Distribution and Persistence of *Staphylococcus* and *Micrococcus* Species and Other Aerobic Bacteria on Human Skin

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The distribution of *Staphylococcus* and *Micrococcus* species and associated coryneform bacteria, *Acinetobacter, Klebsiella, Enterobacter, Bacillus*, and *Streptomyces* on skin was determined during October 1971 from samples collected on persons living in North Carolina and New Jersey. Persistence of these organisms on skin was estimated in temporal studies conducted during the period from June 1971 to June 1972 on persons living in North Carolina. *Staphylococci* and coryneforms were the most predominant and persistent bacteria isolated from the nares and axillae. *Staphylococci*, coryneforms, micrococci, and *Bacillus* were the most predominant and persistent bacteria isolated from the head, legs, and arms. *Acinetobacters* were most frequently isolated during the warmer months of the years. *Staphylococcus aureus* and *S. epidermidis* were the most predominant and persistent *staphylococci* isolated from the nares, whereas *S. epidermidis* and *S. hominis* were the most predominant and persistent *staphylococci* isolated from the axillae, head, legs, and arms. *S. capitis* was often isolated from the head and arms and *S. haemolyticus* was often isolated from the head, legs, and arms. *S. simulans*, *S. xylosus*, *S. cohnii*, *S. saprophyticus*, *S. warneri*, and an unclassified coagulase-positive species were only occasionally isolated from skin. *Micrococcus luteus* was the most predominant and persistent *Micrococcus* isolated from skin in preferred regions of the head, legs, and arms. *M. varians* was the second most frequent *Micrococcus* isolated. *M. lylae*, *M. sedentarius*, *M. roseus*, *M. kristinae*, and *M. nishinomiyaensis* were only occasionally isolated from skin. *M. lylae* was most frequently isolated during the colder months of the years.

Numerous studies have been reported on the composition of the bacterial flora of human skin (12, 16, 17, 21, 24, 26, 30, 32). Most have resolved cutaneous bacterial populations into major groups or genera, e.g., *Micrococcaceae* (coagulase-positive and coagulase-negative *staphylococci*, micrococci, and/or *Sarcina*), streptococci, *Mimae*, nonlipophilic and lipophilic diphtheroids or coryneform bacteria, propionibacteria (or *Corynebacterium acnes*), enterics and other gram-negative bacilli, *Bacillus*, *Neisseria*, *Streptomyces*, and mycobacteria. Some have been more specific and have attempted to resolve species or even strains.

As taxonomic and other systematic studies of microorganisms have progressed rapidly over the past few years, cutaneous bacteria have undergone changes in classification and new species have been recognized. Recent systematic studies of *Staphylococcus* and *Micrococcus* species isolated from human skin have amended descriptions of *Staphylococcus epidermidis*, *S. saprophyticus*, and *Micrococcus sedentarius* and characterized new species including *S. cohnii*, *S. haemolyticus*, *S. xylosus*, *S. warneri*, *S. capitis*, *S. hominis*, *S. simulans*, *M. kristinae*, and *M. lylae* (13–15, 27). Previously, certain staphylococci that failed to produce or produced only small amounts of acid from glucose under anaerobic conditions were misclassified as micrococci, and aerobic *Sarcina* were erroneously separated from the genus *Micrococcus* (2, 3, 10, 20, 21, 32, 33). Also several of the new species of *staphylococci*, if isolated, would have been misclassified as *S. epidermidis* (2–4, 6), and *M. lylae* and *M. sedentarius* would have been either misclassified as *M. luteus* or left unclassified (6). Since methods
are now available to clearly separate members of the genera *Staphylococcus* and *Micrococcus* (15, 27, 28) and new species have been recognized, we have proceeded in the present study to determine the distribution of *Staphylococcus* and *Micrococcus* species on several major areas of human skin and to estimate their degree of persistence over a 1-year period. Other predominant genera or groups of bacteria were also investigated for making comparisons with staphylococci and micrococci.

**MATERIALS AND METHODS**

**Sampling.** Twenty people living within a 10-mile area in Raleigh, N.C., and 20 people living within a 15-mile area in Somerville and New Brunswick, N.J., were sampled once during October 1971. All people sampled were Caucasians. Individuals from Raleigh included 12 children ranging from 3 to 12 years of age and eight adults ranging from 14 to 40 years of age. Individuals from Somerville and New Brunswick included nine children ranging from 2 to 11 years of age and 11 adults ranging from 27 to 61 years of age. Ten of the people from Raleigh were also sampled at monthly intervals over a 1-year period from June 1971 to June 1972. Individuals in the temporal study included six children and four adults.

Samples were taken from healthy skin at two adjacent sites on the forehead and at one site from one cheek, one anterior and external naris, and underside of the chin, the apex of each axilla, and the hairy portion of each upper and lower arm and each upper and lower leg.

**Sampling procedures.** Sterile cotton swabs were moistened with a detergent containing 0.1% Triton X-100 (Packard) in 0.075 M phosphate buffer, pH 7.9 (37), and rubbed vigorously, with rotation, over approximately 8-cm² sites. Swabbing was performed for 5 s on sites of the forehead, cheek, chin, nares, and axillae that usually contained large populations of bacteria and for 15 s on sites of the arms and legs that usually contained relatively small populations. Swabs taken from the forehead, cheek, chin, external nares, arms, and legs were immediately applied directly on agar media by rubbing, with rotation, over the entire surface for two consecutive times. Swabs taken from the anterior nares and axillae were immediately rinsed once in 5 ml of detergent, and the rinse was applied to the surface of agar media (31). Later, during the course of the study, we observed that adults often contained populations of bacteria on the forehead, cheek, chin, and external nares that were too large to be analyzed by inoculating swabs directly onto media. In these instances, samples taken from a single swab rinse proved to be more satisfactory and produced well-isolated colonies.

