Gibraltar Biological Laboratories, Inc.
23 JUST ROAD, FAIRFIELD, NEW JERSEY
07006
TELEPHONE (201) 227-6882
REPRINTS

Studies on the aerobic axillary microflora employing a standardized swabbing technique (total counts, speciation and ecological drift)

By Herbert N. Prince, Ph.D., Gibralter Biological Laboratories, Inc., Fairfield, N.J., and
James A. Rodgers, B.S., The Mennen Company, Morristown, N.J.

The introduction of gram negative rods (especially pseudomonads) to the skin via topical drugs or cosmetics is now generally considered by industry and FDA scientists as an avoidable "potential" hazard.[1] Equally undesirable is the colonization of gram negative rods as a function of repeated applications of limited spectrum antimicrobial drugs which tend to inhibit the usual gram positive flora (Marples),[2] leading to possible overgrowth with gram negative rods and/or *Candida*. However, little is known about the frequency with which one can isolate gram negative rods from normal skin or the types residing thereon (excepting the anogenital areas where they exist as transient autocontaminants).[3] In addition, little is known about the microbial ecosystems which preclude or tolerate the proliferation of gram negative bacilli on the skin.

Borick and Sarra[4] reported that 64% of their subjects harbored gram negative rods in the axillae; Ulrich[5] indicated that gram negative organisms are rarely found on normal skin. Rosebury[3] stressed that data were insufficient but suggested that gram negative rods are not part of the normal skin flora, except as fecal autocontaminants.

Since it was our feeling that the truth resided somewhere between 64% and "rarely," an analysis was made of the frequency with which gram negative rods could be isolated from the skin of normal volunteers who had participated as controls in studies involving the effect of topical agents on cutaneous microorganisms. The axillary region was chosen since it is a semioccluded moist area harboring dense and varied flora.[2]

Numerous methods exist to quantitatively and qualitatively determine the microbial flora of the skin. Among these are mechanical scrubbing with a cup template (Williamson and Kligman),[6] gauze swab enrichment (Borick and Sarra),[4] tape stripping and contact plates (Ulrich),[5] and the serial scrubbing basin techniques of Price.[7] The current paper describes a simple method that approaches the precision of the glass cylinder scrub technique while obtaining better acceptance on the part of subjects who must make frequent visits to the laboratory. While employing this method, additional information was obtained on the total aerobic count per unit volume of axillary wash as well as on the relationship of the *Staphylococcus epidermidis*⇌*Staph. aureus* equilibrium and the transient or persistent appearance of gram negative rods.

Methods and materials

Subjects. Normal male and female volunteers, ages 19 to 48, were studied. Subjects were instructed to discontinue use of all medicated soaps, underarm deodorants, and antiperspirants. Normal living and personal habits were allowed except that all subjects were instructed to bathe or shower with Ivory soap for one week prior to the study according to their custom twice daily and then for the

duration of the study (two to four weeks). Volunteers visited the laboratory each morning 2-4 hours after their morning shower or bath. Subjects who presented axillary rashes or lesions or who were on oral or parenteral antimicrobial therapy were not allowed to enter these studies.

Axillary wash fluid. Tubes containing 5 ml of sterile 0.85% saline with 0.1% Triton-100 were employed. A sterile cotton swab was moistened in this fluid.

Swabbing technique. An area of the axilla 3 cm by 7 cm was swabbed with rotation a total of 20 times with firm pressure. A plastic template was employed at first but with experience, reproducible counts could be obtained without it. Ten strokes with the premoistened swab were made vertically, with the exfoliate expressed by twirling onto the tilted side of the tube after each of five excursions. Similarly, 10 excursions were made horizontally within the same area followed by expression of the exfoliate after each of five excursions. The swab was then discarded and the mildly clear to cloudy suspension mixed vigorously with a vortex blender. The 5 ml saline-Triton exfoliates were then diluted, plated and streaked immediately or held in the refrigerator for no more than two hours. A preliminary study revealed that saline-0.1%-Tween 80 or saline-0.1%-Triton-100 washes could be held for up to three hours at room temperature without decrease in count, but that saline alone or distilled water was less stabilizing:

