Preliminary Studies on the Characterization and Distribution of Staphylococcus and Micrococcus Species on Animal Skin

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A total of 221 strains of staphylococci and 98 strains of micrococci isolated from the skins of Eastern gray squirrels, Southern flying squirrels, raccoons, opossums, squirrel monkeys, swine, sheep, horses, cattle, and dogs were characterized in a preliminary attempt to resolve their natural relationships and distribution in nature. Staphylococci demonstrating the widest host range included Staphylococcus xylosus and unnamed Staphylococcus sp. 3. Unnamed Staphylococcus sp. 2 was isolated only from sheep, Staphylococcus sp. 4 only from opossums, Staphylococcus sp. 5 only from squirrel monkeys, and Staphylococcus sp. 6 only from swine. The predominant species isolated from human skin, including S. epidermidis, S. hominis, S. haemolyticus, and S. capitis, were either not isolated or only rarely isolated from animal skin. Micrococcus varians was the predominant Micrococcus species isolated from animal skin. M. luteus was only occasionally isolated. M. lylae, M. sedentarius, M. roseus, M. kristinae, and M. nishinomyaensis, species occasionally isolated from human skin, were not isolated from animal skin.

Numerous studies have characterized animal strains of coagulase-positive Staphylococcus aureus biotypes and possible subspecies. The majority of these studies have been conducted with strains isolated from bovine (8, 27, 29) and canine (4, 18, 26) sources. A few studies have been conducted with strains isolated from swine (9, 28), horses (24), hares (10, 25), mink (11, 25), sheep (21), and other animals. S. aureus strains isolated from humans and certain animals could be distinguished on the basis of specific biochemical, nutritional, phage typing, and serological properties (19, 22, 24, 31).

Studies characterizing animal coagulase-negative staphylococci and their distribution on animal skin have been reported infrequently in the literature. Most investigators used the system of biotyping proposed by Baird-Parker (1, 2, 12, 23) which is not aimed at resolving the majority of staphylococci at a species or subspecies level. Also, by using this system, many staphylococci producing weak acid from glucose under anaerobic conditions would have been misclassified as micrococci. Since methods are now available to clearly separate staphylococci from micrococci (16, 32, 33) and comprehensive systematic studies have resolved up to 12 Staphylococcus and 8 Micrococcus species isolated from human skin (14–17, 32; W. E. Kloos, K. H. Schleifer, and R. F. Smith, Int. J. Syst. Bacteriol., in press), we have proceeded in the present study to begin to explore the characterization and distribution of staphylococci and micrococci isolated from animal skin.

MATERIALS AND METHODS

Sampling. A total of 14 Eastern gray squirrels (Sciurus carolinensis), nine from Sunbury and five from Raleigh, N.C.; five Southern flying squirrels (Glaucomys volans) from Raleigh, N.C.; three raccoons (Procyon lotor) and 11 opossums (Didelphis virginiana) from Sunbury, N.C.; and four horses (Equus caballus) and five cattle (Bos taurus) from farms in Chapel Hill, N.C., were sampled once during August through December, 1972. Five pigs (Sus scrofa) from farms in Chapel Hill and three pigs and five sheep (Ovis aries) from the North Carolina State University farms in Raleigh, N.C., were sampled once in November, 1972, and March, 1975, respectively. Two dogs (Canis familiaris), one from Raleigh, N.C., and one from New Brunswick, N.J., were sampled once in November and October, 1971, respectively. Two squirrel monkeys (Saimiri sciurea), freshly captured from Columbia, South America, and maintained in cages at the Burroughs Wellcome Company, Research Triangle Park, N.C.,
were sampled once in March, 1973.

Samples were taken from healthy skin at one site on the forehead, one anterior naris, inner and outer sides of the forelimbs and hindlimbs, abdomen, and back of each animal. Samples were also taken from the marsupium (ventral pouch) in female opossums.

**Sampling procedures.** Sterile cotton swabs were moistened with a detergent containing 0.1% Triton X-100 in 0.075 M phosphate buffer, pH 7.9 (35), and rubbed vigorously, with rotation, over approximately 8-cm² sites. Sites on the body were exposed for swabbing by parting the pelage away from the site area. Swabbing was performed for 5 s on sites in the anterior nares and ventral pouch (opossums only) that usually contained large populations of bacteria and for 15 s on sites of the forehead, forelimbs, hindlimbs, abdomen, and back that usually contained relatively small populations (13). Swabs taken from the forehead, forelimbs, hindlimbs, abdomen, and back were immediately rinsed once in 5 ml of detergent and then applied directly on agar media by rubbing, with rotation, over the entire surface for two consecutive times. Swabs taken from the anterior naris and ventral pouch were immediately rinsed once in 5 ml of detergent and then applied to the surface of agar media.