**Isolation medium.** The isolating medium (P agar) (19) was nonselective and had the following composition: peptone (Difco), 10 g; yeast extract (Difco), 5 g; sodium chloride, 5 g; glucose, 1 g; agar (Difco), 15 g; and distilled water, 1,000 ml.

In this study, we did not make a distinction between lipophilic and nonlipophilic dipherthoids (co-ryneforms) by the routine use of Tween 80-supplemented media. On the above, unsupplemented medium, lipophilic co-ryneforms produced distinctive small (0.5 to 1.0 mm in diameter), dry, greyish colonies in numbers that were comparable to those obtained on the same medium supplemented with 0.5% Tween 80.

**Bacteriological analysis.** Inoculated agar media were incubated under aerobic conditions at 34 C for 4 days, at which time colonies were counted and recorded according to morphology and pigment. In most instances, a portion of one representative colony of each colony type per site was picked with a needle and isolated on P agar. Subcultures were stored at 4 C. For convenience, the original isolation plates could be stored at 4 C for 2 to 3 weeks prior to the isolation of cultures (except for the isolation of *Neisseria*).

The occasional molds and yeasts isolated were characterized on the basis of colony and cell morphology and pigment, but were omitted from further analysis.

**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 (6, 7, 13-15, 27, 28). Additional characters used for the identification of specific genera (or groups) and species (in *Staphylococcus* and *Micrococcus* only) are described in the following paragraphs.

(i) *Staphylococcus.* Suspected staphylococci were further tested for lysostaphin and erythromycin susceptibility, acid production from glycerol under aerobic conditions, and growth at 45 C (28). *Staphylococcus* species were characterized on the basis of a wide variety of characters that were described previously (13, 14, 29).

(ii) *Micrococcus.* Suspected micrococci were further tested for a comparison of lysozyme and lysostaphin susceptibilities, erythromycin susceptibility, acid production from glycerol under aerobic conditions, and growth at 45 C (28). *Micrococcus* species were characterized on the basis of a wide variety of characters that were described previously (13).

(iii) *Coryneforms.* Suspected members of the "co-ryneform group of bacteria," presently including the genera *Corynebacterium*, *Arthrobacter* (with the related *Brevibacterium* and *Microbacterium as genera incertae sedis), *Cellulomonas*, and *Kurthia* (6), were further tested for lysozyme susceptibility, methylene blue staining properties, and acid fastness (1, 15). Several representative strains were analyzed for the presence of *meso*-diaminopimelic acid, arabinose, and galactose in whole-cell hydrolysates (kindly performed by R. E. Gordon, Rutgers University, New Brunswick, N.J.) and alphatic hydrocarbons (kindly performed by D. Takemoto and T. G. Tornabene, Colorado State University, Fort Collins, Colo.). Classification of coryneform bacteria into specific genera was made on several selected strains but was not routinely performed. Reference strains in-
cluded Corynebacterium minutissimum American Type Culture Collection (ATCC) 23346, C. melasse-
cola ATCC 17965, and Arthrobacter globiformis (atro-
cyanus) ATCC 13752; C. hoangii (species incertae 
sedis) AJ 1408 and C. equi AJ 1402, obtained from K.
Yamada, Ajinomoto Co., Inc., Kawasaki, Japan; C.
xerosis Czechoslovak Collection of Microorganisms 
(CCM) 1729, A. citreus CCM 1647, A. globiformis 
CCM 1651, A. simplex CCM 1652, A. variabilis (spe-
cies incertae sedis) CCM 1565, Brevisbacterium linens 
CCM 47, B. luteum CCM 2298, B. heliolum CCM 
1923 (all Brevisbacterium as species incertae sedis), 
Microbacterium lacticum CCM 1584, and Cellulio-
monas flavigena CCM 1926, obtained from M. Ko-
cur, Czechoslovak Collection of Microorganisms, J.
E. Purkyne University, Brno, Czechoslovakia.

(iv) Mycobacterium and Nocardia. Suspected mycobacteria and nocardias were further tested for 
acid fastness (6). Several representative strains were 
analyzed for the presence of meso-diaminopimelic acid, 
arabinose, and galactose in whole-cell hydrolysates 
(kindly performed by R. E. Gordon), lipid composition 
(kindly performed by D. Takemoto and T. G. 
Tornabene), and deoxyribonucleic acid base 
composition. Reference strains included Mycobacterium 
smegmatis CCM 2067, obtained from M. Kocur; M.
phlei Institute of Microbiology-Rutgers University 
(IMRU) 5, Nocardia otitidis-caviarum IMRU 1342, 
and members of the Nocardia "rhodochrous com-
plex" including N. salmonicolor IMRU 561, N. rubro-
pertinctum IMRU 388, N. lutea National Collection 
of Type Cultures (NCTC) 576, and Nocardia sp. 
IMRU 372, obtained from R. E. Gordon; additional 
members of the Nocardia "rhodochrous complex" 
including N. rubra JA-SD1, N. corallina JA-SD8, 
and N. erythropolis JA-SD7, obtained from J. N. 
Adams, University of South Dakota, Vermillion, 
S.D.