	Saline-Tween 80	Saline-Triton	Saline	Water
Zero-time	$3x10^7$/ml	$1x10^7$/ml	$2x10^7$/ml	$1x10^7$/ml
Three hours	$3x10^7$/ml	$1x10^7$/ml	$3x10^6$/ml	$2x10^6$/ml

Total aerobic plate count. Saline-Triton-100 washes were diluted in 10-fold increments in Butterfield's phosphate buffer pH 7.0. Counts were performed in duplicate (10^{-1} to 10^{-5}), with trypticase soy agar pour plates incubated at 35 C for 48 hours.

Gram negative rod enumeration. 0.1 Aliquots of undiluted and diluted axillary washes were spread in duplicate onto the surface of tempered plates of Difco MacConkey's agar and incubated at 35 C for 48 hours so that the following were obtained:

Source	Volume	Final surface dilution
Undilute	0.1	10^{-1}
10^{-1}	0.1	10^{-2}
10^{-2}	0.1	10^{-3}
(etc.)		

After incubation, observations were made of colonial morphology, lactose fermentation, and bile precipitation. Isolated colonies were picked to TSI slants and speciations rendered by biochemical and microscopic techniques as per BAM (FDA), Diagnostics Methods (APHA), and the schema of King, Edwards and Ewing. In addition to conventional biochemical tests, rapid screening methods were also employed with particular benefit derived from the Pathotec® and API® systems.*

Staphylococcal enumeration. The method of surface streaking onto tempered agar plates employed for gram negative enumeration was also employed for staphylococcal enumeration except that mannitol-salt agar was employed as the selective and differential medium. Typical smooth, convex, white or yellow butyrous colonies (48 hours at 35 C) were scored as members of the genus *Staphylococcus,* if growth was moderate to luxuriant and gram stains revealed typical morphology. Those colonies fermenting mannitol, as shown by a yellow zone, were scored as *Staph. aureus;* nonfermenters were scored as *Staph. epidermidis.* Small colony salt-tolerant

*Pathotec, Chilcott Laboratories, Morris Plains, N.J.; API, Analytab Products, Inc., Carle Place, N.Y.

Table I. Total axillary aerobic counts—serial determinations in 10 volunteers in a 2-week period—saline-Triton mechanical swabbing technique.

