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Effects of an Antibacterial Soap on the Ecology of Aerobic Bacterial Flora of Human Skin

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The effects of ad lib use of an antibacterial soap containing 1.0% trichlorocarbanilide and 0.5% trifluoromethyldichlorocarbanilide on the bacterial flora of six skin sites of 132 subjects were measured by comparison with the flora of 93 control subjects who avoided the use of topical antibacterials. Each subject was examined once. The test soap produced significant reductions in geometric mean counts of the total aerobic flora on the back, chest, forearm, calf, and foot; counts were also reduced in the axilla, but not to a significant extent. The overall reduction by the test soap on all sites was 62% ($P < 0.001$). Neither age nor sex influenced the effect of the soap on the flora. The antibacterial soap also reduced the prevalence of *Staphylococcus aureus* on the skin, mostly by virtually eliminating it from areas other than the axilla. Partial inhibition of the gram-positive flora was not accompanied by an increase in gram-negative species. The latter were found principally in the axilla; *Klebsiella pneumoniae* and *Enterobacter aerogenes* were the species most frequently found.

The great majority of the studies on the effects of antibacterial soaps on the flora of human skin have been carried out on hands because of the interest in degerming in surgical scrub procedures and because hands carry large numbers of microorganisms and permit the demonstration of sizable reductions in numbers. Several methods based on the original observations of Price have been used (4, 21, 22). Various combinations of methods have been applied to the study of the efficacy of antimicrobials applied to the skin in reducing the aerobic flora (4, 5, 9, 10, 18, 27). However, these methods have generally involved exaggerated exposure to the antibacterial soaps or surgical scrub products, far beyond that to be expected in normal ad lib use for personal hygiene, and have often ignored the effects on the flora on sites other than hands.

Other studies (7, 13, 15) have shown a beneficial effect of antibacterial soaps containing agents active against gram-positive organisms in reducing the incidence of pyogenic skin infections attributed primarily to *Staphylococcus aureus*. However, intensive use of such agents has sometimes been reported to be associated with an increased susceptibility to skin infections with gram-negative species (9, 11, 14). Aside from such observations, there has been little attention paid to the qualitative effects of agents selected for bacteriostatic activity against gram-positive cocci on the nature of the skin flora.

This report describes the study of the influence of ad lib use of an antibacterial soap on the total aerobic bacterial flora on six skin sites. We wished to learn whether the primarily gram-positive flora and the prevalence of *S. aureus* would be effectively reduced under these conditions and whether such an inhibition of the gram-positive flora would be accompanied by an increased prevalence of gram-negative species.

The role of streptococci as potential causative organisms in pyodermas has gained considerable interest recently (1, 2, 6, 23, 25). At the time of this work, however, such interest was relatively low and the streptococci were not followed in this study.

MATERIALS AND METHODS

Subjects. Control subjects consisted of a group of 93 adults (60 males and 33 females, with average ages of 36 and 29 years, respectively) who routinely abstain from the use of topical antibacterial products. They were provided with soap and shampoo devoid of added antibacterials, for their exclusive use. Each was examined once. There were 132 test subjects (91 males and 41 females, with average ages of 35 years in each group) who were provided with the same shampoo as the control subjects, but who were given the test soap and instructed to use it exclusively for normal personal hygiene. They were also instructed to avoid the use of topical products (e.g., shaving creams) which they knew contained antibacterial compounds. Each test subject was examined once after he had used the test soap for 2 to 7 months.

Test soap. The test soap bars contained 1.0% 3,4,4'-trichlorocarbanilide (triclocarban) and 0.5% 3-trifluoromethyl-4,4'-dichlorocarbanilide (cloflucarban) in a mixture of equal parts of tallow and coconut oil.

Skin sampling. The axillary vault, back (interscapular area next to the spine), chest (presternal area), forearm (flexor surface), calf (medial aspect), and foot (medial aspect of the heel) were sampled by a modification of the method of Williamson and Kligman (26). A sterile glass cylinder with an inner area of 16.6 cm^2 (8.6 cm^2 for use on the foot) was applied firmly to the skin surface, and 5 ml of a sterile solution of 0.1% Triton X-100 (octyl phenoxy polyethoxy ethanol; Rohm and Haas Co.) in 0.075 M phosphate buffer (pH 7.9) was pipetted onto the skin. The surface of the skin was scrubbed vigorously for 60 s with a sterile, flattened glass rod having a surface 16 mm wide. The turbid sample was pipetted into a test tube, and the operation was repeated; both samples were pooled and mixed vigorously before plating.

To minimize the possible antibacterial effects of deodorants and antiperspirants in the axilla, the subjects were requested to abstain from their use for 3 days before sampling.

