Characterization of Coagulase-Positive Staphylococcus intermedias and Staphylococcus aureus Isolated from Veterinary Clinical Specimens

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Staphylococci were the most frequent isolates from clinical specimens submitted to a large referral and teaching veterinary hospital. In this study a total of 160 isolates were examined by a wide range of biochemical tests and modifications of basic procedures. An attempt was made to test the validity of these procedures for use in characterization of clinical isolates of coagulase-positive staphylococci. Of the isolates examined, some 27 were Staphylococcus aureus, 115 were Staphylococcus intermedias, and the rest were coagulase-negative staphylococci and were not characterized further. The most useful discriminatory tests were acid production from maltose incubated overnight on maltose purple agar (W. E. Kloos and K. H. Schleifer, J. Clin. Microbiol., 1:82–88, 1975), acetoin production detected by the Barritt method, and detection of hyaluronidase activity. These gave accurate and fast results. Supplemented with the tellurite reduction test and the direct staphylocoagulase assay using Chromozym TH (Engels et al.; J. Clin. Microbiol. 14:496–500, 1981), these tests should eliminate the possibility of false identifications of these two species.

In 1976 Hájek (10) proposed the name of Staphylococcus intermedias for coagulase-positive staphylococci which differed from Staphylococcus aureus in many biochemical reactions and in cell wall composition (11, 18). Briefly, it was found that they (i) did not produce acid from maltose, (ii) did not utilize mannitol anaerobically, (iii) did not produce acetyl-methylcarbinol, (iv) did not produce pigment, (v) reduced tellurite only occasionally, (vi) grew as the positive type (E type) on crystal violet agar, (vii) lacked cell wall polysaccharides A and B, (viii) had peptidoglycan of the L-Lys-Gly4.7-L-Ser0.2.1.0 type, and (ix) were not typable by the basic set of human and bovine phages.

Staphylococci are among the most frequent isolates from clinical specimens submitted from the Sydney University Veterinary Hospital and Clinic. In this study a total of 160 isolates were examined. Of these, 76% were from dogs, 6% were from horses, 6% were from cats, and the remainder came from other small animals and cattle. The purpose of this paper was to provide details of isolates of staphylococci classified as S. intermedias from the animals described and to compare their characteristics with those of other isolates of S. intermedias reported to date. A range of tests and modifications of basic procedures were included to try to test their validity for use in characterization of clinical isolates. Many of the procedures reported in the literature have been tested previously only on small numbers of isolates. There does not appear to be any report of large numbers of clinical isolates having been tested previously by the variety of procedures described in this paper.

MATERIALS AND METHODS

Bacterial strains. A total of 160 strains were included in this study. They had been isolated from a variety of clinical disease situations, including bacterial otitis externa (39.5%), purulent dermatitis (29%), reproductive tract infections (14%), cystitis (7.9%), abscesses (7%), osteomyelitis (2.6%), metritis, and mastitis. A total of 123 strains had been isolated from dogs, 10 from horses, 10 from cats, and 17 from other small animals and cattle.

All bacterial strains were isolated on agar plates (Oxoid blood agar base no. 2) to which 5% (vol/vol) defibrinated sheep blood had been added (BAB). After initial isolation, organisms were stored lyophilized in skim milk until required for characterization. Inocula for biochemical tests were grown overnight at 37°C in brain heart infusion broth (Difco Laboratories).

Morphology and atmospheric requirements. Colonial morphology was observed on P-agar (13), and hemolysis was observed on BAB incubated aerobically at 37°C. Cultures from liquid media were examined for motility in wet hanging-drop preparations. Gram stains were performed on organisms grown on solid and in liquid media. Atmospheric requirements were tested by growth on BAB aerobically and anaerobically in an
anaerobic jar (Oxoid Anaerobic System Gas Generating Kit). Organisms were grown also in tetrahionate broth (Oxoid Ltd.).

**Biochemical tests.** Catalase, oxidase, and lecithinase activities were determined by the methods described by Cowan (3). Organisms were tested in the modified benzidine test (6) for nitrate reduction and for acetyl-methylcarbinol (acetoin) production by the Barratt method (2) as well as by the rapid paper disk system of Davis and Hoyling (5).