Subject	1 Mon.	2 Tues.	3 Weds.	4 Thur.	5 Fri.	6 Mon.	7 Tues.	8 Weds.	9 Thurs.	10 Fri.	Mean Log-arithmic Count	Δ Log
1	$1x10^6$* 6.0	$3.6x10^6$ 6.5	$2.7x10^6$ 6.4	$2.8x10^6$ 6.4	$5x10^6$ 6.7	$1.5x10^6$ 6.2	$1.5x10^6$ 6.2	$1.1x10^5$ 5.0	$9x10^5$ 5.9	$1.3x10^6$ 6.1	6.1 (5.0-6.7)	1.7
2	$8.6x10^5$ 5.9	$5x10^6$ 6.6	$1x10^7$ 7.0	$3.6x10^6$ 6.5	$2.4x10^6$ 6.4	$6.4x10^6$ 6.8	$6x10^6$ 6.7	$5x10^6$ 6.7	$5.5x10^6$ 6.7	$1x10^6$ 6.0	6.5 (5.9-7.0)	1.1
3	$1.5x10^6$ 6.2	$6x10^6$ 6.7	$2.7x10^6$ 6.4	$1.2x10^6$ 6.1	$5.5x10^6$ 6.7	$2x10^6$ 6.3	$3.6x10^6$ 6.5	$3x10^6$ 6.5	$1.1x10^6$ 6.1	$7x10^6$ 6.8	6.4 (6.1-6.8)	0.7
4	$4x10^6$ 6.6	$1x10^7$ 7.0	$7.4x10^5$ 5.9	$4.6x10^5$ 5.7	$5x10^5$ 5.7	$5.5x10^4$ 4.7	$1.7x10^5$ 5.2	$1x10^6$ 6.0	$5x10^5$ 5.7	$5x10^5$ 5.7	5.8 (4.7-7.0)	2.3
5	$1x10^5$ 5.0	$7x10^4$ 4.8	$1.4x10^4$ 4.2	$2.2x10^5$ 5.3	$7x10^4$ 4.8	$1.1x10^5$ 5.0	$8x10^5$ 5.9	$1.7x10^5$ 5.2	$1x10^5$ 5.0	$1.5x10^4$ 4.2	4.9 (4.2-5.9)	1.7
6	$9x10^6$ 6.9	$7.5x10^6$ 6.8	$7.5x10^6$ 6.8	$8.5x10^6$ 6.9	$1.8x10^6$ 6.3	$7x10^6$ 6.8	$2x10^6$ 6.3	$5x10^6$ 6.7	$5x10^6$ 6.7	$8x10^6$ 6.9	6.7 (6.3-6.9)	0.6
7	$1x10^6$ 6.0	$4.3x10^5$ 5.6	$1.4x10^6$ 6.1	$9x10^5$ 5.9	$1.7x10^5$ 5.2	$2x10^6$ 6.3	$3.8x10^5$ 5.6	$1.6x10^6$ 6.2	$1.4x10^6$ 6.1	$1.9x10^5$ 5.3	5.8 (5.2-6.3)	1.1
8	$1.2x10^6$ 6.1	$1x10^6$ 6.0	$6x10^6$ 6.7	$4x10^6$ 6.6	$9x10^5$ 5.9	$3.2x10^6$ 6.5	$2x10^6$ 6.3	$2x10^7$ 7.3	$5x10^6$ 6.7	$3x10^6$ 6.5	6.5 (5.9-7.0)	1.1
9	$6.7x10^4$ 4.8	$5.5x10^4$ 4.7	$3x10^5$ 5.5	$6.5x10^5$ 5.8	$1.5x10^5$ 5.2	$1x10^5$ 5.0	$2x10^5$ 5.3	$5x10^4$ 4.7	$4x10^5$ 5.6	$6.8x10^4$ 4.8	5.1 (4.7-5.8)	1.1
10	$2.8x10^5$ 5.5	$7.4x10^5$ 5.9	$7.4x10^5$ 5.9	$3.4x10^6$ 6.5	$4x10^6$ 6.6	$2x10^6$ 6.3	$3.6x10^6$ 6.5	$1x10^6$ 6.0	$1.2x10^6$ 6.1	$4x10^5$ 5.9	6.1 (5.5-6.6)	1.1

*Counts expressed exponentially and as $\log_{10}$

( ) = range per subject

Grand $\bar{x}$ = 6.0

Average Variation (Δ log) = 1.3

diphtheroids were excluded. Coagulase tests were not uniformly run. When tests were performed, fresh plasma was drawn from adult rabbits by cardiac puncture with a heparinized needle and syringe, and inoculated tubes (0.5 ml plasma) were incubated at 35 C and read at 4 and 24 hours as per the criteria set forth in BAM (FDA). Additional data on animal inoculations are given in a later section.

Identification of gram negative rods. Isolated colonies on MacConkey's agar were scored as to morphology, color and consistency and transferred to triple sugar iron agar slants (TSI). Cultures fermenting glucose were further speciated by the API diagnostic system. Previous studies in one of our laboratories (GBL) had shown a good correlation between this system and the conventional BAM (FDA) multiple media method.