In addition, the anterior nares were sampled by rubbing with sterile Calgiswabs (Colab).

Microbiological examination. Total aerobic counts were made on Trypticase soy agar (BBL) plus 0.5% Tween 80 (polyoxyethylene sorbitan monooleate; Atlas Chemical Inc.), which served both as a neutralizer for the possible carry-over of antibacterial activity and as a substrate for the growth of lipophilic diphtheroids. Axillary washings were diluted 1:100 to 1:10,000, and washings from other sites were tested both undiluted and at a 1:10 dilution. Portions of 0.1 ml were spread evenly over the surface of the plates. Counts were made after 2 days at 35 C and calculated as numbers per square centimeter of skin surface.

Samples (0.1 ml) of undiluted washings were spread on eosin methylene blue agar (BBL) plates that were incubated as above for isolation of gram-negative organisms. Isolates were indentified by using an API 20 Enteric system (Analytab Products Inc.). When it was possible, initial numbers on the eosin methylene blue plates were estimated and converted to numbers per square centimeter.

Early trials demonstrated that the commonly used mannitol salt agar was quite inhibitory to *S. aureus* and markedly reduced recovery of the organism from the nose. Therefore, for isolation and identification of *S. aureus*, nasal swabs and 0.1-ml portions of undiluted washings were spread on Baird-Parker agar base (BBL) plus 5% EY tellurite enrichment (Difco) or Baird-Parker agar base plus 5% egg yolk enrichment (BBL) plus 1% tellurite solution (BBL), 1%; comparative trials showed these two media to be similar in efficacy. Counts of colonies that showed a zone of clearing of the egg yolk after 2 days at 35 C were estimated, and selected colonies were stabbed into plates containing Trypticase soy agar plus 0.3% yeast extract (BBL) and 20% sterile, heparinized pig plasma for determination of coagulase production by a modification of the method of Parisi et al. (19). Plates were read after 24 h at 35 C for the appearance of a hazy zone of precipitated fibrin around the colonies. These were recorded as coagulase-positive *S. aureus*. Comparative trials showed excellent agreement between this method and the usual tube method using lyophilized rabbit plasma.

Data on the total aerobic flora on the various sites were converted to $\log_{10}$ to minimize the influence of great variability in counts. This transformation has both a statistical (3) and a microbiological (17) rationale. Means were examined for the influence of the test soap and for the relation to sex and age by analysis of variance. When one of these factors appeared to be significant by the F test, the significance of the difference between means of subgroups divided by age and sex was determined by the Student's *t* test.

RESULTS

Effect of test soap on total aerobic flora. Geometric means of counts on the six sites sampled on the test and control subjects, and on all sites combined, are shown in Table 1. It is evident that the test soap reduced the total aerobic flora on all sites. The difference between control and test subjects was significant on all sites except the axilla; only in the case of the back was the difference significant at a confidence level < 99%. The overall reduction of the geometric means of the flora on all sites with the use of the test soap was 62%.

Table 2 shows the corresponding means when the subjects were subdivided by sex. The test soap produced significant reductions on the chest, forearm, foot, and all sites combined in the females. The effect was significant at the 0.05 confidence level on the chest and forearm of the males and on the calf of the females, and

TABLE 1. *Effect of antibacterial soap on geometric means of total aerobic bacterial counts per square centimeter on six skin sites of 93 control and 132 test subjects*

Site	Total counts/cm^2	
	Control soap	Test soap
Axilla	180,000	95,000
Back	470	170[a]
Chest	680	230[b]
Forearm	210	49[b]
Calf	190	91[c]
Foot	390	170[c]
All sites	1,000	380[b]

[a] Significantly different from controls ($P < 0.05$).
[b] Significantly different from controls ($P < 0.001$).
[c] Significantly different from controls ($P < 0.01$).

TABLE 2. *Relation of sex to geometric means of total aerobic bacterial counts per square centimeter on six skin sites of 93 control and 132 test subjects*

Site	Total counts/cm²			
	Males		Females	
	Control[a]	Test[b]	Control[c]	Test[d]
Axilla	340,000	160,000	97,000	55,000
Back	540	230	400	130
Chest	1,200	390[e]	400	130[e]
Forearm	200	83[e]	230	29[f]
Calf	310	170	110	50[e]
Foot	890	260[e]	170	110
All sites	1,500	590[e]	670	240[f]

[a] Number of subjects (N) = 60.
[b] N = 92.
[c] N = 33.
[d] N = 40.
[e] Significantly different from controls ($P < 0.05$).
[f] Significantly different from controls ($P < 0.001$).
[g] Significantly different from controls ($P < 0.01$).

it was significant at the 0.01 or 0.001 confidence levels on the foot of males and forearm of females, and on all sites combined. Overall reductions from control levels on all sites were 61% for males and 64% for females. It will also be noted that the control males had higher counts than the females on all sites except the forearm; this difference was significant at the 0.001 level on the axilla, calf, foot, and on all sites combined, and at the 0.002 level on the chest.