(v) Streptomyces. Suspected Streptomyces were 
further tested for mycelium and spore morphology 
(6). The reference strain used in this study was 
Streptomyces aureofaciens Northern Regional Re-
search Laboratory (NRRL) 2209, obtained from J. 
J. Perry, North Carolina State University, Raleigh, 
N.C.

(vii) Acinetobacter. Recent systematic studies have 
indicated that many oxidase-negative strains 
previously reported as members of the genera Herel-
lea, Mima, Bacterium, Acinobacter, Alcaligenes, 
Moraxella, Neisseria, and Micrococcus are different 
strains of the single species Acinetobacter calcoaceti-
cus (5, 11, 25, 35). Suspected acinetobacters were 
further tested for motility, oxidase activity, nitrate 
reduction, reactions in triple sugar iron agar (Difco) 
and D(+)glucose- and α-lactose-supplemented 
phenol red agar base (Difco), and characteristics of 
growth and color on eosin methylene blue agar 
(Difco) (6, 25, 36). Reference strains included Acineto-
bacter calcoaceticus ATCC 9955, ATCC 19003, and 
ATCC 19004 received as Herellea vagincola, ATCC 
19194 received as H. saponiphilum, ATCC 19002 
received as H. caseolytica, ATCC 17903 and ATCC 
15150 received as Bacterium anitratum, ATCC 17906 
received as Achromobacter haemolyticus, ATCC 
17988 received as Alcaligenes haemolyisans, ATCC 
17923 received as Acinetobacter alcaligenes, and 
ATCC 17986 received as Moraxella lwoffi, and Mor-
axella osloensis D-1, obtained from E. J. Ordal, Uni-
versity of Washington, Seattle, Wash.

(vii) Neisseria. Suspected Neisseria were further 
tested for motility, oxidase activity, nitrate reduc-
tion, and acid production from D(+)-glucose, β-D- 
fructose, and maltose under aerobic conditions (6).
Original isolation plates that were stored at 4°C for 
more than 1 to 2 weeks usually produced a high 
proportion of nonviable Neisseria colonies. There-
fore, when complete characterization is desired, we 
would recommend that cultures be picked for iso-
lation immediately after the 4-day incubation period. 
The reference strain used in this study was Neis-
seria subflava ATCC 14799.

(v) Enterics. Suspected members of the family 
Enterobacteriaceae were further tested for character-
istics of growth and color on eoin methylene blue 
agar, oxidase, deoxyribonuclease, and urease activi-
tes, nitrate reduction, growth on acetate differen-
tial agar (Difco), Simmons citrate agar (Difco), and 
KCN, phenylalanine deamination, methyl red test, 
production of acetyl-methyl-carbinol, reactions in tri-
ple sugar iron agar and malonate broth (Difco), acid 
production from D(-)-glucose, dulcitol, β-mannitol, 
β-sorbitol, raffinose, and L(-)-rhamnos, and reac-
tions in the r/b enteric differential system (diagnos-
tic research, Inc., Roslyn, N.Y.), including indole, 
motility, lysine and ornithine decarboxylase, lact-
ose, and H2S (6, 8, 18). Reference strains included 
Escherichia coli K-12 and B, Salmonella typhimu-
erium LT-2, Klebsiella pneumoniae ATCC 13883, En-
terobacter aerogenes ATCC 13048, Serratia marces-
cens Noma, and Proteus mirabilis ATCC 14273 and 
P. morganii ATCC 25880, and, in addition, Alcali-
genese faecalis CCM 1052 and Pseudomonas aerugi-
nosa CCM 1960, obtained from M. Kocur.

(ix) Chromobacterium and Flavobacterium. Sus-
pected chromobacteria and flavobacteria were fur-
ther tested for motility, nitrate reduction, acid pro-
duction from D(+)-xylose under aerobic conditions, 
and oxidase activity (6). Reference strains included 
Chromobacterium violaceum ATCC 12472 and C. lio-
dum (amethystinum) ATCC 6915 and Flavobacteri-
um aquatile CCM 1948, obtained from M. Kocur.

(x) Bacillus. Representative suspected Bacillus 
strains were further analyzed for the presence of 
meso-diaminopimelic acid in whole-cell hydrolys-
ates, characteristics of sporulation, and lysozyme 
susceptibility (kindly performed by R. E. Gordon).
Reference strains included Bacillus subtilis CCM 
2216, B. firmus CCM 2213, B. lentus CCM 2214, B.
brevis CCM 2050, B. sphaericus CCM 2120, B. pan-
thotenticus CCM 2049, B. licheniformis CCM 2143, 
B. pumilis CCM 2144, B. atrocatenaceus CCM 2051, B. 
cereus CCM 2010, and B. megaterium CCM 2007, 
obtained from M. Kocur.

RESULTS
Estimation of the composition and density of 
bacterial populations on skin. Preliminary
experiments were conducted to evaluate the swabbing technique used in this study for estimating the composition and density of aerobic bacterial populations on skin. In a 3 × 3 × 3 factorial analysis using arc sin transformations (kindly performed by H. E. Schaffer), we found no significant difference at the 5% level in the proportion of colony-forming units (CFU) of different genera or Staphylococcus and Micrococcus species isolated on different agar plates inoculated from the same swab or on plates inoculated from different swabs taken from the same site. Hence, it would appear that the action of swabbing for 5 to 15 s dispersed the aerobic bacterial population on skin to a uniform composition and, therefore, would provide a representative sample for determining the proportion of types of bacteria present on skin. These results are in agreement with those of a comparable study by Smith (31) showing that proportions of diphtheroids, gram-negative bacilli, and gram-positive cocci did not change significantly by repeated washings of swabs.