Results and discussion

Total axillary counts and normal variation. The data in Table I (all subjects bathing twice daily with Ivory soap and not using deodorants or antiperspirants or other topical or systemic antimicrobials) indicate that the swabbing-scrubbing technique employed recovered aerobic axillary bacteria at a high and consistent rate when analyzed either serially (culture to culture variations) or consecutively (subject to subject variation). The overall mean logarithmic count was 6.0. The overall variation as expressed by the mean of 10 ranges was 1.3 logs. Since the Δ log about the mean sometimes approached 2.0 (1.7 for subjects 1 and 5, and 2.3 for subject 4), 1-2 log declines are within the limits of sensitivity of the method. Such declines or increases are considered normal variation generated by the variability of three components of the system: 1) day-to-day variation of the skin flora with respect to activity and hygiene; 2) variation implicit in the sampling technique; and 3) variation intrinsic to the pour plate method itself. It should be stressed that any study purporting to calculate percent reductions of skin bacteria as a function of topical antimicrobials should take into consideration the fact that a decline of 90% and sometimes 99% can occur spontaneously and/or be produced by normal hygiene and bland soap. An effective topical antimicrobial can, therefore, only be evaluated in reference to nonmedicated controls. It has been our experience (unpublished data, GBL clinical files) that once base-lines are established in a population washing or showering for one week with *warm water only*, a nonmedicated soap can produce consistent 1 log (90%) declines with minimal effect on axillary odor and that medicated soaps can produce consistent 2 log (99%) declines with noticeable reduction in axillary and foot odor. Such an effect is shown graphically in Figure 1.

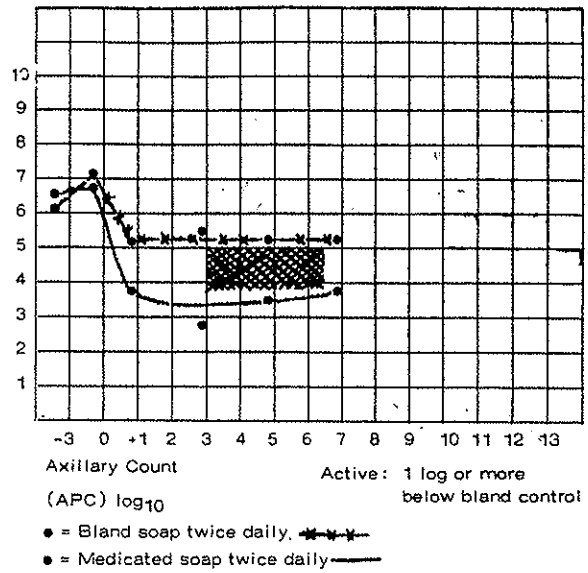

Fig. 1.

Dual controls (warm water and placebo formulation) are required with medicated formulations to demonstrate *in vivo* decline in bacteria and odor reduction.

The mechanical swabbing technique as employed for the data collated in Tables I and II found good

Table II.

Season	No. of subjects[2]	Recovery of organisms (% of population)[1] Staph. epidermidis[3]	Staph. aureus[4]	Gram neg. rods[4]	Aerobic diphtheroids[3]
Winter	92	92/92 (100)	32/92 (35)	34/92 (37)	92/92 (100)
1972	33	33/33 (100)	16/33 (48)	7/33 (21)	33/33 (100)
Total	125	125/125 (100)	48/125 (38)	41/125 (33)	125/125 (100)
Spring 1972	10	10/10 (100)	6/10 (60)	3/10 (33)	10/10 (100)
Summer 1972	31	31/31 (100)	24/31 (77)	23/31 (74)	31/31 (100)
Summer 1972	16	16/16 (100)	13/16 (81)	9/16 (56)	16/16 (100)
Summer 1973	20	20/20 (100)	12/20 (60)	16/20 (80)	20/20 (100)
Total	67	67/67 (100)	49/67 (73)	48/67 (72)	48/48 (100)
Fall 1972	33	33/33 (100)	18/33 (55)	12/33 (36)	33/33 (100)