Growth on 10 and 15% NaCl agar and crystal violet agar, detection of lipase, caseinase, and tellurite reduction, and pigmentation of 30% (vol/vol) full cream milk agar were performed as described by Hájek (10). Heat-stable DNase production (Tnase) was tested by the method of Lachica et al. (14), and hyaluronidase was tested according to the method of Murray and Pearce (15), using capsulated *Streptococcus equi* as the substrate for enzyme activity.

Acid production from carbohydrates was tested by the method recommended by the Subcommittee on Taxonomy of Staphylococeci and Micrococeci (19) and also by the method described by Cruickshank et al. (4). In the latter instance phenol red was used as an indicator. Acid production from maltose (aerobically) was performed also on maltose purple agar (12), and results were recorded after overnight incubation.

Clumping factor was determined with citrated rabbit plasma. Negative strains were retested with citrated dog plasma. The tube coagulase test was performed as recommended (19), using citrated rabbit plasma. Readings were made after 1 and 4 h of incubation at 37°C and after 18 and 24 h at room temperature. Negative strains were retested with citrated dog plasma. All strains were screened for coagulase activity by the rapid and direct assay described by Engels et al. (8), which used Chromozym TH and disodium EDTA as an inhibitor of the coagulase of *S. intermedius*.

Sensitivity to lysostaphin, which enables detection of difference in staphylococcal cell wall glycine cross bridges, was tested by using the disk method (17), with lysostaphin at 5 μg per disk, and the approach of Gramolli and Wilkinson (9). In the latter method, cultures at the beginning of the exponential growth phase were used, and concentrations of lysostaphin were 0.6 and 0.175 mg/ml. The initial turbidity was taken as 100%, and changes in turbidity were recorded. Lysozyme sensitivity was evaluated by the disk method of Poutrel and Caffin (17), using a concentration of 1 mg per disk for the lysozyme.

**RESULTS AND DISCUSSION**

All organisms studied were gram-positive, non-motile, benzidine- and catalase-positive cocci which grew on 15% NaCl agar, aerobically and anaerobically on BAB, and in thioglycolate broth medium. All were resistant to lysozyme and were oxidase negative.

All strains were subjected initially to the range of tests described by Hájek (10). From these results it was possible to divide the strains into two groups, the coagulase-positive, Tnase-positive group and the coagulase-negative, Tnase-negative group. Organisms in the first group were divided further by the following set of tests, which enabled rapid and reliable differentiation of organisms with least variability of results and inaccuracy of test interpretation: acid production from maltose and acid formation anaerobically from mannitol, production of acetyl-methylcarbinol (acetoin), tellurite reduction, pigmentation of colonies, coagulase activity as assayed by Chromozym TH and EDTA, and hyaluronidase production.

The results shown in Fig. 1 divided further the coagulase-positive, Tnase-positive strains into two groups. In Fig. 1 the coagulase-negative, Tnase-negative group has been included for comparison of their reactions also. Of the various methods used to detect coagulase, the coagulase assay with the chromogenic substrate was quite specific when EDTA was added to the reaction mixture (8). On the basis of these discriminatory tests, the coagulase-positive strains were designated *S. aureus* and *S. intermedius*. Of the strains studied, 27 were *S. aureus* and 115 were *S. intermedius*. Eighteen strains were not identified according to species.

With maltose purple agar (12), some 96% of the *S. aureus* strains produced acid overnight, whereas *S. intermedius* strains were negative at that time. After incubation for up to 5 days, some 52% became weakly positive or had colonies that showed a greenish yellow tinge. Phillips and Kloos (16) have suggested that the type of colonial pigmentation of this medium could be used to help differentiate among staphylococcal species. Some 50% of the coagulase-negative strains were positive also by day 5. When detection of acid from maltose was performed with phenol red as an indicator (4), most *S. intermedius* strains gave positive results. This is in agreement with results obtained with the API Staph System, using phenol red as an indicator (1).

The most suitable method for detection of acetoin production was the Barratt method, and cultures were tested first after 24 h of incubation.

![FIG. 1. Discriminatory characters of staphylococcal species tested. Symbols: ■. *S. aureus*; ○, *S. intermedius*; □, other staphylococcal species.](http://jcm.asm.org/Downloaded from October 26, 2017 by guest)
tion. Some 92% of *S. aureus* strains were positive at this time, whereas *S. intermedius* strains were always negative in this period (Fig. 1) and required 5 to 7 days for up to 31% to become positive or weakly positive. By day 5 some 66% of the other staphylococcal species showed acetoin production also. With the rapid disk micro-method for acetoin production, the majority of *S. intermedius* strains gave positive results. This was in agreement with findings of Bornstein and Fleurette (1). Although this finding thus invalidated the usefulness of acetoin production as a discriminatory test, they recommended the rapid disk micromethod because it seemed a more sensitive test of acetoin production.