The results from examining the effect of age are given in Table 3, in which subjects 20 to 30 years of age and those over 30 were treated separately. The younger subjects showed significant effects of the test soap on the chest, forearm, and on all sites combined ($P < 0.05$), whereas the older subjects showed significant reductions on the chest and foot ($P < 0.05$) and on the forearm, calf, and all sites combined ($P < 0.001$). The older control subjects yielded significantly higher counts on the chest ($P < 0.01$) and forearm ($P < 0.05$) than did the younger controls, but significantly lower counts on the foot ($P < 0.01$). Overall reductions from control levels on all sites were 57% for younger subjects and 66% for older subjects.

These data demonstrate that the effects of the test soap were comparable both on males and females and on younger and older subjects.

The $\log_{10}$ means at intervals during the course of study of the control and test groups (Table 4) did not reveal any significant trends; i.e., there was no evident increase in counts, as might have been expected (8), during the summer months.

Occurrence of S. aureus on the skin. Table 5 summarizes the data with respect to the prevalence of *S. aureus* on the skin and in the nose, and the frequency and relative numbers in which the organism was found on the various skin sites in the test and control groups. As expected, the anterior nares yielded *S. aureus* more frequently than did the skin. Prevalence in the nose was similar to the values of about 30% or more that have been frequently reported in the literature (16, 20). Of the 26 subjects from whom *S. aureus* was recovered on the skin, 14 (54%) also carried the organism in the nose, again indicating that there is a positive rela-

TABLE 3. *Relation of age to geometric means of total aerobic bacterial counts per square centimeter on six skin sites of 93 control and 132 test subjects*

Site	Total counts/cm²			
	20–30 years		30+ years	
	Control[a]	Test[b]	Control[c]	Test[d]
Axilla	20,000	10,000	160,000	86,000
Back	660	220	330	140
Chest	470	130[e]	960	390[e]
Forearm	130	47[e]	340	52[f]
Calf	150	110	230	79[f]
Foot	580	300	270	100[e]
All sites	970	420[f]	1,000	340[f]

[a] N = 39.
[b] N = 58.
[c] N = 54.
[d] N = 74.
[e] Significantly different from controls ($P < 0.05$).
[f] Significantly different from controls ($P < 0.001$).

TABLE 4. *$\log_{10}$ means of total aerobic bacterial counts on all sites of control and test subjects at various periods*[a]

Treatment	$\log_{10}$ total counts						
	March	April	June	July	August	September–October	November–January
Control	3.00 (35)	3.00 (33)		3.17 (17)			3.37 (8)
Test soap			2.69 (47)		2.69 (41)	2.68 (29)	2.61 (15)

[a] Numbers in parentheses indicate number of subjects.

tion between nasal carriage and occurrence of the organism on the skin.

Table 5 shows that *S. aureus* was isolated from the axillae of only nine subjects and from the calf of only one subject in the group using the antibacterial soap. On the other hand, it was isolated from 26 sites (including all sites examined) on a total of 16 subjects in the smaller control group. The differences between the control and test groups in both numbers of subjects and numbers of sites carrying *S. aureus* are significant ($P < 0.05$ and $P < 0.001$, respectively), demonstrating that the test soap had achieved a real reduction in the carriage of the organism on the skin. Inspection of Table 5 shows that this reduction was achieved by eliminating the organism from the relatively dry skin of the back, chest, forearm, calf, and foot; carriage of the organism in the moist environment of the axilla was not significantly affected although its prevalence was reduced in the test group. In all areas, including the axilla, the numbers of *S. aureus* found on the normal skin were very low.

Occurrence of gram-negative species on the skin. The results of the examinations for gram-negative species on the various sites are summarized in Table 6. It is evident that partial suppression of the gram-positive flora by the carbanilides in the test soap was not sufficient to promote increased colonization of the skin by gram-negative bacteria; prevalence of these organisms was almost identical in the two groups. As in the case of *S. aureus*, they were found primarily in the moist environment of the axilla; 82% of the isolations of these organisms were from this site. *Klebsiella pneumoniae* was the species most frequently encountered, followed in frequency by *Enterobacter aerogenes*. Other genera and species were recovered much less frequently. In the axillae of two control subjects, *K. pneumoniae* was recovered in association with *E. aerogenes* or *Escherichia coli;* in all other cases, only one gram-negative species was found at a site. Means of estimated counts were similar in the control and test groups. It is clear that the use of an antibacterial soap led to neither increased frequency of colonization nor increased numbers of gram-negative organisms in the sites examined.