Total subjects - 235

1. An organism was considered to have been recovered if it was isolated at any dilution on trypticase soy agar, MacConkey's agar or mannitol-salt agar at 35°C for 48 hours.
2. All subjects had at least six serial cultures over a two-week period using Ivory soap only.
3. Staphylococcus epidermidis and aerobic diphtheroids were recovered 100% of the time (all dilutions, all serial cultures, all consecutive subjects).
4. Staphylococcus aureus and gram negative rods were not ubiquitous but were recovered at at least one dilution from at least one of the six to 11 serial cultures per subject. When Staph. aureus was recovered it was usually from all of the six to 11 serial cultures per subject. When gram negative rods were recovered they ranged from <60% of the serial cultures (transient) to 60% more of the serial cultures ("permanent").

acceptance among volunteers in deodorant and antimicrobial panels. Participants willingly visited the laboratory and followed the protocol. Dropout rates, even when panelists were well paid, were high when subjects (housewives, students, office and factory workers) were asked to be sampled by stripping or glass-cup aspiration techniques. Normal "in-use" evaluation of products demands the use of this type of open population.

Seasonal ecological findings. The seasonal recovery of cutaneous staphylococci, gram negative rods and aerobic diphtheroids from a total of 235 subjects is summarized in Table II. These data are further portrayed as a frequency distribution in Figure 2. The study was conducted in northern New Jersey. While variation exists in the number of subjects studied per season, our greatest interest was in the summer-winter contrast, for which a minimum number of 60 subjects was felt to be required.

The data in Table II suggest that regardless of season, the staphylococci and diphtheroids, not unexpectedly, were found to be ubiquitous. Differentiation between lipophilic and nonlipophilic diphtheroids (Marples)[8] was not attempted. All were gram positive club-shaped, pleomorphic or rudimentary branched curved rods that were catalase positive of variable colonial morphology and pigmentation. The staphylococci all grew well on mannitol-salt agar (occasionally confirmed on Vogel-Johnson's agar) and were categorized as S. *epidermidis* or S. *aureus* on the basis of pigment and mannitol fermentation. Coagulase tests were not routinely performed. Occasional mouse virulence tests (mucin-potentiated intraperitoneal injection) indicated that mannitol-positive, coagulase-positive strains produced fatal septicemia in 24 hours whereas strains of mannitol-negative, coagulase-negative staphylococci did not.

Seasonal effects were clearly seen when one analyzes the recovery data for both the gram negative rods and S. *aureus*. A winter-summer cycle seems apparent when one examines the percent recovery of S. *aureus* and gram negative rods independently of the total staphylococcal and diphtheroidal flora (the staphylococci represented 10 to 50% of the total flora, diphtheroids, 90 to 50%). The winter results indicated that 33% of the subjects harbored gram negative rods whereas the summer data revealed an incidence as high as 72% (compare with Borick at 64%).

Between these two seasons antipodal to living habits and temperature of a temperate zone, we noted a rate of 33% and 36% for gram negative rods in spring and fall respectively.

A similar rise in the rate of recovery of S. *aureus* was noted when one plotted summer frequency versus winter frequency. For S. *aureus* it can be noted that subsequent to winter (38%) a gradual increase in recovery was seen through the spring culminating in the summer at 73% and then declining through the fall at 55%.

The cause of such ecological drift is not clear in the case of S. *aureus*. For the gram negative rods it may be assumed that among other factors the stimulatory moisture effect of Blank and Dawes[9] was operative. The rise in gram negative rods was not due to a breakdown in the S. *epidermidis*⇌ S. *aureus* equilibrium, which tends to inhibit gram negative rods.[2,8] In all cases where gram negative rods were isolated either transiently or persistently in a series of six or more cultures, no correlation existed with respect to recovery of S. *aureus*. Thus decline in S. *epidermidis* and rise in S. *aureus* were not ecological precursors to appearance by enterobacteriaceae or nonfermentative gram negative rods of the Acinetobacter/Achromobacter/Moraxella group.

Gram negative rods. Additional attention was directed to the winter-summer recovery of gram negative rods from the points of view of: 1) persistence of isolation over a two-week period; and 2) characterization of the organisms isolated. These data are summarized in Tables III and IV.

