

The Environment and the Microbial Ecology of Human Skin

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Received for publication 3 May 1976

Microbial flora of the skin of three human population groups representing different natural environments was examined quantitatively and qualitatively to determine whether environmental differences in temperature and humidity can influence the microbial flora of normal skin. Five anatomical skin sites—hands, back, axillae, groin, and feet—were sampled from 10 subjects working in a high-humidity, high-temperature environment, 10 subjects from a low-temperature, high-humidity environment, and 10 subjects working in a moderate-temperature and low-humidity environment. Bacterial populations were significantly larger from the back, axillae, and feet in individuals from the high-temperature and high-humidity environment as compared to the moderate-temperature, low-humidity environment. High humidity and low temperature had no significant effect on total populations, but this group showed a higher frequency of isolation of fungi, and gram-negative bacteria from the back and feet. Although there was an indication that increase in the environmental humidity could result in an increased frequency of isolation of gram-negative bacteria, there was no evidence that an increase in either temperature or humidity altered the relative proportions of gram-negative bacteria in the predominantly gram-positive microbial flora found on normal skin. It was concluded that, although climatic changes may cause fluctuation in microbial populations from certain sites, they are not a major influence on the ecology of the microbial flora of normal skin in the natural environment. The variables introduced by studying individuals in their natural environment and the influence of these on the results are discussed.

The microbial flora of normal skin presents a distinct ecosystem which consists of a characteristic microflora. Whereas the literature has classically described the bacteria present on skin as various species of *Staphylococcus*, *Micrococcus*, and *Corynebacterium*, the complexity of the system has only recently gained attention (10, 12). The difficulty of classifying and identifying the numerous gram-positive cocci and diphtheroid-like organisms found on the skin has masked the individuality and diversity of its microbial flora. A comprehensive study on the taxonomy of gram-positive cocci isolated from skin (5, 6, 13) has shown the diversity of these organisms, and the *Corynebacterium* have been found to be equally as varied (11, 17). Indications are, however, that normal healthy individuals maintain a stable microbial flora which consists of predominantly gram-positive bacteria (4, 5, 12), even though there have been reports that in certain disease states there is an increase in the frequency of isolation of gram-negative bacteria (8, 18). Many of the factors controlling microbial flora

of human skin are obscure owing to the complexity of the physiology of the skin, complicated by interaction with the environment with its variations in temperature and humidity. The relationship of the cutaneous microflora to the physiology of the skin on one hand and to the environment on the other has caused speculation as to the extent the environment can control the microbial flora of the skin.

The effect of temperature and humidity on the microbial flora of the skin has been studied experimentally in a climate-controlled chamber where a combination of high temperature and high humidity resulted in an increase in total microbial populations (3) and the frequency of isolation of gram-negative bacteria (9). Although these results clearly showed that temperature and humidity can influence cutaneous microbial flora under controlled conditions, the extent of this influence in the natural environment has not been examined.

The present study was done to determine to what extent high temperatures and humidities in the natural environment can influence cuta-

neous microbial flora in a normal healthy population. Three groups of subjects were chosen by occupation to examine this hypothesis: medical center personnel, working in an air-conditioned, low-humidity environment; laborers working in a high-humidity, high-temperature environment; and Coast Guard personnel from a high-humidity, low-temperature environment. Five different anatomical skin sites—hands, back, axillae, groin, and feet—were sampled. Samples were studied qualitatively and total microbial populations were determined as well as the relative proportions of gram-positive to gram-negative bacteria.