**Isolation medium.** The original isolating medium (P agar) (14) was nonselective for bacteria, but contained the mold inhibitor cycloheximide (50 μg/ml). Later, we also employed an additional isolating medium that was similar in composition to the above, but contained 7.5% NaCl to inhibit the development of common large or spreading *Bacillus* colonies.

**Bacteriological analysis.** Inoculated agar media were incubated aerobically at 34 C. After 4 days, colonies were counted and their morphology and pigmentation were recorded. In most instances, one representative of each colony type per site was picked and isolated on P agar. Subcultures were stored at 4 C.

**Identification of bacteria.** Subcultures of bacteria were first tentatively identified or grouped on the basis of colony and cell morphology, pigmentation (when present), Gram stain, catalase activity, growth pattern in a semisolid thioglycolate medium, and growth rate (3, 7, 13, 14, 16, 33). Those suspected of being staphylococci or micrococci were tested further for specific genus and species characteristics by previously described procedures and current schemes (14-16, 32-34; Kloos et al., in press).

**RESULTS**

Characterization of *Staphylococcus* species isolated from animal skin. The five coagulase-positive *S. aureus* strains isolated from animal skin were similar in most characteristics to strains isolated from human skin (14, 15). A second group of eight coagulase-positive staphylococci isolated from animal skin that were distinguishable from the above *S. aureus* strains by a number of characters were tentatively designated as unnamed *Staphylococcus* sp. 1. These strains were similar to those previously described by Reeder and Ekstedt (30), Oeding (24), and Kloos and Schleifer (14, 15) which differed from typical *S. aureus* strains by having glycerol and galactosamine as components of teichoic acid, usually weaker anaerobic growth in a thioglycolate medium, no or only weak hemolysin activity on bovine blood agar, usually no or only weak acid produced, aerobically, from maltose and D-mannitol, and lack of pigmentation.

Thirty *S. sciuri* strains isolated in this study were described earlier in a proposal for new species status (Kloos et al., in press). Strains isolated from members of the squirrel family *Sciuridae* could be distinguished from strains isolated from most other animals in having a higher resistance to lysostaphin.

Sixty-four *S. xylosus* strains isolated from animals had very similar character parameters to those isolated from human skin (14, 32; Kloos et al., in press). Five strains of staphylococci isolated from sheep skin appeared to be related to *S. xylosus* and *S. sciuri* and were tentatively designated as unnamed *Staphylococcus* sp. 2. These strains could be distinguished from either of the above species by their orange pigment and small colony diameter, unique umbonate colony profile, lack of acid production from D(+)-galactose, and a combination of other characteristics. Like *S. sciuri*, they produced acid aerobically from D(+)-fucose, D(+)-cellobiose, and β-gentiobiose.

Twenty *S. cohnii* strains isolated from animals were similar to those isolated from human skin in many character parameters (14, 16, 32); however, several noteworthy discrepancies were observed that might suggest some divergence and a separate subspecies status. Hence, their classification here as *S. cohnii* is only tentative. Strains isolated from several different animals produced slightly larger colonies and usually more intense anaerobic growth in a thioglycolate medium, and more strains produced acid aerobically from D(+)-galactose, D(+)-mannose, maltose, α-lactose, and D-mannitol as compared to strains isolated from human skin.

Forty-eight strains of staphylococci isolated from a variety of animal species that appeared to be somewhat intermediate in relationship between *S. xylosus* and *S. cohnii* were tentatively designated as unnamed *Staphylococcus* sp. 3. These strains were different from *S. xylosus* in having only glistening colonies with an entire edge, no or occasionally very weak anaerobic growth in a thioglycolate medium, no reduction of nitrates, no or only weak pro-
duction of acid aerobically from D(+)-galactose, and no acid from D(+)-mannose, D(+)-xylose, α-lactose, sucrose, or D(+)-turanose. On the other hand, these strains were different from S. cohnii on the basis of pigmentation pattern, no or occasionally very weak anaerobic growth (as mentioned above), weak-to-moderate phosphatase activity, no lecinthinase activity or hydrolysis of Tween 80, no or only weak production of acid aerobically from D(+)-galactose, no acid from D(+)-mannose, α-lactose, or xylitol, and often producing acid from L(+)-arabinose and D(-)-ribose.

The three S. saprophyticus strains isolated from animal skin were very similar to those isolated from human skin (14, 32), with the one exception that they produced acid aerobically from D(+)-galactose.