The data in Table III indicate that in a random

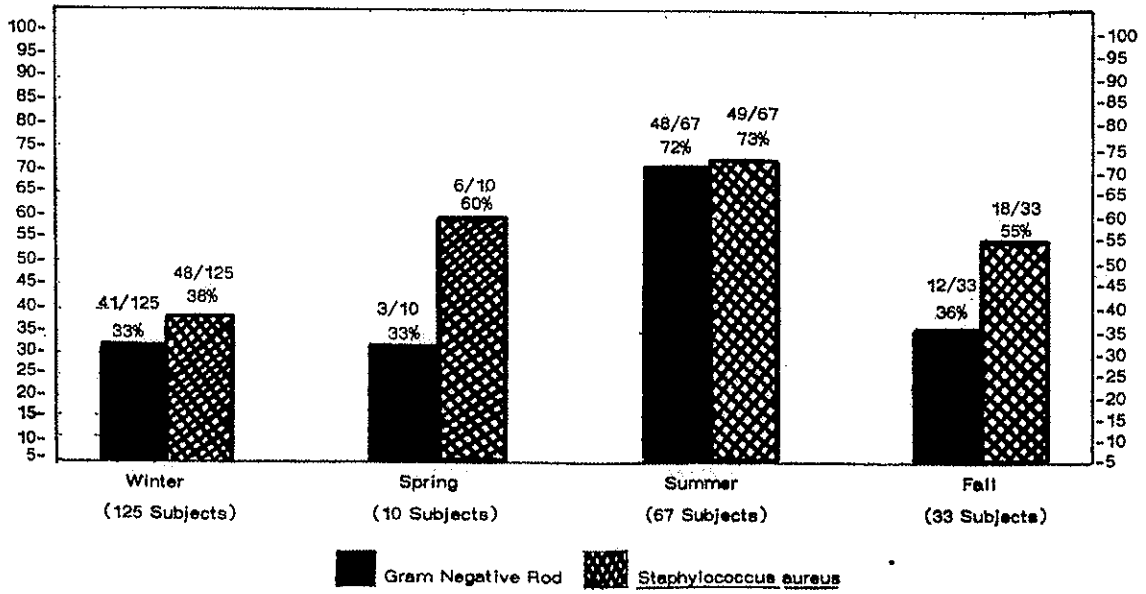

Fig. 2. Frequency distribution of gram negative rods and Staph. aureus recovered in axillary cultures in respect to season of the year.

summer population of 20 subjects (ages 19-45, male and female) 80% harbored gram negative rods (16/20 subjects).

The data in Table IV indicate that in a random winter population of 33 subjects (ages 20-48, male and female), only 21% harbored gram negative rods (7/33 subjects).

A subcollation of the data in these two tables revealed the breakdown of gram negative rod incidence as is shown in Table V.

It can be noted that the recovery rate for gram negative rods was four times greater in the summer than in winter. During both seasons there was a slightly greater tendency for the organisms to be "transient" than "permanent." However, the T/P ratio for both seasons was identical. Given the

Table III. Summer recovery of gram negative rods—serial axillary determinations in 20 volunteers in a 3-week period—saline-Triton swabbing technique.