MATERIALS AND METHODS

Three groups of 10 subjects each were studied and their description is shown in Table 1. Laborers were predominantly gardeners who were sampled in the summer when temperatures in Houston range between 23.9 to 35.6°C (75 to 98°F) and humidities range from 80 to 100%. Coast Guard personnel were sampled in the winter and temperatures at the time of sampling ranged from 8.89 to 22.2°C (30 to 72°F), with a mean of 12°C (53.6°F), and the humidity varied between 56 and 100%, with a mean of 72%. The environment of the medical center group, who worked and lived in air-conditioned buildings, varied only slightly from day to day, between 21.1 and 23.9°C (72 and 75°F) and between 30 and 60% humidity. Age range, although overlapping, was not comparable between the groups, with the Coast Guard subjects being the youngest and laborers being the oldest. This difference should not influence the results, however, since evidence indicates that once puberty is reached, microbial flora remains stable until the seventh decade (16). Use of antibacterial soaps and deodorants was also recorded, and Coast Guard and medical personnel were found to be the heaviest users of deodorants (9 out of 10) as compared to 2 out of 10 laborers. Fewer laborers (four) used antibacterial soaps than medical (six) or Coast Guard (six) personnel. No effort was made to select subjects by the use of antibacterial soaps and deodorants since it was rare to find anyone in the medical center environment not using these preparations and the converse was true for laborers; therefore, this was considered to be normal for these popula-

tions. All subjects were sampled in their natural environment and were not receiving treatment of any kind, including antibiotics.

Sampling procedures. Sites cultured were palms of hands, soles of feet, axillae, groin, and back over the scapulae. Each area was cultured in duplicate, samples being taken from both the right and left sides of the body. The skin sampling method chosen was evaluated statistically and compared favorably with other quantitative skin sampling methods, although it gave slightly lower values (14). Specimens were collected by delineating a 16-cm² area with a sterile template and scrubbing the skin in that area with calcium alginate swabs moistened in phosphate-buffered saline (pH 7.2) containing 0.1% Triton X-100. These swabs were suspended in 2.5 ml of the same buffer solution in test tubes (13 by 100 mm) and shaken on a wrist-action shaker for 5 min. After suitable dilutions, bacterial counts were made by using pour plates of Trypticase soy agar containing 0.1% Tween 80 and drop plates by inoculating the surface of Casman sheep blood agar with a calibrated dropping pipette. McConkey, phenylethyl alcohol agar, and other selective media were used when necessary. Of the original suspension, 0.25 ml was used to inoculate Casman sheep blood agar for qualitative studies and 0.5 ml was inoculated into thioglycolate broth to detect organisms present in low concentrations. Plates were incubated for 3 days at 31°C. Routine diagnostic media and procedures were used for identification (2).

Statistical procedures. The mean bacterial population was calculated from duplicate cultures taken from right and left sides of the body from each site in each subject. Significant differences in bacterial skin populations between groups were determined by an analysis of variance followed by calculation of least-significant differences when $P < 0.05$.

RESULTS

Quantitative results. Total bacterial populations were determined from the five skin sites. There was no difference in populations between the environmental groups from the hands and groin but statistically significant differences were found in populations from the axillae, back, and feet (Fig. 1). Bacterial populations of the back and axillae from the medical center personnel proved to be significantly lower than

TABLE 1. Subject description

Subject group	Subject descriptor								
	No.	Age		Environmental temp (°C)		Environmental RH ^a (%)		No. using antibacterial soaps	No. using deodorants
		Range	Median	Mean	Range	Mean	Range		
Medical personnel	10	27-32	30	22.2	21.1-23.9	47	30-60	7	9
Laborers	10	17-58	38	28	23-35.6	90	70-100	4	2
Coast Guard	10	19-23	21	12	8.89-22.2	92	72-100	6	9

^a RH, Relative humidity.

