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# Use of the Clumping Factor Reaction for the Identification of Encapsulated Strains of *Staphylococcus aureus* from Human Sources

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Using the clumping factor test on 815 strains of *Staphylococcus aureus*, 775 positive strains were of compact morphology. Of 40 negative strains, 39 were diffuse in serum-soft agar. The test may be used to detect capsulated *S. aureus* strains.

Encapsulated strains of *Staphylococcus aureus*, which had negative clumping factor reactions, have been found to be highly virulent for mice. A simple screening procedure, the slide coagulase test (1), now used extensively as a preliminary differential method in clinical work for the rapid speciation of *S. aureus*, was proposed for possible use as a screening method to detect encapsulated strains of this organism.

The 815 strains of *S. aureus* used in this experiment were isolated from clinical materials at the 406th Medical Laboratory, Kanagawa, Japan. Suspect colonies were screened by Gram staining, catalase testing, and utilization of glucose both aerobically and anaerobically. Of those strains determined to be staphylococci, those exhibiting a positive tube coagulase and deoxyribonuclease were speciated as *S. aureus* (3). Bacteriophage and serotyping were accomplished on the indicated strains, as reported previously, and those showing a negative clumping factor reaction or a diffuse colony type in serum-soft agar (4) had India ink preparations made for light microscope examination.

With these strains, positive reactions with tube coagulase, deoxyribonuclease, mannitol fermentation, and clumping factor reactions were 97.3, 98.4, 95, and 95%, respectively, for the 815 strains shown in Table 1.

Strains positive for clumping factor reaction were also positive both in tube coagulase and deoxyribonuclease production; however, 3.3% of 775 strains were negative for mannitol attack under any conditions of aerobiasis. Growth in serum-soft agar of these mannitol-negative strains was compact without exception. Bacteriophage and serotypabilities were 71.6 and 97.5%, respectively.

Of the negative clumping-factor reaction strains, 55, 32.5, and 37.5% of 40 strains gave negative tube coagulase, negative deoxyribonuclease, and negative mannitol fermentation, respectively, as shown in Table 2, suggesting a lower production of these enzymes than reported previously (5). These strains were of a diffuse type growth in serum-soft agar, nontypable by phage and serology, and, except for one strain, capsules were visible under the light microscope. This exceptional strain, designated 5928, was negative for clumping factor reaction even in undiluted fresh plasma but gave a typical compact type growth in serum-soft agar, and a capsule could be demon-

TABLE 1. *Coagulase, deoxyribonuclease, mannitol fermentation, and clumping factor reactions of 815 strains of human source Staphylococcus aureus*

| Test | Reaction | No. of strains | Per cent |
|---|---|---|---|
| Coagulase (tube) | + | 793 | 97.3 |
| | − | 22 | 2.7 |
| Deoxyribonuclease | + | 802 | 98.4 |
| | − | 13 | 1.6 |
| Mannitol fermentation | + | 774 | 95.0 |
| | − | 41 | 5.0 |
| Clumping factor | + | 774 | 95.0 |
| | − | 41 | 5.0 |

TABLE 2. *Coagulase, deoxyribonuclease, mannitol fermentation, colonial morphology in serum-soft agar, phage, and serotypabilities of positive and negative clumping factor reactions in human source Staphylococcus aureus*

| Test | Reaction | Clumping factor reactions | |
|---|---|---|---|
| | | Positive (775 strains) | Negative (40 strains) |
| Coagulase (tube) | + | 775 | 18 |
| | − | 0 | 22 |
| Deoxyribonuclease | + | 775 | 27 |
| | − | 0 | 13 |
| Mannitol fermentation | + | 749 | 25 |
| | − | 26 | 15 |
| Diffuse | | 0 | 39 |
| Compact | | 775 | 1 |
| Phage typable | | 554 | 1 |
| Phage nontypable | | 221 | 39 |
| Serotypable | | 773 | 1 |
| Nonserotypable | | 2 | 39 |

strated by using the light microscope. The phage type of strain 5928 was 53/86 and serotype was Oeding bc, Pillet 10 (4).

No reports on the clumping factor reaction for encapsulated strains of *S. aureus* have appeared in the literature. Lipinski et al. (2) described the mechanisms for the clumping factor reaction and suggested that the compact colonial morphology in serum-soft agar was produced by clumping factor. In our experimental results, negative clumping factor *S. aureus* strains were, with one exception, encapsulated strains. This exception, negative for clumping factor but positive for encapsulation, was not of the mucoid type encapsulation reported previously (4, 5). Rather, it represents a new kind of encapsulation and further investigation is underway on this strain.

The problem also remains to determine if there are indeed encapsulated strains of *S. aureus* that are clumping factor positive. The use of the clumping factor test as a significant screening procedure to determine and identify encapsulated strains of *S. aureus* is thus proposed, and those strains which are clumping factor-negative may then be further tested in serum-soft agar and by India ink for true encapsulation, whereas the clumping factor-positive strains are never encapsulated and need no further testing.

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