Different clusters of eight strains of staphylococci isolated from opossum skin, eight strains from squirrel monkey skin, and six strains from pig skin could not be identified with currently recognized Staphylococcus species or the unnamed species mentioned above and were tentatively designated as unnamed Staphylococcus spp. 4, 5, and 6, respectively. Staphylococcus sp. 4 was resistant (≥1.6 μg/ml) and Staphylococcus sp. 5 was slightly resistant (0.4 to 0.8 μg/ml) to novobiocin, which together with certain other characteristics would suggest a relationship to the novobiocin-resistant species group composed of S. sciuri, S. xylosus, S. cohnii, and S. saprophyticus. Staphylococcus sp. 4, isolated from opossums, possessed the unique characteristic combination of nitrate reduction, resistance to novobiocin, weak-to-moderate deoxyribonuclease, caseinolytic, and gellatinase activities, moderate phosphatase activity and lipolytic activity on triolein, production of acid aerobically from D(+)-mannose, sucrose, and β-gentiobiose, but no acid produced from maltose, D(+)-cellobiose, D(+)-fucose, D(+)-xylose, or L(+)-arabinose. Staphylococcus sp. 5, isolated from squirrel monkeys, demonstrated a unique colony morphology and pigment pattern and possessed the unique characteristic combination of nitrate reduction, slight resistance to novobiocin, moderate caseinolytic, gellatinase, lecinthinase, and phosphatase activities and lipolytic activities on triolein and tributyrin, production of acid aerobically from maltose, sucrose, D-mannitol, D(+)-turanose, D(+)-trehalose, and usually L(+)-arabinose, but no acid was produced from D(-)-ribose, D(+)-xylose, or β-gentiobiose. This species also contained a cell wall peptidoglycan similar to the L-Lys-Gly3, L-Ser0.6-1.5 type found in predominately human Staphylococcus species (K. H. Schleifer, personal communication). Strains of Staphylococcus sp. 6 appeared to be similar to a group of porcine staphylococci described by Baird-Parker (2) and Smith and Bettg (34).

The seven S. haemolyticus, two S. warneri, and three S. epidermidis strains isolated from animal skin were very similar to those isolated from human skin (14, 33).

Characterization of Micrococcus species isolated from animal skin. M. luteus strains isolated from animal skin were very similar to those isolated from human skin in most character parameters (16); however, one group of 10 strains isolated predominantly from Eastern gray squirrels and occasionally from opossums living in the Dismal Swamp area around Sunbury, N.C., were different from human strains in that they produced orange-yellow to orange pigmented colonies and all strains tested produced acid aerobically from D(+)-xylose. The latter strains may represent a distinct geographical race.

Distribution of Staphylococcus species on various animal skins. The most widely distributed Staphylococcus species isolated from animal skin in this study was S. xylosus, followed by Staphylococcus sp. 3, S. sciuri, and Staphylococcus sp. 1 (Table 1). Other staphylococci were isolated from only one or, at most, three different animal species and, therefore, appear to be exhibiting some degree of host specificity.

S. aureus was isolated from only two out of 14 Eastern gray squirrels and one out of eight pigs. The closely related species Staphylococcus sp. 1 was isolated from a slightly larger variety of animal species including Eastern gray squirrels, raccoons, squirrel monkeys, and dogs. In general, the coagulase-positive staphylococci were found on a relatively small-to-moderate percentage of individuals within a species and usually composed only a small-to-moderate percentage of the total staphylococci isolated from skin.

S. sciuri was one of the major species isolated from the Eastern gray squirrel and Southern flying squirrel. All of the squirrels sampled contained moderate to relatively large populations of this species. S. sciuri was also occasionally isolated from opossums, raccoons, sheep, and dogs. The related species Staphylococcus sp. 2 was only found in sheep. S. xylosus was isolated from a wide variety of animal species and was often present in a large percentage of individuals within a species. It also usually composed a moderate-to-large percentage of the total staphylococci isolated from skin. Very high percentages of this
species were found in sheep, horses, and cattle.

Staphylococcus sp. 3 was isolated primarily from wild animals and occasionally from domestic animals. This species usually composed a moderate percentage of the total staphylococci isolated from skin in wild animals and a small percentage in domestic animals.

Strains tentatively identified as various S. cohnii subspecies were found on three different animal species, namely, opossums, squirrel monkeys, and pigs. These organisms composed only a small-to-moderate percentage of the total staphylococci isolated from skin.

S. saprophyticus was isolated from only three out of the 59 animals sampled in this study and in each case was represented by a single isolated colony. For this reason, a serious question should be raised as to the original source of this species, i.e., whether it is indigenous or represents a contaminant from another source.