Subject	Culture Day	1 0	2 3	3 4	4 7	5 10	6 11	7 14	8 17	9 18	10 21	11 24	Category
1		+	+	0	0	+	+	+	++	++	++	+	Persistent* (A)
2		0	0	0	0	0	0	0	+	0	0	0	Transient (D)
3		0	0	0	0	0	0	0	0	0	0	0	Absent
4		0	0	0	0	0	0	0	0	0	0	0	Absent
5		0	0	0	0	+	0	0	0	+	+	0	Transient (D)
6		+++	+++	+++	+	+++	+++	++	++	++	++	++	Persistent (A)
7		0	0	+	0	0	0	++	0	+	+	0	Transient (D)
8		0	0	0	0	0	0	0	0	0	0	0	Absent
9		+	++	++	++	++	++	++	++	++	++	++	Persistent (B)
10		++	++	++	++	++	++	+	+	++	++	++	Persistent (C)
11		+	+	0	0	+	0	+	+	+	0	+	Persistent (D)
12		++	0	0	0	0	0	0	++	+	0	0	Transient (D)
13		0	0	+	+	0	0	++	++	++	+	+	Persistent (C)
14		+	0	0	+	0	+	0	0	0	+	+	Transient (A)
15		0	0	0	0	+	0	0	0	0	0	0	Transient (C)
16		0	0	0	0	0	0	0	0	0	0	0	Absent
17		++	0	0	0	0	+	0	+	0	0	0	Transient (D)
18		0	+	0	0	+	0	0	0	0	0	0	Transient (D)
19		+	0	0	0	+	++	0	0	0	0	+	Transient (D)
20		++	+	++	++	++	+++	++	+	++	++	++	Persistent (E)

$\xi = \frac{16}{20} = 80\%$

+ = 10^1 per ml axillary wash
++ = 10^2 per ml axillary wash
+++ = 10^3 per ml axillary wash
0 = <10 per ml axillary wash

A = Acinetobacter > + coliforms
B = Acinetobacter + Achromobacter > + Proteus
C = Coliform
D = Acinetobacter
E = Coliform > + Acinetobacter

*Persistent = recovered ≥ 60% of subjects cultures

Table IV. Winter recovery of gram negative rods—serial axillary determinations in 33 volunteers in a 2-week period—saline-Triton 100 swabbing technique.

Subject	Culture Day	1 0	2 4	3 5	4 8	5 9	6 12	Category
1		0	0	0	0	0	0	Absent
2		0	0	0	0	0	0	Absent
3		0	0	0	0	0	0	Absent
4		0	0	0	0	0	0	Absent
5		0	0	0	0	0	0	Absent
6		0	0	0	0	0	0	Absent
7		0	0	0	0	0	0	Absent
8		0	0	0	0	0	0	Absent
9		0	0	0	0	0	0	Absent
10		0	0	0	0	0	0	Absent
11		0	0	0	0	0	0	Absent
12		0	0	0	0	0	0	Absent
13		0	0	0	+	0	+	Transient (A)
14		0	0	0	0	0	0	Absent
15		0	0	0	0	0	0	Absent
16		0	0	0	0	0	0	Absent
17		0	0	0	0	0	0	Absent
18		0	0	0	0	0	0	Absent
19		0	0	0	0	0	0	Absent
20		0	+	0	++	++	++	Persistent* (A)
21		++	++	++	+	+	+	Persistent (B)
22		0	0	0	0	0	++	Transient (C)
23		0	0	0	0	0	0	Absent
24		0	0	0	0	0	0	Absent
25		0	0	0	0	0	0	Absent
26		0	0	0	0	0	0	Absent
27		+	++	0	0	0	0	Transient (A)
28		0	0	0	0	0	0	Absent
29		0	0	0	0	0	0	Absent
30		+	0	0	0	0	++	Transient (B)
31		0	0	0	0	0	0	Absent
32		++	++	+	0	+	0	Persistent (A)
33		0	0	0	0	0	0	Absent

+ = ca. 10^1 per ml axillary wash
++ = 10^2 per ml axillary wash
0 = <10 per ml axillary wash

A = Acinetobacter
B = Acinetobacter > + Coliform
C = Coliform (Enterobacter, Citrobacter, Escherichia)

$\xi = 7/33 = 21\%$

*Persistent = recovered ≥ 60% of subjects cultures

Table V.

