Acta Dermatovener (Stockholm) 58: 501–504, 1978

QUANTIFICATION OF ANAEROBIC DIPHTHEROIDS ON THE SKIN

Raza Aly, Howard I. Maibach and Ernest Bloom

Department of Dermatology, University of California Medical School, San Francisco, California, USA

Abstract. The widely used technique for sampling skin bacteria employs a detergent (Triton X-100) in buffer solution to remove and suspend the microorganisms. The fraction of the total population of anaerobes on the forehead removed by scrubbing with this solution was estimated. We calculated that approximately 10% of the resident anaerobes were removed by one minute of scrubbing with the detergent solution and two 1-min washes remove approximately 19.5% of the resident anaerobes ($8.8\times10^5/cm^2$).

Key words: Anaerobic diphtheroids; Propionibacterium acnes; Acne vulgaris; Quantification of anaerobic diphtheroids

The widely used technique for quantitative sampling of the skin bacteria is that of Williamson & Kligman (6). Their method utilizes a detergent, Triton X-100, in buffer solution to remove and suspend the bacteria. They estimated that approximately 85% of the resident aerobes are removed by one minute of scrubbing with this solution. They did not record the anaerobic flora. The present study estimates the fraction of the total resident population of anaerobes on the forehead removed by scrubbing with this solution.

MATERIALS AND METHODS

Wash solution. Triton X-100 (0.1%) solution in 0.075 M phosphate buffer. Triton X-100 is octyl phenoxy polyethoxyethanol. Because of its non-ionic structure it is estimated to be less toxic to bacteria than most detergents (6).

Removal of anaerobes from the skin

Sampling method. We studied 9 young adult volunteers with low-grade acne to normal skin. The technique of Williamson & Kligman (6) was modified in that five serial 1-min washings were performed on a single site. All washings were done on the forehead. An appropriate 10-fold dilution of the wash solution was quantitatively plated (0.1 ml) on BHI agar with 1% glucose added. The plates were incubated anaerobically for 6 to 7 days.

Identification of anaerobic diphtheroids was based on three criteria: 1) colony morphology, 2) cellular morphology, and 3) a preference for anaerobic conditions.

Mathematical model. For processing the data we assumed a model in which the amount of bacteria removed per wash is directly proportional to the amount of bacteria remaining on the skin.

$$\text{Amount removed per cm}^2\text{ per wash} = \text{Amount remaining per cm}^2\text{ on skin} \times k \qquad \text{(I)}$$

where

k = the fraction of the population on skin removed per wash.

Integration of equation I gives:

$$\log_{10} Y_i = s \times W + Y_0 \qquad \text{(II)}$$

where

Y_i = amount removed per cm² on wash no. 1
S = wash no. 1, 2, 3, ... etc.
δ = slope = $\log_{10} Y_i$/wash, found from least squares fit of data
Y_0 = an integration constant.

The data were plotted with the X-axis giving the wash number, viz. wash no. 1, wash 2, ... etc., and the Y-axis being the $\log_{10}$ of the number of *P. acnes* per cm² removed per wash. The best statistical fit for the slope of the data, s, was found using the least squares method.

The fraction of the total population removed per wash, k, was estimated using the following formula:

$$\text{fraction removed per wash} = k = 1 - 10^s \qquad \text{(III)}$$

The total original population was computed by extrapolating from the five washes using the following formula:

$$\text{total original population} = (10Y_0)/k \qquad \text{(IV)}$$

The standard deviations were estimated from the appropriate formulae.