Staphylococcus sp. 4 was only isolated from opossums, Staphylococcus sp. 5 only from squirrel monkeys, and Staphylococcus sp. 6 only from pigs. These species were among the major staphylococci isolated from each of the respective animal species and all individuals contained moderate-to-large populations of these organisms.

S. haemolyticus, S. epidermidis, and S. warneri were isolated from only three, two, and one of the 59 animals sampled, respectively. S. haemolyticus was represented by a single isolated colony in one individual and two colonies in another. This species was represented by 28 colonies in a head site and 12 colonies in a naris site of one pig. S. epidermidis and S. warneri were each represented by two colonies in one individual and in one pig; S. epidermidis was represented by 49 colonies in a naris site. As previously mentioned above for S. saprophyticus, very small frequencies of isolation and numbers of isolates do raise a question as to the original source of these species. By comparison, out of 40 humans sampled in an earlier study (13), S. haemolyticus was isolated from 78%, S. epidermidis from 100%, and S. warneri from 52% of the individuals.

Most of the various Staphylococcus species found on animal skin in this study did not appear to exhibit significant skin site specificity in those areas sampled. However, S. aureus was isolated predominantly from the nares and abdomen, and S. sciuri was only occasionally...

### Table 1. Occurrence of Staphylococcus and Micrococcus species on the skins of various animals

<table>
<thead>
<tr>
<th>Genus and species</th>
<th>Animal host</th>
<th>Total wild and domestic animals studied</th>
<th>Humans studied</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Eastern gray squirrel (14)</td>
<td>Sheep (5)</td>
<td>Horse (4)</td>
</tr>
<tr>
<td><strong>Staphylococcus</strong></td>
<td>Southern flying squirrel (5)</td>
<td>Swine (8)</td>
<td>Horse (4)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Racoon (3)</td>
<td>Opossum (11)</td>
<td>Squirrel monkey (2)</td>
</tr>
<tr>
<td>S. sciuri</td>
<td>100</td>
<td>100</td>
<td>33</td>
</tr>
<tr>
<td>S. xylosus</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. cohnii</td>
<td>86</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>S. saprophyticus</td>
<td>7</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>S. epidermidis</td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Micrococcus</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. luteus</td>
<td>21</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>M. varians</td>
<td>64</td>
<td>0</td>
<td>67</td>
</tr>
</tbody>
</table>

* Numbers of individuals sampled per animal host species.
* References 13, 14, 16, and Kloos et al., in press.
* Percentages of individuals from which Staphylococcus or Micrococcus species were isolated. Parentheses around percentages denote that only one or two colonies of the species were isolated from an individual. Capital letters preceding percentages signify different suspected unnamed subspecies within the designated species. S. simulans, S. hominis, and S. capitis were not isolated from animals but were isolated from 12, 100, and 65%, respectively, of the 40 humans studied previously. M. luteus, M. sedentarius, M. kristinae, M. roseus, and M. nishinomiyensis were not isolated from animals but were isolated from 35, 20, 35, 10, and 38%, respectively, of the 40 humans.
isolated from the nares. We believe that a more comprehensive study involving additional animals and skin sites would be required to clearly resolve any subtle site specificity that might be present.

Distribution of Micrococcus species on various animal skins. Micrococi were commonly isolated as relatively large populations from the nine Eastern gray squirrels living in the Dismal Swamp area around Sunbury, N.C., but were found as very small populations on the five Eastern gray squirrels living in Raleigh, N.C., and the three raccoons, eleven opossums, two squirrel monkeys, four horses, five cattle, and two dogs. Members of the genus Micrococcus were not isolated from Southern flying squirrels, pigs, or sheep in this preliminary study. The most widely distributed Micrococcus species isolated from animal skin was M. varians (Table 1). M. luteus was isolated from only three different animal species.

Micrococi were isolated from a wide variety of skin sites, but were seldom isolated from the nares.

DISCUSSION

Staphylococcus species populations isolated from animal skin were remarkably different from those isolated from human skin in earlier studies (13, 14, 32). Differences were observed in the predominant species present and species characteristics. The predominant species isolated from animal skin, including S. xylosus, Staphylococcus sp. 3, and S. sciuri, as well as several species with a limited host range, were resistant to novobiocin and produced acid from D-mannitol under aerobic conditions, and representative strains contained the cell wall peptidoglycan types of L-Lys-Gly\textsubscript{5,4} or L-Lys-L-Ala-Gly\textsubscript{4} (16; K. H. Schleifer, personal communication). Also, certain species produced acid from D-(+)-xylose, D-(+)-fucose, L-(+)-arabinose, \(\beta\)-gentiobiose, and/or D-(+)-cellobiose under aerobic conditions. On the other hand, the most predominant species isolated from human skin, including S. epidermidis, S. hominis, and S. haemolyticus, were susceptible to novobiocin, usually failed to produce acid from D-mannitol, failed to produce acid from D-(+)-xylose, D-(+)-fucose, L-(+)-arabinose, \(\beta\)-gentiobiose, and D-(+)-cellobiose, and contained the cell wall peptidoglycan type of L-Lys-Gly\textsubscript{5,3}, L-Ser\textsubscript{0.6-1.5} (14, 32).

