Animal Fecal Carriery and Biotypes of
Staphylococcus aureus

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Staphylococcus aureus was found in 44% of the rectal swabs from 377 pigs and in 12% of the swabs from 147 cows. Seventy-one percent of the isolates from pigs showed fibrinolytic activity and grew in colonies of the negative violet type on crystal violet agar. In this group, 47% of the isolates coagulated both human and bovine plasmas and were characterized as intermediate type, whereas 24% coagulated human plasma and were classified as human biotype A. Among the fibrinolysin-negative isolates, 15, 12, and 2% were identifiable as animal biotypes B, C, and E, respectively. The cow isolates were classified as intermediate type (51%), human biotype A (40%), and animal biotype C (9%).

The involvement of Staphylococcus aureus in human diseases and its great frequency in the environment have justified investigations into methods useful for subdividing this species into varieties or subspecies. The biochemical characteristics such as fibrinolysin production, coagulation of different animal plasmas, hemolysin patterns, growth on crystal violet agar, and pigment formation have been used extensively to divide this organism into varieties or biotypes. The subdivision of S. aureus into six biotypes, one of human origin and five of animal origin (8), seems to be the most acceptable and comprehensive of the proposed divisions (2).

The differences in the characteristics of S. aureus observed in strains isolated from different animals stress the importance of the mammalian host in the development of a specific S. aureus biotype and provide a useful and practical tool for tracing the origin of a strain isolated from either an infection or food product.

Most of the results appearing on the separation of S. aureus into biotypes are based on the characteristics of strains obtained from the upper respiratory tract of the animals. We have extended our investigations to also include strains from the feces of animals that live in close contact with humans and contribute to the distribution of S. aureus in the environment (5).

In this paper we report on the frequency of S. aureus fecal carriery and its biotypes among a large number of pigs and cows originating from different herds located in several parts of the country.

MATERIALS AND METHODS

Animals and sampling. A total of 377 pigs and 147 cows were examined. The animals involved in this study did not suffer from an apparent infectious disease. They had been brought to the Central Market of Athens from several herds and different places of the country. Fecal material was collected from the rectum by a sterile cotton swab immediately after the animals had been slaughtered.

Methods. The rectal swabs were streaked on egg yolk-tellurite-glycine-pyruvate agar (1), and the plates were incubated at 37°C for 18 h. On the same medium, the potassium tellurite reduction and the egg yolk reaction were observed. From each sample more than two black colonies surrounded by a clear halo were picked. Single colonies were inoculated into nutrient broth and incubated at 37°C for 6 h, the broth cultures being used for the tests.

Each culture was Gram stained and examined for catalase activity. The determination of aerobic and anaerobic utilization of glucose was carried out in oxidation-fermentation tubes (10) as modified by the Subcommittee on Taxonomy of Staphylococci and Micrococci (16).

The coagulation of fresh human, rabbit, and bovine plasmas was tested in tubes according to the recommendations of the Subcommittee on Taxonomy of Staphylococci and Micrococci (16). Fibrinolytic activity was noted on medium containing 12% human citrated plasma (18). The determination of the production of α-, β- and δ-hemolysins was with rabbit, sheep, and human erythrocytes, which had been added to heart infusion agar to a final concentration of 5%, by the replica plating technique (9).

Pigment formation was observed on agar (Difco) supplemented with 3% skim milk powder (4).

Growth characteristics of the colonies on crystal violet agar were evaluated by the method of Meyer (13).

The clumping factor was tested by the use of rabbit plasma, and only reactions occurring within 10 s were considered to be positive (3).

RESULTS

Of the total 377 rectal swabs from pigs, 165
(44%) yielded *S. aureus* on Baird-Parker medium. Among the cows, the rectal carriage of *S. aureus* was comparatively low, since out of 147 animals examined, 19 (12%) were found to be positive for *S. aureus*.

The total number of *S. aureus* strains tested was 327, of which 292 were isolated from pigs and 35 were isolated from cows.

Table 1 summarizes the characteristics by which the isolated strains were classified into biotypes. All of the strains coagulated rabbit plasma, reduced tellurite, and exhibited egg yolk and clumping factors, and all but seven from pigs were pigmented.

The majority (206, 71%) of the strains isolated from pigs showed fibrinolytic activity, a criterion considered characteristic of strains of human origin, and grew on crystal violet agar in colonies of the negative violet type (type C). Of these strains, 138 (47%) coagulated both human and bovine plasmas, a property of animal strains. The combination of these characteristics does not represent one of the known biotypes, but rather classifies these strains in an intermediate type.

The remaining 68 (24%) isolates of the fibrinolysin-positive group coagulated human plasma and were classified as human biotype A.

The group of fibrinolysin-negative strains consisted of 86 (29%) isolates. Based on the coagulation of human and bovine plasmas and their growth characteristics on crystal violet agar, 44 (15%) isolates were classified as biotype B and 35 (12%) isolates were classified as biotype C. Seven (2%) fibrinolysin-negative strains grew on crystal violet agar in colonies of the positive white type (type E); they coagulated human plasma, were not pigmented, and were classified as biotype E. This biotype is isolated from dogs and probably from horses.

Thirty-two (91%) of the *S. aureus* isolates from cows were fibrinolysin positive and grew on crystal violet agar in colonies of the negative violet type. Eighteen of them coagulated both human and bovine plasmas and were classified as the intermediate type, whereas 14 coagulated human plasma and were classified as human biotype A.

Only three isolates from cows had the properties characteristic of biotype C; i.e., they were fibrinolysin negative and coagulated both human and bovine plasmas, and their colonies on crystal violet agar were of the negative violet type.

The hemolysin patterns encountered among the pig and cow isolates of *S. aureus* are presented in Table 2.

