Comparision of methods to differentiate Staphylococcus and Micrococcus species isolated from bovine mammary glands

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(Received 15. September, 2002)

The hemolytic pattern of colonies, the slide coagulase (clumping factor) test, the tube coagulase test and the thermonuclease test were studied for their capability to differentiate Staphylococcus aureus from other Staphylococcus and Micrococcus species. A total 93 strains of Staphylococcus aureus isolated from bovine udder glands were tested. The hemolytic pattern of colonies and the slide coagulase test were found to be less reliable than other tests. No important differences could be found between the tube coagulase test and the thermonuclease test. The API-Staph test was used as a reference.

Key words: cow, laboratory methods, mastitis, Staphylococcus aureus

Introduction

Staphylococcus aureus (S. aureus) is a common cause of contagious mastitis in dairy herds and also an important pathogen in humans (Barkema et al. 1998). In the control of staphylococcal mastitis antibiotic therapy continues to play an important role (Craven et al. 1986). However, despite a variety of available antibiotics, the success of treatment of S. aureus mastitis, particularly during lactation, is still very low (Owens et al. 1997). Preventive measures are therefore essential to reduce the prevalence of S. aureus mastitis in a given herd. For a successful mastitis control program, accurate laboratory diagnostic procedures are crucial (Eberhart et al. 1987).

The tube coagulase test is a valid means of identifying S. aureus (Hogan et al. 1986). However, observing haemolysis and performing the slide coagulase test is quicker, cheaper and easier. Most clinical laboratories still depend entirely upon the coagulase test for distinguishing S. aureus from other species in the genera Staphylococcus (Devriese 1980, Hodges et al. 1984). Inaccurate results with the coagulase test would lead to an identification error, which could have serious clinical implications. Such technical errors could go undetected unless the procedure is controlled by use of a separate test to confirm each identification (Martin et al. 1987). For this purpose, the inexpensive, simple and rapid toluidine blue deoxyribonucleic acid (DNA) agar (TDA) technique of Lachica et al. (1971) was adapted to permit detection of thermonuclease (heat-stable nuclease) at the same time that a coagulase test is performed. The TDA technique is more specific than the
conventional test on DNA agar plates because the heat-labile enzymes of other *Staphylococcus* and *Micrococcus* species are inactivated before testing (Barry *et al.* 1973).

### MATERIAL AND METHODS

A random sample of 93 strains of *Staphylococcus aureus* isolated from bovine udder glands was collected and tested with the methods described below. In all tests, positive and negative controls were used. The slide coagulase test was used to confirm the presence of bound coagulase or “clumping factor”. The test was performed as described by the manufacturer of the coagulase plasma (Bacto coagulase plasma EDTA, 0803 Difco Laboratory, Detroit, USA). If a clumping reaction could be observed within 10 seconds, the sample was considered to be positive. If the reaction was weak or if it occurred after 10 seconds the sample was judged as doubtful. Free coagulase production was determined by the tube method. Approximately 0.1 ml of the Brain heart infusion (BHI) suspension was added to 0.5 ml of reconstituted rabbit plasma (EDTA, Difco). Tubes were incubated at 35°C and results were read after 4 and 24 hours. No reaction or a flocculent or fibrous precipitate was considered as a negative result (Kloos 1980). For the thermonuclease test, which detects for the presence of heat-stable nuclease, DNA agar was used. The bottom of a petri plate (90 mm diameter) was filled with toluidine blue DNA agar. Twelve small wells (3 mm diameter) were cut in this agar. After being allowed to cool, 10 µl of the BHI broth that had been heated at 100°C for 15 minutes was dispensed into each of the wells. The plates were incubated at 35°C and the results were read at 4 and 24 hours. Positive reactions were bright pink zones, indicative of nuclease activity.

### RESULTS AND DISCUSSION

The objective of the present study was to quantify the reliability of diagnostic tests and combinations of tests that can be used as a standard laboratory technique to differentiate *S. aureus* from other *Staphylococcus* and *Micrococcus* species. For this purpose several techniques were compared to the results of the API-Staph test and to each other.

Of the 93 cultures tested by the API-Staph system, 57 (61.3%) were identified as *S. aureus* and 36 as coagulase negative *Staphylococcus* (CNS) species. Of the identified CNS species, *Staphylococcus hyicus* was found most frequently (*n=12; 12.9%*), followed by *S. hominis* (*n=10; 10.7%*), *S. xylosus* (*n=6; 6.5%*) and *S. simulans* (*n=5; 5.3%*). All other species were found only once. The differentiation of these cultures to the species level is presented in table 1. Of the species that were found more than once, the positive results in the tests performed are presented in table 2.

The data obtained in our study and data from the literature (Jasper *et al.* 1966) indicate that the slide coagulase test and the hemolytic patterns are not as reliable as the other tests used. On the other hand they are quick and cheap meth-
ods and therefore common practice in applied bovine mastitis bacteriology (Devriese et al. 1979).

Table 1. Frequency distribution for all cultures (n=93) tested, according to the results of the API-Staph test

<table>
<thead>
<tr>
<th>Species</th>
<th>Number</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>S. aureus</td>
<td>57</td>
<td>61.3</td>
</tr>
<tr>
<td>S. hyicus</td>
<td>12</td>
<td>12.9</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>10</td>
<td>10.7</td>
</tr>
<tr>
<td>S. hominis</td>
<td>6</td>
<td>6.5</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>5</td>
<td>5.3</td>
</tr>
<tr>
<td>S. simulans</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>S. lentus</td>
<td>1</td>
<td>1.1</td>
</tr>
<tr>
<td>S. warneri</td>
<td>1</td>
<td>1.1</td>
</tr>
</tbody>
</table>

Table 2. Number of isolates diagnosed correctly for each of the tests used (n=93)

<table>
<thead>
<tr>
<th>Method</th>
<th>Correct diag. (n)</th>
<th>Correct diag. %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hem</td>
<td>73</td>
<td>78</td>
</tr>
<tr>
<td>CF</td>
<td>83</td>
<td>89</td>
</tr>
<tr>
<td>C - 4</td>
<td>88</td>
<td>95</td>
</tr>
<tr>
<td>C - 24</td>
<td>89</td>
<td>96</td>
</tr>
<tr>
<td>TN - 4</td>
<td>89</td>
<td>96</td>
</tr>
<tr>
<td>TN - 24</td>
<td>90</td>
<td>97</td>
</tr>
</tbody>
</table>

Hem – presence of alpha – beta or beta hemolysis  
CF – slide coagulase  
C-4 – tube coagulase test read after 4 hours  
C-24 – tube coagulase test read after 24 hours  
TN-4 – thermonuclease test read after 4 hours  
TN24 – thermonuclease test read after 24 hours

Considering the results of this study it could be concluded that either the coagulase (tube method) or thermonuclease test may be used for routine identification of S. aureus. However with regard to our experience, very little additional effort would be required to perform both tests routinely on all isolates. Because false negative or false positive results could not be detected with only one test, the routine use of both tests is recommended for the clinical laboratory.

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REFERENCES


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SADRŽAJ