Staphylococcus sp. 2 through 6 were not isolated from human skin, and S. simulans, S. capitis, and S. hominis, isolated from human skin (14), were not isolated from animal skin. The very rare isolation of S. sciuri and uncommon isolation of S. xylosus from human skin, except when contact with pets or farm animals was notable, would suggest that these species are usually contaminants rather than residents on humans. Since S. sciuri and S. xylosus grow very poorly or fail to grow on an agar medium of pH 5.3 or below (6), we might not expect to find colonization of these species on human skin of about pH 5 (18). Both species grow well at pH 7.0 or slightly above, which is characteristic for the skin of several different mammals other than man (5). Conversely, the rare isolation of S. saprophyticus, S. haemolyticus, S. epidermidis, and S. warneri from animal skin would suggest that these species are usually contaminants rather than residents on animals.

Differences in host range suggest to us the possibility that many Staphylococcus species may have evolved as a result of adaptation to different cutaneous environments. Of the 12 species reported to occur on human skin (13-16, 32; Kloos et al., in press), seven species appear to be limited in range to man, though we have not yet extended our studies to staphylococci of other closely related primates. Based on this finding and earlier studies illustrating certain species preferences for specific habitats (e.g., nares, axillae, head, legs, etc.) within the human cutaneous environment (13), we would hypothesize that different human Staphylococcus species may have evolved from one or at most a few ancestral species as a result of selective pressures imposed by different niches in the cutaneous environment or, alternatively, became adapted to human skin as a result of secondary contact. Future deoxyribonucleic acid hybridization and genetic recombination studies with the proposed species should help to provide an estimate of genetic relationships.

One uncommon human species, S. warneri, appears to be somewhat intermediate between S. haemolyticus and S. hominis and is occasionally confused with these species when using only simple characters for differentiation. Perhaps it represents an ancestral form or a group of staphylococci that is only slightly divergent (or convergent) from S. haemolyticus or S. hominis. Another species that should prove interesting in studying the evolution of human staphylococci is S. saprophyticus. This human species appears to be somewhat intermediate between typical coagulase-negative animal and human staphylococci. Like most animal staphylococci, it is resistant to novobiocin, produces acid from D-mannitol and xylitol under aerobic conditions, and grows well at 15°C but not at 45°C; however, like major human staphylococci, it contains significant levels of L-serine in the cell wall peptidoglycan (14, 32). The
amino acid requirement profile of this species is also intermediate between typical animal and human species and, like human species, it grows well at pH 5.3 (6). Since S. saprophyticus appears to link human and animal staphylococci, it might conceivably represent an ancestral form of certain other human species; this, of course, is based on the reasonable assumption that the more archaic forms of staphylococci first appeared on animals.

Staphylococcus sp. 5, isolated from only squirrel monkeys in this study, appears to be like typical coagulase-negative human staphylococci in that it contains significant levels of L-serine in the cell wall peptidoglycan, but like certain animal staphylococci it is slightly resistant to novobiocin and produces acid from L(+)-arabinose and D-mannitol under aerobic conditions. It would be interesting to study additional primates, especially the Pongidae, to see if their staphylococci are more closely related to those of humans than other mammals.

Individual animal hosts in this study did not contain as large a variety of different Staphylococcus species as human hosts (13) and most animal staphylococci did not demonstrate pronounced skin site specificity. According to our hypothesis, these results may be related to (i) increased habitat uniformity, and/or (ii) restricted skin area of the small animals. The several examples of host specificity reported in this study should provide an impetus to explore additional animal species in search of new Staphylococcus species or subspecies.

Micrococcus species populations isolated from animal skin contained only the two species M. varians and M. luteus. M. lyleae, M. sedentarius, M. roseus, M. kristinae, and M. nishinomiyaensis, isolated from human skin in earlier studies (16), were not isolated from animals.

This preliminary study should be followed by more comprehensive studies for more complete characterization of the staphylococci and micrococci of specific animal host species. As in this study, only a small number of individuals were sampled per host species; some of the uncommon Staphylococcus and Micrococcus species or subspecies may not have been isolated.

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