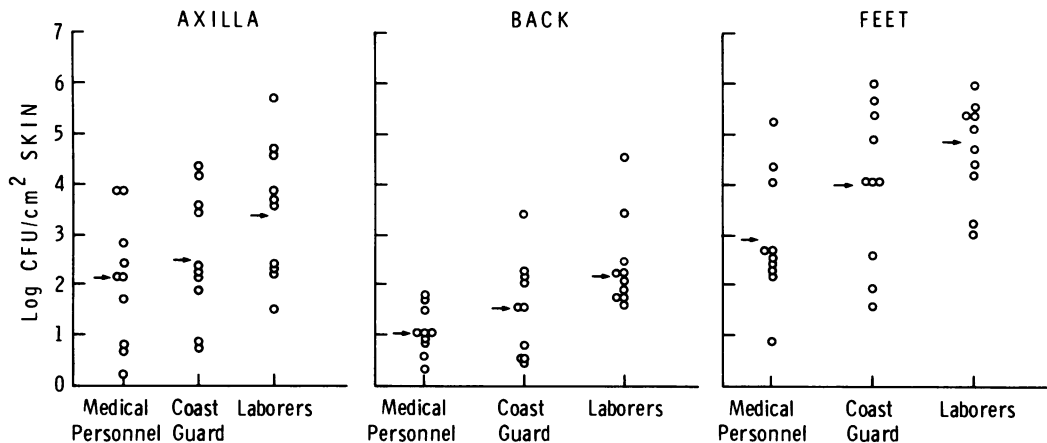


FIG. 1. Total bacterial populations of the skin of the axillae, back, and feet from subjects from different environments. Symbol: ○, mean CFU per square centimeter of skin duplicate samples from each subject; →, mean CFU per square centimeter of skin for each group.

those of the laborers, with P values <0.02 . Similarly, the bacterial populations of the feet of the medical center personnel proved to be significantly lower than those of both of the other groups (P values <0.01). The means, standard deviations, and P values are shown in Table 2. Hence, high temperatures and humidities can result in an increase in microbial skin populations in certain anatomical sites.

Qualitative results. The frequency of isolation of different genera of bacteria from the five anatomical sites in each subject group is shown on Table 3. With groups containing only 10 subjects, it is not possible to make a statistical evaluation of difference in the frequency of isolation between the three environmental groups; however, trends can be seen. Although all of the organisms were speciated, when possible the microorganisms were grouped into the major types encountered on normal skin, e.g., gram-positive cocci (other than streptococci and *Staphylococcus aureus*) were considered together and include species of both micrococci and staphylococci. Similarly, the aerobic species of corynebacteria were grouped together as were the anaerobic species (all of which proved to be *Propionibacterium acnes*). Gram-negative bacteria were separated into two categories: (i) non-fermentative, including species such as *Acinetobacter* and *Moraxella*, which are considered to be water or environmental in origin, and (ii) fermentative, all of which were species of *Enterobacteriaceae*, the majority belonging to the genus *Enterobacter*.

Results (Table 3) reveal that, despite differences in environment, the presence of species of micrococci and staphylococci remain stable in 100% of the population of all environmental groups.

TABLE 2. Comparison of bacterial populations on skin in subjects under different environmental conditions

Skin site	Log ₁₀ CFU/cm ² of skin		
	Mean	SD	P
Axillae			
Medical personnel	2.05	1.27	<0.02
Laborers	3.41	1.48	
Coast Guard	2.59	1.27	
Back			
Medical personnel	1.08	0.42	<0.02
Laborers	2.16	1.09	
Coast Guard	1.55	0.98	
Feet			
Medical personnel	2.94	1.29	<0.01
Laborers	4.81	0.97	
Coast Guard	4.07	1.57	

There was a high frequency of isolation of fungi from all subject groups. It is with this particular group of microorganisms that we can see differences between the environmental groups. Coast Guard personnel had a considerably greater incidence and variety of fungi than the other two subject groups. Frequently, two or more species of fungi were isolated from each culture site. Species isolated are listed in Table 4 and are common to air and soil microflora. Yeasts isolated were all species of *Candida*, but not *Candida albicans*, and occurred randomly in each environmental group.

S. aureus was isolated solely from the Coast Guard group, a member of which appeared to be a persistent skin carrier.

Since the presence of gram-negative bacteria is considered to be an abnormal occurrence on all sites except the axilla, the relative propor-

TABLE 3. Frequency of isolation of bacterial species from five skin sites in subjects in different environmental conditions of temperature and humidity^a