The swabbing technique also provided on a single isolation plate a relatively constant percentage of the total aerobic CFU recoverable at sites on the forehead, cheek, and chin (x̄ and sₓ = 2.1 ± 0.3%) and legs and arms (x̄ and sₓ = 22 ± 1%). We were unable to obtain uniform estimates of the percentage of total aerobic CFU isolated from swabs taken from the nares and axillae due to the irregular topography and fluids present at these sites. Although the swabbing technique does not appear to be quite as accurate as the more elaborate glass cup method of Pachtman et al. and Williamson and Kligman (22, 37, 38) in estimating bacterial density, it nevertheless can provide a reasonably good estimate devoid of gross errors.

The actual CFU isolated from sites on the legs and arms and adjusted CFU (normalized according to percent isolation and/or dilution) isolated from sites in the nares, axillae, and on the head (including forehead, cheek, and chin) are shown in Fig. 1. Data from external naris sites were omitted, as they were distinctly different from those obtained from anterior naris sites and were often intermediate between ante-

**Fig. 1.** Density of bacterial populations on human skin. Data for the leg and arm sites represent the actual number of CFU isolated, whereas data for the nares, axilla, and head sites were adjusted by multiplying the actual CFU isolated by 6 × 10^5, 1.2 × 10^5, and 5 × 10^5, respectively, for normalization to leg and arm sites. Solid symbols represent data from persons living in North Carolina; open symbols represent data from persons living in New Jersey. Symbols representing data for the same individual are connected by a vertical line. The position of each individual is the same along the abscissa in each major skin area. Symbols (e.g., solid): (●), single naris, left and right axilla, adjacent forehead sites, left and right upper leg, left and right upper arm; (■), cheek, left and right lower leg, left and right lower arm; (▲), chin.
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rior naris and cheek sites. The estimated bacterial densities determined in this study were in close agreement with those reported by others for similar sites (16, 17, 34, 37), and serve as a reference for correlations between site, density, and composition to be discussed later in the paper.

Distribution of genera (groups) on skin. The most predominant and persistent aerobic bacteria isolated from human skin in this study were members of the coryneform group and the genera Staphylococcus, Micrococcus, and Bacillus, followed by Acinetobacter, Klebsiella, Enterobacter, and Streptomyces (Fig. 2 and 3). Bacteria that were only occasionally isolated included Neisseria (sometimes in large numbers), Flavobacterium, Mycobacterium, Nocardia, Escherichia, Pseudomonas, Serratia, Moraxella, Streptoverticillium, Chromobacterium, and some unclassified strains. The extremely small, convex, glistening colonies (≈0.3 mm in diameter) of microaerophilic Streptococcaceae, occasionally isolated in relatively large numbers from the nares and head, were not enumerated.

Staphylococci usually composed greater than 50% of the bacteria isolated from the head, nares, and axillae and from 10 to 70% of those isolated from the legs and arms. Adults from both North Carolina and New Jersey contained larger percentages of staphylococci on the head than most children. Seventy percent of the individuals in the temporal study maintained a relatively constant percentage throughout the 1-year period.

Micrococi usually composed less than 1% of the bacteria isolated from the nares and axillae and from 1 to 20% of those isolated from the head, legs, and arms. Eighty percent of the individuals in the temporal study maintained a persistent population of micrococi on the head, legs, and arms.

Coryneforms (estimated to be predominantly Corynebacterium and small numbers of Arthrobacter) occurred over a wide range of percentages of the bacteria isolated from the nares and axillae. They usually composed from 1 to 40% of bacteria isolated from the head and from 10 to 70% of those isolated from the legs and arms. Adults contained larger percentages of coryneforms on the legs and arms than most children. Sixty percent of the individuals in the temporal study maintained a relatively constant percentage throughout the 1-year period.

Acinetobacters were only occasionally isolated from the nares and axillae and then usually at a proportion less than 1% of the total isolated bacteria. They usually composed from 1 to 30% of bacteria isolated from the head, legs, and arms. These organisms were predominantly isolated from persons living in North Carolina. The occurrence of acinetobacters on individuals in the temporal study followed a seasonal pattern. They were more frequently isolated during the warmer months of the years, from June to September 1971 and then again from April to June 1972.

Klebsiella and Enterobacter species were most commonly isolated from the nares and axillae of adults and only occasionally from children. These organisms were more frequently isolated from the head, legs, and arms of persons living in New Jersey compared to those living in North Carolina. Fifty percent of the adults in the temporal study maintained a persistent population of Klebsiella in the nares and axillae and 25% maintained a persistent population of Enterobacter in the axillae. These organisms were only sporadically isolated from the head, legs, and arms, and then usually only during the warmer months of the years.

Bacillus species usually composed less than 1% of the bacteria isolated from the nares and axillae and from less than 1 to 25% of those isolated from the head, legs, and arms. Children contained larger percentages of Bacillus than most adults. All individuals in the temporal study maintained a relatively persistent population on the head, legs, and arms. Large percentages (≥50% of the total isolated bacteria) of these organisms were only isolated during the warmer months of the year; however, a seasonal pattern related to percentage was not particularly clear.