From the analysis, it may be seen that from the 292 pig isolates of *S. aureus* examined, 138 (47%) were characterized as the intermediate type, 68 (24%) were classified as human biotype A, 44 (15%) were of biotype B, 35 (12%) were of biotype C, and 7 (2%) were of biotype E. Of the 35 isolates of *S. aureus* from cows, 18 (51%)

### Table 1. Characteristics and biotypes of *S. aureus* strains isolated from pigs and cows

<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 isolates</th>
<th>Classification†</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Pigs</strong></td>
<td>+</td>
<td>C</td>
<td>B</td>
<td>68</td>
<td>24</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>A</td>
<td>H</td>
<td>31</td>
<td>11</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>C</td>
<td>H + B</td>
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<td>4</td>
<td>C</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>E</td>
<td>H</td>
<td>13</td>
<td>4</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B + H</td>
<td></td>
<td>24</td>
<td>8</td>
<td>C</td>
</tr>
<tr>
<td><strong>Cows</strong></td>
<td>+</td>
<td>C</td>
<td>B</td>
<td>14</td>
<td>40</td>
<td>A</td>
</tr>
<tr>
<td></td>
<td>-</td>
<td>C</td>
<td>H</td>
<td>18</td>
<td>51</td>
<td>INT</td>
</tr>
</tbody>
</table>

* A, Yellow colonies, positive type (13); C, violet colonies, negative type (13); E, white colonies, positive type (13).
† B, Bovine; H, human.
† A, Human biotype (8); B, C, and E, animal biotypes (8); INT, intermediate type (see text).
were of the intermediate type, 14 (40%) were classified as human biotype A, and 3 (9%) were of biotype C.

DISCUSSION

S. aureus was present in pigs examined by rectal swabs in a relatively high frequency (44%) compared with the frequencies reported by other authors. Tzanetis and Dimitracopoulos (17) found S. aureus in 18% of the stools of 11 pigs, and about the same frequency was reported by Wilsens and Vande Casteele (19). Papavasiliou and Dendrinos (14) reported a frequency of 16%, whereas Smith and Jones (15) did not isolate S. aureus from the stools of healthy pigs. The same discrepancy in the carrierness of S. aureus among pigs is observed with the strains isolated from the upper respiratory tract. Hajek and Marsalek (7) examined 71 pigs and found nasal carriage of S. aureus in 78.9% of them. The same authors cited results of other investigators who found that frequencies of nasal carriage of S. aureus ranged from 1 to 64%. The differences observed among the aforementioned results might be attributed to differences in the environmental conditions of the animals examined.

The frequency of S. aureus in the rectal swabs of cows was low (12%) and exactly the same as that found by Tzanetis and Dimitracopoulos (17), who examined a considerably smaller number of animals. It is interesting to note here that about the same frequency (12.5%) was reported by Hajek and Marsalek (6) for strains of S. aureus isolated from the external nares of healthy cattle. It is possible that neither the respiratory tract nor the rectal environment of cattle is favorable for staphylococci.

By the existing nine biochemical tests, S. aureus strains may be subdivided into six distinct biotypes, each one denoting the source of isolation. Fibrinolysin production determines strains of human origin, and it does not occur in any animal biotype. In our experience at least strains isolated from rectal swabs of domestic animals express the character of fibrinolysin production in a high frequency, but in respect to other properties they resemble strains of animal biotypes. Since the criteria used for the differentiation of S. aureus into biotypes are based solely on strains from the upper respiratory tract, we cannot compare our results with those of others.

Biotype B includes strains of pig origin (8); however, biotype C strains have also been isolated from pigs (6). It seems difficult to distinguish the staphylococci isolated from pigs from those obtained from cattle. Our results show that, among the pig isolates, biotypes B and C are encountered in about the same frequency.

The isolation of human biotype A strains and intermediate-type strains from the rectal swabs of pigs and cows correlates well with our findings among the isolates of S. aureus from sheep and goats (5). This might be explained by the transfer of staphylococci from human to animals through food contaminated by human hands. The isolation of S. aureus strains of human origin in a high percentage from canine carriers of the bacteria has been also reported by Live (12).

Until more data are available, the criteria used so far for the subdivision of S. aureus serve the purpose for a realistic means of tracing the source of an S. aureus isolate and reflect to a great extent the influence of the mammalian host in the development of a specific S. aureus phenotype. Besides, according to Live (11), additional progress in determining the characteristics of S. aureus peculiar to different animal species will contribute further to epidemiological studies in assessing the role of staphylococci of animal origin in human staphylococcosis, a recognized zoonosis discussed in the report of the joint Food and Agriculture Organization-World Health Organization Expert Committee on Zoonoses.

LITERATURE CITED

1. Baird-Parker, A. C. 1962. An improved diagnostic and

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TABLE 2. Hemolysin patterns of S. aureus strains isolated from rectal swabs of pigs and cows

<table>
<thead>
<tr>
<th>Source of S. aureus</th>
<th>Biotype*</th>
<th>No. of strains</th>
<th>No. of strains with hemolysin pattern:</th>
<th>α</th>
<th>β</th>
<th>δ</th>
<th>αβ</th>
<th>αδ</th>
<th>αβδ</th>
<th>Negative</th>
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<tr>
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<td>68</td>
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<td>1</td>
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<td>1</td>
<td>62</td>
<td>1</td>
<td>3</td>
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<td></td>
<td>B</td>
<td>44</td>
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<td>1</td>
<td>1</td>
<td>1</td>
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<td>C</td>
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<tr>
<td></td>
<td>INT</td>
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<td>116</td>
<td>19</td>
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* See Table 1, footnote c, for explanation.