Microorganisms	No. of subjects/10														
	Hands			Axillae			Back			Groin			Feet		
	MCP	Lab	CG	MCP	Lab	CG	MCP	Lab	CG	MCP	Lab	CG	MCP	Lab	CG
Micrococci and staphylococci	10	10	10	10	10	10	10	10	10	10	10	10	10	10	10
<i>Bacillus</i> spp.	6	8	8	2	6	6	5	7	3	3	3	5	4	5	4
Aerobic diphtheroids	7	8	10	4	10	9	5	8	5	8	8	9	5	8	8
Anaerobic diphtheroids	7	4	0	5	2	1	9	3	7	7	2	0	4	0	0
Non-fermentative gram-negatives	0	0	1	1	1	0	1	0	0	0	1	1	0	1	3
<i>Enterobacteriaceae</i>	1	0	0	2	3	5	0	0	4	1	0	0	1	1	4
Yeast	2	0	3	0	0	1	0	0	0	0	2	0	1	0	0
Fungi	3	4	9	0	3	5	1	5	4	2	2	2	6	2	3
<i>Staphylococcus aureus</i>	0	0	2	0	0	0	0	0	0	0	0	1	0	0	2

^a Abbreviations: MCP, medical center personnel (moderate temperature and humidity); Lab, laborers (high temperature and humidity); CG, Coast Guard (moderate temperature, high humidity).

TABLE 4. Frequency of isolation of fungi and aerobic actinomycetes from skin in subjects under different environmental conditions

Genus	No. of subjects/10		
	Coast Guard	Laborers	Medical personnel
<i>Aspergillus</i>	7	1	4
<i>Hormodendron</i>	7	0	0
<i>Streptomyces</i>	4	5	2
<i>Penicillium</i>	4	2	3
<i>Alternaria</i>	3	0	1
<i>Oospora</i>	2	0	0
<i>Curvularia</i>	2	1	1
<i>Mucor</i>	1	0	0
<i>Gliocladium</i>	1	0	1
<i>Monotospora</i>	1	0	0
<i>Paecilomyces</i>	1	1	0
<i>Scopulariopsis</i>	1	0	0
<i>Nigrospora</i>	0	1	0

tion of gram-negative populations to gram-positive populations was determined to establish whether changes in environmental temperature and humidity could result in population shifts. Results are shown in Fig. 2 and 3. Each bar represents the relative proportion of gram-positive to gram-negative populations from a single subject, and since relatively few subjects were carriers of gram-negative bacteria, there are different numbers of bars shown for each environmental group. As was expected, gram-negative bacteria were found predominantly in the axillae (Fig. 2), but even at that site they were not in high populations in a majority of subjects. Furthermore, gram-positive bacteria were present in all subjects except two of the medical center personnel. Gram-negative bacterial populations were found to be low from

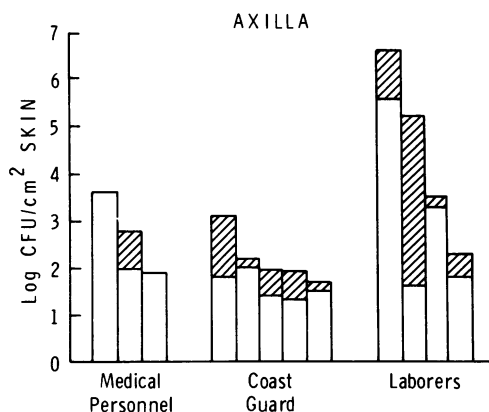


FIG. 2. Relative proportions of gram-negative bacteria to total bacterial populations of the skin of the axilla. Symbols: ▨, gram-positive population; ▩, populations of gram-negative bacteria. Each bar represents the relative populations from a single subject.

hands, back and groin (Fig. 2) in all subjects regardless of environment, but high from the feet.

DISCUSSION

A parallel can be drawn between the changes observed in skin microflora from studies using a climate chamber for an experimentally controlled environment (3) and the natural environment. In each case increased temperature and humidity resulted in an increase in total bacterial population from certain anatomical sites. In the environmental chamber experiments, however, the increase was considerably greater. Similarly, humidity alone proved to have no significant effect on bacterial populations.