Streptomyces species usually composed less than 1% of the bacteria isolated from the nares and axillae and from less than 1 to 4% of those isolated from the head, legs, and arms. Children contained larger percentages of Streptomyces on the axillae, head, legs, and arms (with one exception of persons living in New Jersey) than most adults. These organisms did not show any clear evidence of persistence, except for occasional short periods of 2 to 5 months.

Distribution of Staphylococcus species on skin. The most predominant and persistent staphylococci isolated from human skin in this study included the species Staphylococcus epidermidis and S. hominis, followed by S. haemolyticus, S. capitis, and S. aureus (Fig. 4 and 5). Species that were only occasionally isolated included S. saprophyticus, S. cohnii, S. xylosus, S. simulans, S. warneri, and one unclassified species.

S. aureus was a predominant species isolated from the nares of children and was less frequently isolated from the nares of adults. All
Fig. 2. Frequency of occurrence and relative percentage of predominant aerobic bacteria isolated from human skin. Solid bars represent data for persons living in North Carolina; striped bars represent data for persons living in New Jersey. The left half of each bar represents data for children; the right half represents data for adults.

Populations of the nares of children in the temporal study were persistent and most were maintained at a relatively constant percentage of the total staphylococci for a period of 9 to 12 months. This species was only occasionally isolated from the head, legs, and arms of adults. It
was more frequently isolated from these sites in children. Isolations from axillae were rare.

*S. simulans* was a relatively common species isolated from the legs, arms, and heads of children living in North Carolina, but was only occasionally isolated from the legs and arms of children living in New Jersey. It was isolated from only one out of 19 adults. When present, populations often composed as much as 20 to 80% of the staphylococci isolated from the legs and were isolated at slightly lower percentages on the head and arms. Isolations from the nares and axillae were relatively rare.

*S. xylosus*, *S. cohnii*, and *S. saprophyticus* usually composed from less than 1 to 15% of the staphylococci isolated from the head, legs, and arms. Of the three related species (27), *S. xylosus* was the least and *S. saprophyticus* was the most frequently isolated in this study. These species were usually not persistent; however,
Fig. 4. Frequency of occurrence and relative percentage of Staphylococcus species isolated from human skin. Bar designations as in Fig. 2.
### Temporal Study of Staphylococcus Species Isolated from Human Skin

**Explanation and Symbols:** Symbols indicate the percentage of total Staphylococcus CFUs represented by each species.

| Species          | NARES 1971 | NARES 1972 | AXILLAE 1971 | AXILLAE 1972 | HEAD 1971 | HEAD 1972 | LEGS 1971 | LEGS 1972 | ARMS 1971 | ARMS 1972 |
|------------------|------------|------------|--------------|--------------|-----------|-----------|-----------|-----------|-----------|-----------|
| **S. aureus**    |            |            |              |              |           |           |           |           |           |           |
| **S. simulans**  |            |            |              |              |           |           |           |           |           |           |
| **S. pyogenes**  |            |            |              |              |           |           |           |           |           |           |
| **S. hominis**   |            |            |              |              |           |           |           |           |           |           |
| **S. epidermidis** |          |            |              |              |           |           |           |           |           |           |
| **S. capitis**   |            |            |              |              |           |           |           |           |           |           |

**Fig. 5.** Temporal study of Staphylococcus species isolated from human skin. Explanation and symbols as in Fig. 3. Symbols indicate the percentage of total Staphylococcus CFUs represented by each species.
they occasionally demonstrated some persistence for relatively short intermittent periods. Isolations from the nares and axillae were relatively rare.

\( S. \) haemolyticus usually composed from less than 1 to 2% of the staphylococci isolated from the nares and axillae and from 1 to 30% of those isolated from the head, legs, and arms. Fifty percent of the individuals in the temporal study maintained relatively persistent populations on the legs and arms. 

\( S. \) warneri, a species that appears to be somewhat intermediate in relationship between \( S. \) haemolyticus and \( S. \) hominis (13, 27), was relatively uncommon. This species usually composed from less than 1 to 5% of the staphylococci isolated from the nares, head, legs, and arms. Isolations from the axillae were rare. Persistence was uncommon and, when evident, occurred for short periods of 2 to 6 months.

\( S. \) hominis usually composed from 4 to 20% of the staphylococci isolated from the head and from 10 to 75% of those isolated from the axillae, legs, and arms. Isolations from the nares were uncommon. Seventy percent of the individuals in the temporal study maintained persistent populations on the axillae and head. All populations were persistent on the legs and arms.

\( S. \) epidermidis usually composed from 90 to 100% of the staphylococci isolated from the nares when the sympatric species \( S. \) aureus was not present or only present in small numbers; otherwise, it usually composed from 10 to 20%. This species often composed greater than 75% of the staphylococci isolated from the axillae and head and usually from 10 to 45% of those isolated from the legs and arms. \( S. \) epidermidis together with \( S. \) hominis were the predominant sympatric species of the axillae. Persons living in New Jersey usually had slightly larger percentages of \( S. \) epidermidis compared to those living in North Carolina. Sixty percent of the individuals in the temporal study maintained persistent populations on the legs and arms. All maintained persistent populations on the head, nares, and axillae.