	Summer		T/P Ratio	Winter		T/P ratio
		%			%	
Total gram negative rods	16/20	80	-	7/33	21	-
Transient recovery	9/20	45	-	4/33	12	-
Persistent recovery	7/20	35	-	3/33	10	-
Transient carriers (t)	9/16	56	1.3	4/7	57	1.3
Persistent carriers (p)	7/16	44		3/7	43	

marked increase in incidence of gram negative rods during summer and, furthermore, given the identity of the seasonal T/P ratios, the data would tend to indicate the occurrence in nature of an ecological drift in which transient auto- and/or environmental *contamination* and persistent *colonization* occur at equal and increasing rates during the late spring months, reaching a maximum equilibrium during the summer. The data further suggest that this trend reverses itself in the fall, so that by winter the axillary gram negative rod flora are minimal.

This tandem ecological drift probably originates via autocontamination in the case of members of the enterobacteriaceae and by inoculation via water and soil with respect to members of the neisseriaceae. Undoubtedly the more casual and recreational life style of the warmer months in concert with increased apocrine and eccrine activity account for the creation of ecological conditions conducive to axillary carriage of gram negative rods.

The gram negative rods encountered in this study were primarily of the neisseriaceae, i.e., coccobacillary to pleomorphic gram negative rods primarily oxidative in nature either without swimming motility (flagellation) or with twitching motility (fimbriation). The taxon *Acinetobacter* most nearly describes these forms, the older terms *Mimeae* and *Herellea* being rejected (Henriksen).[10]

When glycolytic, cytochrome oxidase negative forms of the enterobacteriaceae were isolated they were generally coliforms such as *Citrobacter, Enterobacter cloacea, Enterobacter agglomerans* or *Enterobacter aerogenes,* or strains of *Escherichia coli* (IMVIC ∓±==) that did or did not ferment lactose in boric acid broth at 45°C. *Pseudomonas aeruginosa,* the subject of so much regulatory consternation vis-a-vis topical drug or cosmetic formulations, was not isolated in any of the approximately 1,400 axillary cultures derived from 235 healthy subjects over a two-year period. We still regard water, soil, vegetables and the feces of debilitated patients as the primary habitat for this much publicized opportunistic but nonindigenous pathogen.[11]

Summary

A standardized swabbing technique employing a saline-Triton wash has been described which exfoliates high yields of surface bacteria with sufficient precision to permit quantitation of an evaluation of changes in total aerobic flora, gram positive cocci and gram negative rods. Specific data on approximately 1,400 axillary cultures from 235 subjects were presented. Total counts in the order of magnitude of 10^6 bacteria per ml of wash were obtained. The predominant aerobic axillary flora consisted of *S. epidermidis* and diptheroids, with the former existing at 10 to 50% of the whole. The recovery rate of *S. aureus* ranged from a low of 33% of the subjects during the winter months to a high of 72% in the summer. Similar findings were found among the gram negative rods: 38% of the population shedding these organisms in the winter, drifting to a high of 73% in the summer. The carriage of axillary gram negative rods was arbitrarily defined as "transient" or "permanent," depending upon the percentage of recoveries per subject from serial cultures over a two-week period. Maximal transiency was evidenced in certain subjects by the finding of only 1/11 positive cultures. This was taken to indicate autocontamination and rapid "die-off" due to an ecological setting not inducive to colonization. Maximal persistence was evidenced in certain subjects by the finding of 11/11 positive cultures over a two-week period. This was taken to indicate disruption of an ecological equilibrium to allow for colonization. It is suggested that *S. aureus* and gram negative rods drift in and out of the host ecosystem in a manner somewhat cyclic with the seasons, possibly related to variations in summer-winter living habits, increased moisture, or perhaps some undefined circadian effect. The gram negative organisms were members of the enterobacteriaceae (coliforms primarily) or, more frequently, members of the neisseriaceae *(Acinetobacter)* formerly *Herellea. Ps. aeruginosa* was not isolated from any of the 235 subjects. Additional qualitative and quantitative studies on the microbial flora of the axillae seem warranted from the points of view of: 1) developing reproducible experimental models to evaluate the *in vivo* efficacy of topical antimicrobials; and 2) elucidating the dynamics of cutaneous ecosystems as they are affected by extrinsic and/or intrinsic factors.

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As appeared in *Cosmetics and Perfumery* for October, 1974