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NOTES

Staphylococcus aureus Strains Which Are Not Identified by Rapid Agglutination Methods Are of Capsular Serotype 5

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A total of 183 recent *Staphylococcus aureus* clinical isolates were tested with three commercially available rapid agglutination methods. The capsular polysaccharide type and resistance to oxacillin of these isolates were also determined. Seven isolates were not identified correctly by agglutination methods. All isolates not identified by the rapid methods were of capsular serotype 5, and of these isolates, six were resistant to oxacillin. The results suggest that these agglutination kits can be improved by the use of antibodies reactive with *S. aureus* capsular polysaccharide.

Rapid procedures capable of distinguishing *Staphylococcus aureus* from coagulase-negative staphylococci have been developed. These methods use either latex particles coated with human plasma for the simultaneous detection of protein A and clumping factor (8) or sheep erythrocytes sensitized with fibrinogen for the detection of clumping factor (9). Commercially available systems using these methods have been evaluated in clinical situations, and the results have been controversial. Although most of these evaluations have shown good correlation with reference methods (1, 3, 6–8, 12, 14–17, 20, 22), some reports have noted that oxacillin-resistant *S. aureus* isolates may yield false-negative reactions with these kits (1, 4, 18, 21, 25).

Capsular polysaccharides have been characterized in clinical isolates of *S. aureus* in humans (13). Surveys have shown that two capsular serotypes, 5 and 8, account for about 70 to 80% of serological types (2, 11, 23). A predominance of capsular serotype 5 among oxacillin-resistant *S. aureus* isolates has also been described (10).

In this study, we investigated whether a capsular serotype predominates among *S. aureus* isolates which are not identified by rapid agglutination methods.

A total of 183 recent *S. aureus* clinical isolates were collected from the clinical microbiology laboratories of five hospitals (Hôtel-Dieu, Hôpital Saint-Joseph, Hôpital Bichat, and Hôpital Claude-Bernard, Paris, and Hôpital Antoine-Béclère, Clamart, France). The organisms were isolated either from blood cultures or from purulent or inflammatory processes. Each isolate was recovered from a different patient. Each isolate was taken from maintenance medium (Columbia agar; Difco Laboratories, Detroit, Mich.), was streaked onto tryptic soy agar plates (no. 64557; Diagnostics Pasteur, Marnes-la-Coquette, France), and was incubated overnight at 37°C. A single colony was passed into coagulase test broth (no. 53545; Diagnostics Pasteur) and was grown overnight at 37°C. Broth culture was passed onto both Columbia agar (Difco) and Mueller-Hinton agar plates (Difco).

The tube coagulase test was done by mixing 0.5 ml of broth culture with 0.5 ml of oxalated rabbit plasma (no. 56351; Diagnostics Pasteur) in a sterile hemolysis tube. The tube was incubated at 37°C and was examined at 4 and 24 h. Clot formation at either reading was recorded as positive.

Capsular serotyping was done by the detection of type 5 and 8 capsular polysaccharides of bacteria grown on Columbia agar. Bacteria were collected off agar slants by washing with phosphate-buffered saline (pH 7), were transferred to glass tubes, and were autoclaved. After centrifugation, capsular polysaccharide was detected in the supernatant by enzyme-linked immunosorbent assay using monoclonal antibodies as previously described (19).

Resistance to oxacillin was studied on isolates grown on Mueller-Hinton agar. The MICs of oxacillin were determined by twofold dilutions of the drug in Mueller-Hinton agar. Final concentrations ranged from 0.025 to 256 μg of oxacillin (Bristol Laboratories, Paris, France) per ml. Stationary-phase broth cultures were diluted 1 in 100 to deliver ca. 10^4 organisms to each plate with a Steers replicator (24). The plates were incubated at 30°C and were evaluated at 24 and 48 h. *S. aureus* ATCC 25923 was used as a control strain.

The following rapid agglutination methods were tested on isolates grown on Mueller-Hinton agar: Staphyslide test (no. 55081; BioMérieux, Charbonnières-les-Bains, France), which consists of two reagents: (i) sheep erythrocytes sensitized with fibrinogen with 0.1% sodium azide preservative and (ii) nonsensitized sheep erythrocytes with 0.1% sodium azide preservative as a negative control; StaphAurex (Wellcome Diagnostics, Research Triangle Park, N.C.), which consists of latex particles coated with fibrinogen for the detection of clumping factor and with immunoglobulin G for the detection of protein A; and Pastorex Staph (Diagnostics Pasteur), which consists of latex particles sensitized with human plasma for the simultaneous detection of clumping factor and protein A.