In the tellurite reduction test, 84% of the *S. aureus* strains were positive. However, for a specific interpretation of the test, the duration of incubation had to be kept around 17 h. Prolonged incubation resulted in some *S. intermedius* strains (negative at 17 h) beginning to resemble *S. aureus*. The main drawback of this test was the failure of large numbers of *S. aureus* strains to grow on this medium.

Pigment was produced by 100% of the *S. aureus* strains, whereas all *S. intermedius* strains were negative in that test. This method was a useful discriminatory test, but it was time consuming. The same drawback was encountered when testing anaerobic acid production from mannitol, although in that case the results were less clear cut. Some 18% of the *S. aureus* strains failed to produce acid in this test, whereas 4% of the *S. intermedius* strains and 25% of the coagulase-negative strains were positive acid producers. Increased incubation beyond the 5 days recommended (19) resulted in a further 12% of the *S. intermedius* strains and 8% of the other staphylococci giving positive results.

Although only 20 strains of *S. aureus* and 40 strains of *S. intermedius* were tested for hyaluronidase activity, the result was unequivocal and confirmed the results previously published (7). All *S. aureus* strains produced hyaluronidase; none of the *S. intermedius* strains was positive in this test.

With the rest of the tests described (10), the variation among strains of *S. aureus* and *S. intermedius* was too small to be useful for differentiation (Table 1).

Sensitivity to lysostaphin was used to test the difference in the cell wall composition of all strains. According to Hájek and Marsalek (11) the peptidoglycan pattern of *S. intermedius* differs from that of other species by having serine in the interpeptide bridge. The results, as shown in Table 2 where the absolute decrease in turbidity of cultures is recorded, indicate a difference in susceptibility to lysostaphin, with some overlap between *S. aureus* and *S. intermedius*. In general, *S. aureus* strains lysed much faster than others, although there was some variation within the species. Therefore, the rate of lysis might be a better indicator of differences in the composition of the cell walls among the species.

Table 3 presents the results for acid formation aerobically from a wide range of carbohydrates for strains of *S. intermedius* only. These results also were not useful for differentiation.

The results presented here show that *S. intermedius* was the most common staphylococcal species encountered in the urban veterinary hospital from which these organisms were taken. The species could easily be differentiated from *S. aureus* by simple biochemical tests if some conditions described could be met. Acid production from maltose incubated overnight on maltose purple agar and acetoin production detected by the Barritt method gave accurate and fast results. Supplemented with the hyaluronid-

### Table 1. Some other characteristics of staphylococcal species tested

<table>
<thead>
<tr>
<th>Organisms</th>
<th>Egg yolk factor</th>
<th>Caseinase</th>
<th>Glycerol</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>45 (9)</td>
<td>22 (11)</td>
<td>100</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td>47 (10)</td>
<td>87 (8)</td>
<td>95 (3)</td>
</tr>
<tr>
<td>Other staphylococci</td>
<td>40 (10)</td>
<td>40 (20)</td>
<td>90</td>
</tr>
</tbody>
</table>

- *NA* Not attempted.

### Table 2. Comparison of staphylococcal species in a test designed to measure sensitivity to lysostaphin

<table>
<thead>
<tr>
<th>Organisms</th>
<th>% of initial turbidity after 120 min of exposure to the following concn (mg/ml) of lysostaphin:</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>S. aureus</em></td>
<td>0.6 0.175</td>
</tr>
<tr>
<td><em>S. intermedius</em></td>
<td>0.3–5 1–5</td>
</tr>
<tr>
<td>Other staphylococci</td>
<td>25–85 84*</td>
</tr>
</tbody>
</table>

*One culture only.*
dase test (which is very simple and uses organisms readily obtained in a veterinary laboratory) and, if possible, with the tellurite reduction test and the direct staphylocoagulase assay with Chromozym TH. These tests should eliminate the possibility of false identifications.

ACKNOWLEDGMENT

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LITERATURE CITED