslide test, 9 of 100 of the TC-negative strains produced clumping when mixed with both the fibrinogen-sensitized erythrocytes and the negative control (unsensitized erythrocytes); these results were judged to be noninterpretable.

Major differences in susceptibility patterns were noted between strains that tested false-negative in either of the experimental tests and strains that yielded positive results (Table 1). Of 73 oxacillin-resistant *S. aureus* strains, false-negative results were obtained with the StaphAurex and Staphyloslide tests for 17 strains, and 1 additional strain tested false-negative in the Staphyloslide test only; all 18 strains were resistant to trimethoprim-sulfamethoxazole and rifampin, whereas 55 and 52 of the 55 strains which tested positive were susceptible to trimethoprim-sulfamethoxazole and rifampin, respectively. Susceptibility to other drugs (clindamycin, chloramphenicol, erythromycin, gentamicin, and vancomycin) could not be correlated with false-negative test results.

The results we obtained with the StaphAurex and Staphyloslide tests in differentiating oxacillin-susceptible *S. aureus* and coagulase-negative *Micrococcaceae* strains are comparable to the results obtained by other investigators with latex agglutination test systems (1, 5, 10) and the Staphyloslide test system (1, 3, 5, 10). Oxacillin-intermediate *S. aureus* strains have not been tested previously as a separate group by other investigators. Although the total number of oxacillin-intermediate *S. aureus* strains in this study was small, all were detected by both the StaphAurex and Staphyloslide tests. Thus, for oxacillin-susceptible and -intermediate *S. aureus* strains, both test systems demonstrated sensitivities of 100%.

The marked failure to detect 25% of oxacillin-resistant *S. aureus* strains by both of these test methods was the most striking finding. This high rate of failure is particularly noteworthy when compared with results of previous studies with these organisms and these test methods which reported failure rates of 1% (14), 2 to 4% (5), and 0 to 12% (1).

A review of the antimicrobial susceptibility profiles showed that the failure of the two test methods to identify oxacillin-resistant *S. aureus* strains was highly correlated with resistance to trimethoprim-sulfamethoxazole and rifampin. These were the only antimicrobial susceptibility results predictive of test procedure failure.

The resistance of oxacillin-resistant *S. aureus* strains to trimethoprim-sulfamethoxazole or rifampin or both appears to be unusual in other regions of the country (13). Our oxacillin-resistant *S. aureus* isolates that tested negative by the evaluated methods were obtained from patients hospitalized in three separate institutions; it is possible that these highly resistant strains have become endemic in Southern California. Further characterization of these strains is indicated and may help to clarify this issue. In addition, it is interesting to consider that the high degree of antimicrobial resistance of these strains may be related to alterations in cell wall structure that also may reduce the amount or availability of clumping factor or protein A or both for binding to the sensitized particles used in the test methods.

Our findings indicate that the use of new methods for the rapid identification of *S. aureus* should be carefully evaluated in each laboratory that encounters oxacillin-resistant *S. aureus* strains and that strains resistant to trimethoprim-sulfamethoxazole or rifampin or both may be especially likely to give false-negative results.

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