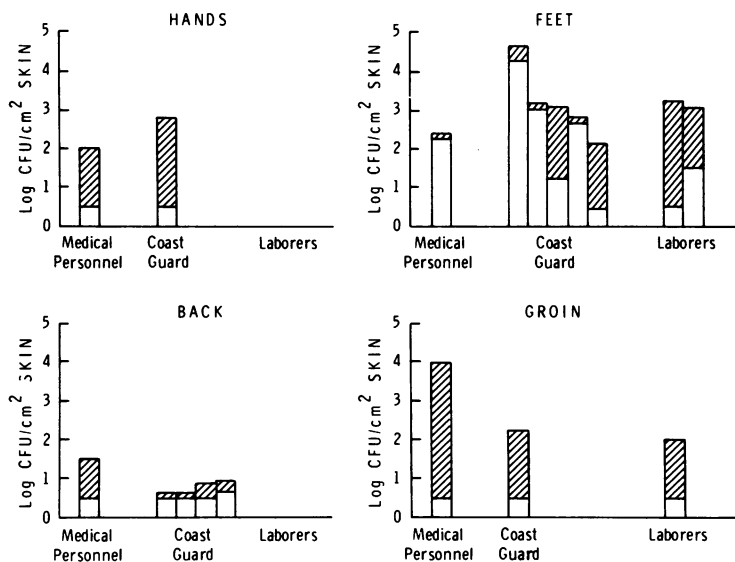


FIG. 3. Relative proportions of gram-negative bacteria to total bacterial populations of the skin of the hands, feet, back, and groin. Each bar represents the populations from a single subject. Striped bar, Total populations; open bar, gram-negative populations.

The presence of gram-negative bacteria, such as *Enterobacteriaceae* and *Acinetobacter* species, was used as an indication for population changes, and there was no increase in either frequency of isolation of these organisms or their populations in the group working in high temperatures and humidity. Humidity alone, however, appeared to cause an increase in the frequency of isolation of gram-negative bacteria.

When one attempts to compare observations made under controlled experimental conditions to those made in a natural environment, it is not possible to eliminate many of the variables which are introduced. Choice of subjects is an important one, and in this study occupation was chosen as the basis of selecting the environment. This, unfortunately, introduced socioeconomic factors over which there was no control. Use of antibacterial soaps and deodorants is a reflection of this since the majority of medical center and Coast Guard personnel used these products, and such use may have been a factor in the lower populations observed. Introduction of this variable may, however, provide additional information. The increased use of antibacterial soaps and cosmetics has caused concern that these compounds may upset the balance between gram-negative and gram-positive populations. Abnormal usage of antibacterials on skin has shown to result in a decline in the gram-positive population and an increase of colonization by gram-negatives (1, 19). The medi-

cal center group, however, was not found to have a higher frequency of isolation of gram-negatives than laborers, although in axillae the relative proportion of gram-negative to gram-positive populations was higher. The Coast Guard personnel, however, had a higher frequency of isolation of gram-negative bacteria from the back, feet, and axillae, and relative roles of antibacterials versus humidity can be debated. The question can only be resolved by studying much larger groups of subjects.

Axilla was the only site where gram-negative bacteria were isolated, and it is of interest that these organisms were isolated with equal frequency from individuals who did not use deodorant as compared to those who did. The main difference between deodorant users and non-users was a lower incidence of aerobic diphtheroids from the former group which is consistent with a recent study (12). Unlike earlier reports (15), gram-negative flora could not be correlated directly with use of deodorants.

Little is known of the incidence of fungi on normal skin (12), but the incidence experienced in this study from the skin of Coast Guard personnel would seem higher than normal. Even the laborers, whose main occupation was gardening and other activities related to soil (which one would assume to be the source of these fungi), had a lower frequency of isolation than Coast Guard personnel.

If a general assessment can be made of the relative roles of the external environment and

the physiological environment in the control of the microbial flora of human skin, the data presented here support the view that changes in environment can cause fluctuations in bacterial populations of the skin, but the gram-positive character of the flora both in types and numbers of organisms is remarkably stable.

ACKNOWLEDGMENT

This study was funded by the U.S. Army Medical Research and Development Command, Office of the Surgeon General, Contract No. DA-49-193-MD-274b.

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