TABLE 5. *Occurrence of S. aureus on skin and in the nose*

Determination	Control soap	Test soap
No. of subjects	93	132
No. with *S. aureus* in nose	32 (34.4%)	31 (23.4%)
No. with *S. aureus* on skin	16 (17.2%)	10 (7.6%)[a]
No. of skin sites with *S. aureus*	26 (4.7%)	10 (1.3%)[b]
S. aureus geometric mean count per cm²		
Axilla	47 (11)[c]	74 (9)
Back	11 (6)	0
Chest	66 (3)	0
Forearm	6 (1)	0
Calf	22 (2)	12 (1)
Foot	26 (3)	0

[a] Significantly different from controls ($P < 0.05$).
[b] Significantly different from controls ($P < 0.001$).
[c] Number of subjects positive for *S. aureus* is in parentheses.

TABLE 6. *Occurrence of gram-negative species on the skin*

Determination	Control soap	Test soap
No. of subjects	93	132
No. of subjects positive	25 (27%)	36 (27%)
No. of sites positive	28 (4.3%)	43 (5.4%)
No. of subjects with positive:		
Axilla	22	36
Back	1	2
Chest	1	2
Forearm	1	2
Calf	1	1
Foot	2	0
Isolations of:		
K. pneumoniae	13	17
K. ozaenae	0	1
E. aerogenes	4	13
E. cloacae	4	2
Enterobacter sp.	0	1
E. coli	2	5
Pseudomonas aeruginosa	1	0
Proteus mirabilis	2	0
Proteus sp.	0	1
Alcaligenes sp.?	1	1
Unidentified or lost	3	2
Estimated geometric mean count per cm²	250	290

DISCUSSION

The nature of the control population dictated that they not use the test soap. Therefore, each subject was examined once after an extended period of use of the assigned soap; before-and-after comparisons were not possible. The assumption must be made that there was no significant difference between the two groups, unrelated to the use of soap; they were well balanced for age, sex and economic status.

The data on the total aerobic flora showed significant inhibition by the test soap on all sites except the axilla. Conditions for growth

are much more favorable there, and a larger population exists there than on the other areas of the skin that were examined. Even in the axilla, the population was smaller on the test subjects than on the controls although the difference was not statistically significant. Although female subjects showed lower counts than did the males, neither age nor sex appeared to influence the effect of the antibacterials on the flora. In spite of the fact that relatively large increases in temperature and relative humidity have been shown to increase the bacterial numbers on the skin (8), there was no indication in this population of subjects living and working in heated and air-conditioned environments over a period of several months that seasonal changes in climate were reflected in the size or nature of the flora.

There is some indication that the effect of the antibacterial soap on the prevalence of *S. aureus* on the skin may be slowly cumulative over a period of months. Eight isolations of the organism were made from 88 subjects after 2 to 4 months use of the soap, whereas only two isolations were made from 44 subjects after 4 to 7 months of use. Other data (unpublished) also suggest that the effect of the antibacterial soap on the prevalence of *S. aureus* on the skin may be cumulative over a period of months.

This study indicates that the regular ad lib use of the antibacterial soap, while reducing the gram-positive flora, does not cause overgrowth of gram-negative species. In unusual cases when the normal gram-positive flora have been sharply depressed by intensive use of antibacterials or antibiotics, it has been reported that this is followed by the overgrowth of gram-negative bacteria (9–11, 14, 24). Undoubtedly, the normal gram-positive flora of the skin serve a beneficial function in inhibiting the growth of other, less desirable organisms. Their complete eradication would create a vacuum by removing competition for space in the ecological niche on the skin, permitting the entrance of other organisms to fill the space vacated by the gram-positive bacteria; gram-negative bacteria and yeast are the other organisms which would be most likely to occupy the vacated niche. It seems preferable to attempt to decrease the prevalence of potential pathogens such as *S. aureus*, which is not as well adapted to growth on the skin as are the normal saprophytes, by methods that will reduce but not eliminate the other gram-positive organisms that serve a useful protective function. Such a reduction yields deodorant effects, as well as possible health benefits. We have shown that it is possible by the normal use of antibacterial soap to achieve a reduction in the numbers of gram-positive organisms and in the prevalence of *S. aureus* without establishing conditions favorable to an increased growth of gram-negative organisms.

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