Testing with the commercial kits was done as recommended by the manufacturers. Each of the strains was tested with each method on the same day by the same operator.

The isolates were examined for susceptibility to typing bacteriophages by the technique of Blair and Williams (5).

Among 183 isolates tested, 79 (43%) contained type 5 capsular polysaccharide, 75 (41%) contained type 8 capsular

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TABLE 1. *S. aureus* isolates not identified by rapid agglutination methods

Isolate no.	Hospital[a]	Source	MIC of oxacillin (μg/ml)	Lysotype
1	CB	Sputum	>256	6/47/54/75/77/84
2	CB	Blood culture	>256	6/47/54/75/77
3	SJ	Pus (abscesses)	64	47/54/75/77
4	AB	Blood culture	0.25	94/96
5	AB	Sputum	>256	77
6	CB	Blood culture	64	Resistant
7	HD	Pus (abscesses)	128	Resistant

[a] CB, Claude-Bernard; SJ, Saint-Joseph; AB, Antoine-Béclère; HD, Hôtel-Dieu.

polysaccharide, and 29 (16%) were nontypeable with antibodies specific for type 5 or 8 capsular polysaccharide. Among the 50 isolates resistant to oxacillin, 46 were of capsular type 5, 2 were of type 8, and 2 isolates were nontypeable.

Of 183 *S. aureus* isolates positive in the tube coagulase test, 172 isolates (68 type 5, 75 type 8, and 29 nontypeable) were positive with Staphyslide, StaphAurex, and Pastorex Staph, and 4 isolates (type 5) were positive with only one ($n = 1$) or two ($n = 3$) of these kits. These 176 isolates of *S. aureus* were considered to be correctly identified by these rapid agglutination methods.

Seven isolates failed to produce latex agglutination in the StaphAurex and Pastorex Staph tests and hemagglutination in the Staphyslide test. All seven isolates were of capsular type 5. Of these isolates, six were resistant to oxacillin (MIC, >64 μg/ml) and also to several other antibiotics such as aminoglycoside, tetracycline, and clindamycin. The source, the MIC of oxacillin, and the phage pattern for each of these isolates are listed in Table 1. The diverse characteristics of these isolates show that they do not constitute a unique clone which could have disseminated in Parisian hospitals.

In our study, rapid agglutination tests correctly identified 132/133 (99%) oxacillin-susceptible *S. aureus* isolates. If we consider the total of oxacillin-susceptible ($n = 133$) and oxacillin-resistant ($n = 50$) *S. aureus* isolates studied, these tests correctly identified 176 (96%) isolates. These results agreed with previous studies with these rapid test methods which reported sensitivities of 95 to 100% (1, 3, 6–8, 12, 14–17, 20, 22). However, if we consider only oxacillin-resistant *S. aureus*, 6 of 50 (12%) isolates were not identified by rapid agglutination methods. Among the 44 oxacillin-resistant isolates which were correctly identified, 40 were of capsular type 5, 2 were of type 8, and 2 isolates were nontypeable. This relatively high rate of failure of these tests to detect oxacillin-resistant *S. aureus* isolates is comparable with previous results of other investigators (1, 4, 18, 21, 25). This failure is particularly noteworthy since infections provoked by these isolates which are often multiresistant necessitate the use of appropriate therapy.

One of the more interesting observations with the capsular serotyping was that all isolates not identified by rapid agglutination methods were of capsular serotype 5. This observation may indicate that the cell wall structures recognized by these reagents (clumping factor or protein A or both) are not exposed on the surface of these isolates. Such modification might be explained by the presence of a large amount of capsular polysaccharide masking other cell wall structures. Electron microscopy examination of the capsular polysaccharide of *S. aureus* has indeed shown the variation in the degree of encapsulation, depending on the culture conditions (11). However, further microscopic studies of the various antigens exposed on the surface of *S. aureus* bacterial cells are necessary to examine this hypothesis.

The observation that *S. aureus* isolates which are not identified by rapid agglutination methods do possess capsular polysaccharide offers the possibility of improving these currently available agglutination kits by adding to these reagents particles sensitized with antibodies which react with *S. aureus* capsular polysaccharide. Such improvement may be applicable for the preparation of rapid agglutination kits reliable for both oxacillin-susceptible and oxacillin-resistant *S. aureus* isolates.

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