\( S. \) capitis was more frequently isolated from the head and arms and only occasionally isolated from the legs. It usually composed from 1 to 30% of the staphylococci isolated from these sites. Twenty percent of the individuals in the temporal study maintained persistent populations on the head and arms.

One unclassified coagulase-positive species, closely related to \( S. \) aureus (14, 23), was isolated in small numbers from two children living in North Carolina and from two children and one adult living in New Jersey.

**Distribution of Micrococcus species on skin.** The most predominant and persistent micrococi isolated from human skin in this study were members of the species Micrococcus luteus and secondly \( M. \) varians (Fig. 6 and 7). Species that were only occasionally included \( M. \) lylae, \( M. \) nishinomiyaensis, \( M. \) kristinae, \( M. \) sedentarius, and \( M. \) roseus.

\( M. \) luteus was only sporadically isolated from the nares and axillae of persons living in North Carolina and the axillae of persons living in New Jersey. However, when it was present, it usually composed greater than 90% of the micrococi isolated from these sites. This species usually composed from 20 to 80% of the micrococi isolated from the head, legs, and arms. It was more frequently isolated from persons living in North Carolina than in New Jersey. Sixty percent of the individuals in the temporal study maintained persistent populations on the head and 70% maintained persistent populations on the legs and arms.

\( M. \) lylae, a closely related species to \( M. \) luteus (9, 15), was most frequently isolated from the head, legs, and arms and occurred over a wide range of percentages of micrococi isolated from these sites. The occurrence of \( M. \) lylae on individuals in the temporal study followed a seasonal pattern. They were more frequently isolated during the colder months of the years, from September to February.

\( M. \) sedentarius was only occasionally isolated from the head, legs, and arms and usually composed 1 to 25% of the micrococi isolated from these sites. This species was predominantly isolated from persons living in North Carolina.

\( M. \) varians and \( M. \) kristinae were usually isolated from the head, legs, and arms and occurred over a wide range of percentages of micrococi isolated from these sites. Of the two related species (15), \( M. \) varians was the most frequently isolated in this study. Both species were more frequently isolated from persons living in New Jersey than in North Carolina. Only one person in the temporal study maintained a persistent population of either species for as long as 9 to 12 months.

\( M. \) roseus, a closely related species to \( M. \) varians (9, 15), was relatively rare. When isolated, this species usually composed from 1 to 10% of the micrococi isolated from the head, legs, and arms.

\( M. \) nishinomiyaensis was more frequently isolated from the head, legs, and arms and less frequently from the axillae. This species usually composed from 5 to 30% of the micrococi isolated from these sites and was predomi-
Fig. 6. Frequency of occurrence and relative percentage of Micrococcus species isolated from human skin. Bar designations as in Fig. 2.
Fig. 7. Temporal study of Micrococcus species isolated from human skin. Explanation and symbols as in Fig. 3. Symbols indicate the percentage of total Micrococcus CFUs represented by each species.

DISCUSSION

The results on bacterial population composition and density presented here have been based on a semiquantitative CFU. Several earlier reports have implied that the enumeration of bacteria from skin can be estimated quantitatively; however, we would question this implication, as techniques were not presented that would insure single cell isolation of all varieties. Systematic studies characterizing species of staphylococci and micrococci isolated from human skin have indicated that the basic cell aggregation unit may vary in size according to species and strain (13, 15, 27). For example, the basic unit may be a single cell or a pair, tetrad, or packet of cells. Large cell clusters were usually dispersed by the action of detergents (37), but tenacious units such as packets, commonly found in strains of *M. luteus* and *M. sedentarius*, and tetrads, found in many species of micrococci and certain species of staphylococci, were

| Species | NARES 1971 | NARES 1972 | AXILLAE 1971 | AXILLAE 1972 | HEAD 1971 | HEAD 1972 | LEGS 1971 | LEGS 1972 | ARMS 1971 | ARMS 1972 |
|---------|------------|------------|--------------|--------------|-----------|-----------|-----------|-----------|-----------|-----------|
| M. luteus | ○          | ○          | ○            | ○            | ∙         | ∙         | ∙         | ∙         | ∙         | ∙         |
| M. lyoe  | ○          | ○          | ○            | ○            | ∙         | ∙         | ∙         | ∙         | ∙         | ∙         |
| M. sedentarius | ○ | ○ | ○ | ○ | ∙ | ∙ | ∙ | ∙ | ∙ | ∙ |
| M. varians | ○ | ○ | ○ | ○ | ∙ | ∙ | ∙ | ∙ | ∙ | ∙ |
| M. roseus | ○ | ○ | ○ | ○ | ∙ | ∙ | ∙ | ∙ | ∙ | ∙ |
| M. kristinae | ○ | ○ | ○ | ○ | ∙ | ∙ | ∙ | ∙ | ∙ | ∙ |
| M. nishimiyae | ○ | ○ | ○ | ○ | ∙ | ∙ | ∙ | ∙ | ∙ | ∙ |
not significantly reduced by such treatment. Information on cell aggregation units was obtained from cultures propagated on P agar media. We do not have any data on the nature of these units in natural environments, such as skin. Until techniques have been devised to provide a single cell or uniform CFU without a significant loss in viability, it would be more accurate to consider the enumeration of bacteria from skin as being semiquantitative rather than quantitative.

