Staphylococci from the Feces of Different Animal Species: Biotypes of *Staphylococcus aureus* Strains of Sheep and Goat Origin

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*Staphylococcus aureus* was found in 96% of the rectal swabs from 133 sheep and in 80% of the swabs from 125 goats. Seventy-seven percent of the isolates from both hosts exhibited the fibrinolytic and growth characteristics of human biotype A on human plasma and crystal violet agar, respectively, but 99% of these isolates resembled *S. aureus* of animal origin in their other properties. Only 21% of the sheep and 24% of the goat isolates were clearly identifiable as human biotype A and animal biotypes B and C.

In recent years several studies have focused attention on the biological properties of *Staphylococcus aureus* isolated from different animal species. Meyer (9) described two varieties of *S. aureus* of animal origin, *S. aureus* var. *bovis* and *S. aureus* var. *canis*, whereas Hájek and Marsálek (5) developed a new classification scheme for *S. aureus* based on certain physiological properties. In this scheme there exist five biotypes of animal origin and one of human origin.

Since most of the strains studied so far have been isolated from the upper respiratory tract, we were interested in extending the investigation to another source of staphylococci, namely feces. Therefore, the present paper is primarily concerned with the classification of such strains for a better understanding of the characteristics exhibited by staphylococci isolated from different mammalian hosts.

MATERIALS AND METHODS

Rectal swabs were taken from 133 sheep and 125 goats immediately after slaughter. The animals originated from several herds and different places of the country. None of the animals examined suffered from an apparent infectious disease.

The swabs were transferred to nutrient broth with 7.5% NaCl and incubated at 37°C for 24 h (12). Isolation of the *S. aureus* strains was made on egg yolk-tellurite-glycine-pyruvate agar (1) after observation of potassium tellurite reduction and egg yolk reaction.

Black colonies surrounded by a clear halo were Gram stained and examined for catalase activity and nitrate reduction. Aerobic and anaerobic utilization of glucose and mannitol was determined in O/F tubes (8) according to the recommendation of the Subcommittee on Taxonomy of Staphylococci and Micrococci (15).

Fresh human, rabbit, and bovine plasmas were used as substrates in tube tests (15) for coagulase production, and the results were recorded after 1, 2, 4, 8, and 24 h.

Production of α-, β-, and δ-hemolysins was noted on rabbit, sheep, and human blood agar plates, respectively (7).

Fibrinolytic activity was examined on medium containing 12% human citrated plasma (16).

Colonial pigmentation was observed on agar (Difco) containing 3% skim milk powder (3).

Growth characteristics of the colonies on agar containing crystal violet in a final concentration of 1:300,000 was evaluated by the method of Meyer (10).

The clumping factor was established by the use of rabbit plasma (2). Only reactions occurring within 10 s were considered to be positive.

RESULTS

Ninety-six percent of the sheep and 80% of the goats showed rectal carriage of *S. aureus*.

The total number of *S. aureus* strains tested was 496, of which 257 were isolated from sheep and 239 were isolated from goats.

Table 1 summarizes the characteristics by which the isolated strains were biotyped. All strains also coagulated rabbit plasma, reduced tellurite, were pigmented, and exhibited egg yolk and clumping factors.

By virtue of fibrinolytic activity and on the basis of their growth as violet colonies on crystal violet agar, the majority (384 [77.0%]) of the strains from both animals might be classified as human biotype A. However, 380 coagulated both the human and bovine plasmas, and 4 coagulated the bovine plasma only, properties of *S. aureus* biotype C strains. In this group of isolates, the production of α- and δ-hemolysins
was encountered in 240 strains; 83 strains produced δ-hemolysin; 51 produced α-, β-, and δ-hemolysins; 6 produced α-hemolysins; 1 produced β- and δ-hemolysins; and 3 were not hemolysin producers. One strain was of type A on crystal violet agar. The properties expressed by the strains of this group do not allow their classification in one of the known biotypes but, rather, characterize strains of an intermediate type.

Another group of 47 (9.0%) strains also produced fibrinolysin, coagulated human plasma, and grew on crystal violet agar in colonies of the negative violet type (type C). The production of α- and δ-hemolysins was found in 32 strains; 7 strains produced δ-hemolysin; 4 produced α-, β-, and δ-hemolysins; 2 produced δ-hemolysin; and 2 were hemolysin negative. All of these strains were classified as human biotype A.

In the group of fibrinolysin-negative strains, 20 isolates were classified as biotype B, and 45 had properties corresponding to biotype C.

The biotype B strains coagulated human plasma and grew in violet colonies as the negative type on crystal violet agar. Eight of them produced α- and δ-hemolysins; three produced δ-hemolysin; three produced α-, β-, and δ-hemolysins; three produced α-hemolysin; and three were hemolysin negative.