Samples taken from the nares and axillae usually contained fewer varieties of bacteria than those taken from the head, legs, or arms. This may be due, in part, to the small proportion of total bacteria analyzed from the nares and axilla sites, but if additional varieties were actually present they clearly represented only a very small percentage. In contrast, numerous varieties of bacteria were often isolated in moderate percentages from the head, legs, and arms. The nares and axillae, because of their location, are more naturally protected from environmental contamination and this may be a factor in limiting the number of varieties present. In addition, these sites usually contain very dense populations of bacteria, whose presence may ultimately destroy or limit the colonization of incoming, less-adapted varieties.

With only a few interesting exceptions, results obtained from persons living in North Carolina and New Jersey were rather similar, and, together, provide an estimate of the distribution of various bacteria on human skin. Generally, *Staphylococcus* species showed less geographical variation than *Microoccus* species (Fig. 4 and 6). Most staphylococci are believed to be capable of inhabiting the deeper, more anaerobic, portions of follicles (16) and the relatively sheltered areas of the nares, axillae, and groin, whereas most micrococci appear to be only capable of inhabiting the more exposed surface areas of skin. Such a habitat would place micrococci in greater contact with the external environment and possibly subject them to the influence of environmental factors that vary among geographical regions. An alternate explanation for our results would be that the percentages of different *Microoccus* species in the external environment might vary among geographical regions and by frequent contamination aid in determining the composition of species on skin. Determinations have not yet been made relating the proportion of species in the surrounding environment to the proportion found on skin. Our results on the distribution and relative percentage of coryneforms, enteric bacteria, *Bacillus*, and *Streptomyces* (Fig. 2) and *S. aureus* (Fig. 4) on skin are in general agreement with those reported by others (10, 12, 16, 17, 20, 24, 30, 32) and, therefore, serve as an adequate reference in this study for making comparisons with various *Staphylococcus* and *Microoccus* species not previously reported in detail.

The results of temporal studies presented here provide an estimate of the ability of various bacteria to occur or persist in certain cutaneous habitats. The terms persist or persistence are used throughout the text in a relaxed sense, since we cannot clearly distinguish between continued contamination from other sources and colonization. Some examples of contaminating sources would include other skin sites, such as headgear regions containing dense bacterial populations or surrounding areas, plants, fomites, soil, airborne particles, water, food, and other human or animal contact. Interpretation of results becomes somewhat difficult in temporal studies taken at the genus or species level, where certain species and strains, respectively, may be appearing on skin sites from another source while others may be disappearing, without causing a marked change in the total percentage or an interruption in isolation. We can reasonably conclude, however, that genera or species that are persistently isolated in large numbers in relatively sheltered areas such as the nares and axillae have established colonization or residency. Persistence in more exposed areas such as the head, legs, and arms is obviously more difficult to interpret. On the other hand, if a genus or species fails to demonstrate persistence in isolation, we can surmise that it was unable to establish an effective colonization, notwithstanding the small numbers of members that might have been missed in the sampling procedures. Colonization would be best estimated in temporal studies where individual strain or clonal populations are followed; however, this is beyond the scope of the present paper and will be reported in another communication.

The remarkable seasonal variations observed in the occurrence of acinetobacters and *M. lylae* on skin offer some interesting preliminary data for future ecological studies relating to mechanisms of adaptation. In the case of acinetobacters (many previously referred to as *Mimeae*), Taplin et al. (36) and Kligman (12) have suggested a high moisture requirement for survival and multiplication. This requirement may be, at least, one of the reasons why acinetobacters were more prevalent on skin during the warm and humid months in North Carolina, at which time the skin might be especially moist
due to high sweating activity and reduced evaporation.

The regions of the body sampled in this study represent several major habitats that include approximately 60% of the total surface area of skin (16). Several special areas of skin that were not studied here include the scalp, external auditory meatus, palm of the hands, sole and interdigital spaces of the feet, and the inguinal and perineal area. These areas provide rather unique habitats (16) and might contain species populations that differ in composition from those reported above.

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LITERATURE CITED

1. Bailey, W. R., and E. G. Scott. 1966. Diagnostic bacteriology. The C. V. Mosby Co., St. Louis.
2. Baird-Parker, A. C. 1965. Staphylococci and their classification. Ann. N.Y. Acad. Sci. 128:4–25.
3. Baird-Parker, A. C. 1965. The classification of staphylococci and micrococcii from world-wide sources. J. Gen. Microbiol. 38:383–387.
4. Baird-Parker, A. C. 1974. The basis for the present classification of staphylococci and micrococcii. Ann. N.Y. Acad. Sci. 236:7–14.
5. Baumann, P., M. Doudoroff, and R. Y. Stanier. 1968. Study of the Moraxella group. II. Oxidase-negative species (genus Acinetobacter). J. Bacteriol. 95:1520–1541.
6. Buchanan, R. E., and N. E. Gibbons. 1974. Bergey’s manual of determinative bacteriology. The Williams & Wilkins Co., Baltimore.
7. Evans, J. B., and W. E. Kloos. 1972. Use of shake cultures in a semisolid thioglycolate medium for differentiating staphylococci from micrococcii. Appl. Microbiol. 23:326–331.
8. Ewing, W. H. 1970. Enterobacteriaceae infectious. p. 227–250. In H. L. Bodily, E. L. Updyke, and J. O. Moxon (ed.). Diagnostic procedures for bacterial, mycotic and parasitic infections. American Public Health Association, Inc., New York.
9. Farriror, J. W., and W. E. Kloos. 1975. Amino acid and vitamin requirements of Micrococcus species isolated from human skin. Int. J. Syst. Bacteriol. 25:36–82.
10. Glass, M. 1973. Saricina species on the skin of the human forearm. Trans. St. John’s Hosp. Dermatol. Soc. 59:56–60.
11. Junit, E. A. and A. Janik. 1969. Transformation of Acinetobacter calcoaceticus (Bacterium anthratus). J. Bacteriol. 98:281–288.
12. Klugman, A. M. 1965. The bacteriology of normal skin, p. 13–31. In H. I. Maibach and G. Hildick-Smith (ed.), Skin bacteria and their role in infection. McGraw-Hill Book Co., New York.
13. Kloos, W. E., and K. H. Schleifer. 1975. Isolation and characterization of staphylococci from human skin. II. Descriptions of four new species: Staphylococcus warneri, Staphylococcus capitis, Staphylococcus hominis, and Staphylococcus simulans. Int. J. Syst. Bacteriol. 25:82–79.
14. Kloos, W. E., and K. H. Schleifer. 1975. Simplified scheme for routine identification of Staphylococcus species. J. Clin. Microbiol. 1:82–88.
15. Kloos, W. E., T. G. Tornabene, and K. H. Schleifer. 1974. Isolation and characterization of micrococcii from human skin, including two new species: Micrococcus lylae and Micrococcus kristinae. Int. J. Syst. Bacteriol. 24:79–101.
16. Marples, M. J. 1965. The ecology of the human skin. Charles C Thomas, Publishers, Springfield, Ill.
17. Marples, R. R. 1969. Diphtheroids of normal human skin. Br. J. Dermatol. 81(Suppl. 1):47–54.
18. Martin, W. J. 1970. Enterobacteriaceae, p. 151–174. In J. E. Blair, E. H. Lennette, and J. P. Truant (ed.), Manual of clinical microbiology. American Society for Microbiology, Bethesda, Md.
19. Naylor, H. B., and E. B. Ferguson. 1956. Observations on abortive infection of Micrococcus lysodeikiticus with bacteriophage. Virology 2:577–593.
20. Noble, W. C. 1969. Distribution of the Micrococcaceae. Br. J. Dermatol. 81(Suppl. 1):27–32.
21. Noble, W. C. 1969. Skin carriage of the Micrococcaceae. J. Clin. Pathol. 22:249–253.
22. Pachtman, E. A., E. E. Vicher, and M. J. Brunner. 1954. The bacteriologic flora in seborrhoic dermatitis. J. Invest. Dermatol. 22:389–397.
23. Reeder, W. J., and R. D. Ekstedt. 1973. Unique teichoic acid isolated from the cell walls of a strain of Staphylococcus aureus. Infect. Immun. 7:586–588.
24. Rosebury, T. 1962. Microorganisms indigenous to man. McGraw-Hill Book Co., New York.
25. Samuels, S. B., B. Pittman, H. W. Tatum, and W. B. Cherry. 1972. Report on a study set of Moraxellae and allied bacteria. Int. J. Syst. Bacteriol. 22:19–38.
26. Sarkany, I., and C. C. Gaylard. 1968. Bacterial colonization of the skin of the newborn. J. Pathol. Bacteriol. 95:115–122.
27. Schleifer, K. H., and W. E. Kloos. 1975. Isolation and characterization of staphylococci from human skin. I. Amended descriptions of Staphylococcus epidermidis and Staphylococcus saprophyticus and descriptions of three new species: Staphylococcus cohnii, Staphylococcus haemolyticus, and Staphylococcus xylosus. Int. J. Syst. Bacteriol. 25:50–61.
28. Schleifer, K. H., and W. E. Kloos. 1975. Simple test system for the separation of staphylococci from micrococcii. J. Clin. Microbiol. 1:337–338.
29. Schleifer, K. H., and M. Kocur. 1973. Classification of staphylococci based on chemical and biochemical properties. Arch. Mikrobiol. 93:65–85.
30. Smith, R. F. 1969. Characterization of human cutaneous lipophilic diphtheroids. J. Gen. Microbiol. 55:433–445.
31. Smith, R. F. 1970. Comparative enumeration of lipophilic and nonlipophilic cutaneous diphtheroids and coccii. Appl. Microbiol. 19:254–258.
32. Somerville, D. A. 1969. The normal flora of the skin in different age groups. Br. J. Dermatol. 81:248–258.
33. Somerville, D. A., and M. Lancaster-Smith. 1973. The aerobic cutaneous microflora of diabetic subjects. Br. J. Dermatol. 89:395–400.
34. Somerville, D. A., and C. T. Murphy. 1973. Quantitation of Corynebacterium acnes on healthy human skin. J. Invest. Dermatol. 60:231–233.
35. Subcommittee on the Taxonomy of Moraxella and Allied Bacteria. 1971. Minutes of meeting. Int. J. Syst. Bacteriol. 21:213–214.
36. Taplin, D., G. Rebell, and N. Zais. 1963. The human skin as a source of Mima-Herellea infections. J. Am.
37. Williamson, P. 1965. Quantitative estimation of cutaneous bacteria, p. 3–11. In H. I. Maibach and G. Hildick-Smith (ed.), Skin bacteria and their role in infection. McGraw-Hill Book Co., New York.
38. Williamson, P., and A. M. Kligman. 1965. A new method for the quantitative investigation of cutaneous bacteria. J. Invest. Dermatol. 45:498–503.