Of the 45 strains of the C biotype, only 3 produced orange pigment on crystal violet agar. All of the strains of this group coagulated human and bovine plasmas; 22 produced α- and δ-hemolysins; 7 produced δ-hemolysin; 7 produced α-, β-, and δ-hemolysins; 6 produced α-hemolysin; 2 produced α- and β-hemolysins; and 1 was hemolysin negative.

In summary, clear-cut results were obtained with 55 (21.0%) of the sheep strains and 57 (24.0%) of the goat strains. Of the sheep strains, 22 were of biotype A, 14 were of biotype B, and 19 were of biotype C. Among the goat strains, 25 were of biotype A, 6 were of biotype B, and 26 were of biotype C.

**DISCUSSION**

The data presented indicate that *S. aureus* is present in sheep examined by rectal swab at high frequency (96%). Papavassiliou and Dendrinos (11) found that, among 50 sheep examined, 5 carried *S. aureus* in their stools, but no more reports were available to us for a detailed correlation of our findings with those of other authors. Pulverer and Entel (13) also studied the staphylococci of sheep, but the isolation site of the strains was the upper respiratory tract and the frequency was 56%.

The frequency of *S. aureus* in the rectal swabs of the goats was also high (80%), but again we lacked reports from other laboratories for comparison. The frequencies found illustrate clearly the importance of sheep and goat feces as a reservoir of staphylococci and hence as a distribution source to the environment.

Most of the strains were fibrinolysin positive, a criterion considered reliable for characterization of human strains, but, at the same time, they coagulated bovine plasma, a character possessed by strains of animal biotypes (5). These two properties led us to classify these strains into an intermediate type without taking into account the hemolysin pattern as a key criterion for subdivision of the staphylococci. Although β-hemolysin production is indicative of animal strains (9), we do not consider that this property is acquired only after colonization of the animal, since there exist reports of β-hemolysin production alone or in combination with α- and δ-hemolysin by strains found in the nasal areas of healthy humans (4, 14). Until

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**Table 1. Characteristics and biotypes of *S. aureus* strains isolated from sheep and goats**

<table>
<thead>
<tr>
<th>Source of <em>S. aureus</em></th>
<th>Fibrinolysin</th>
<th>Reaction on crystal violet agar (type)*</th>
<th>Plasma coagulation</th>
<th>No. of strains</th>
<th>% of total isolates</th>
<th>Classification</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sheep</td>
<td>+ A</td>
<td>B, H, B + H</td>
<td>1</td>
<td>0.2</td>
<td>A</td>
<td>INT</td>
</tr>
<tr>
<td></td>
<td>+ C</td>
<td>B, H</td>
<td>3</td>
<td>0.6</td>
<td>B</td>
<td>INT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>22</td>
<td>4.4</td>
<td>C</td>
<td>INT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>198</td>
<td>40</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Goats</td>
<td>+ C</td>
<td>B, H</td>
<td>1</td>
<td>0.2</td>
<td>A</td>
<td>INT</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>25</td>
<td>5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>181</td>
<td>38.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- A</td>
<td>B, H</td>
<td>2</td>
<td>0.4</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>- C</td>
<td>B, H</td>
<td>6</td>
<td>1.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* A, Yellow colonies, positive type (10). C, Violet colonies, negative type (10).
B, Bovine; H, human.

* A, Human biotype (6); B and C, animal biotypes (6); INT, intermediate type (see text).
more data are available, the aforementioned strains might be considered of human origin but also possessing characters of animal isolates.

The usual association of man and domestic animals suggests that the 47 strains from both identified as biotype A probably originated in man. These strains exhibited properties similar to those of human isolates. Besides their fibrinolytic activity, they coagulated only the human plasma and grew in colonies of the negative violet C type on crystal violet agar.

About 13.0% of all the strains tested were found to belong to B and C biotypes. In the classification scheme of Hájek and Marsálek (5), the sheep strains are of the C biotype; this was also true with our strains, although some of them possessed the characters of the B biotype, a fact easily explained by the interchange of staphylococci among different animals due to their frequent contact. Since their properties were quite similar to those of the sheep strains, the present data furnished evidence for classification of the goat strains in the same biotype as cattle and sheep strains (C biotype).

With respect to the hemolysin pattern, we observed that animal strains were α-hemolysin producers, although Meyer (9) reserves this character only for the human strains. The hemolysin pattern of the staphylococci is in agreement with the statement of Hájek et al. (6) that hemolysins are not reliable basic criteria for subdivision of the staphylococci into biotypes.

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LITERATURE CITED