Table I. *Summary of results from washings on 9 subjects*

The slope represents the average rate of decrease of a semi-log plot of the data (equation II). The computed total represents the estimated total population of anaerobes on the forehead based on an extrapolation from five serial 1-min washes. "k" is calculated from the slope using equation III

Subject	Slope ($\log_{10}$ units/ wash)	1st Wash (organisms/cm^2)	Computed total (organisms/cm^2)	Computed k (fraction of computed tot. removed/wash)
1	$-.074\pm.055$	6.1×10^5	3.4×10^6	16%
2	$-.079\pm.020$	1.1×10^6	7.4×10^6	17%
3	$-.072\pm.071$	3.8×10^5	3.2×10^6	15%
4	$-.336\pm.089$	4.9×10^5	3.3×10^6	54%
5	$-.004\pm.027$	2.0×10^5	1.9×10^7	1%
6	$-.027\pm.027$	1.0×10^5	1.7×10^6	6%
7	$\pm.015\pm.053$	6.0×10^4	–	–
8	$+.043\pm.028$	1.2×10^5	–	–
9	$+.043\pm.028$	1.3×10^5	–	–
$\bar{X}$	$-.047$	3.4×10^5	6.3×10^6 ($n=6$)	18% ($n=6$)
Geometric mean	–	1.8×10^5	4.5×10^6 ($n=6$)	–

RESULTS

Removal of anaerobes from the skin

95% of the organisms recovered were anaerobic diphtheroids. Thus, within the accuracy of our computations, the computed total population of anaerobic diphtheroids approximated the computed total population anaerobes. Approximately $\frac{1}{3}$ of the anaerobic diphtheroids recovered from the subject 6 grew feebly in air. The anaerobic diphtheroids from all other subjects were obligate anaerobes.

The average slope over the five washes was -0.047 $\log^{10}$ units per wash ($n=9$). This corresponds to an average k of 10% per wash. For those 6 subjects who showed an average decrease per wash we estimated the total population was 6.3×10^6 organisms/cm^2 (geometric mean$=4.5\times10^6$) (Table I). For the 3 subjects who had non-negative slopes, we were unable to compute the total population.

Several subjects were followed for more than five washes. Subject 2 received 14 serial 1-min washes, giving a computed total of 7.8×10^6 organisms/cm^2; subject 1 had nine washes, with a computed total of 2.0×10^6 organisms/cm^2. One subject (not shown) had 19 washes on the malar aspect of the cheek. His data give a computed total of 3.4×10^6 organisms/cm^2 and an average decrease of 21% per wash ($k-0.21$). Subject 7, who showed a slight increase in yield over the first five washes, was followed for 21 washes. He had a computed total of 3.8×10^6 organisms/cm^2 and an average decrease per wash of 13% ($k-0.13$). For these extended wash data, the average computed total population is 4.3×10^6 organisms/cm^2 (geometric mean$\times$ 3.8×10^6). It is interesting to note the similarity between these computed totals and the computed totals based on five washes.

Our model assumes that the quantity of bacteria removed per wash is directly proportional to the total population on the skin. The model might not be valid if there were to exist, not one, but two environments on the skin, each with different removal characteristics. An alternative model has

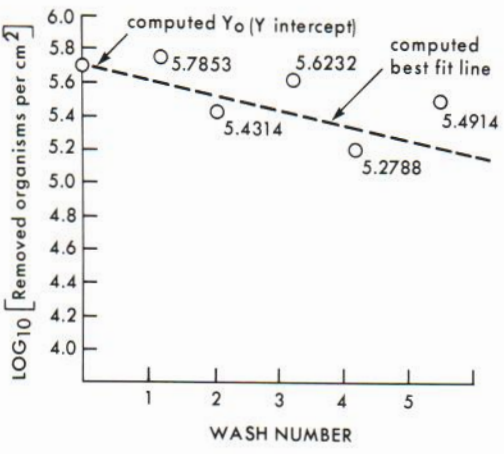

Fig. 1. Example of data from a series of five 1-min washes on a single site on the forehead. For this patient the computed slope was -0.074 ± 0.055 $\log_{10}$ units per wash. This corresponds to approximately 16% of the total population being removed per 1-min wash. The computed total population on this individual was 3.4×10^6 organisms/cm^2. The data are taken from patient 1.

been proposed which postulates, on anatomical grounds, the existence of a non-surface environment deep within the sebaceous duct with a much slower rate of removal. Mathematically, this alternative model can be represented as

$$\text{Amount removed per cm}^2 \text{ per wash} = Y_s \times k_s + Y_d \times k_d \quad \text{(V)}$$

where

Y_s = density of organisms in the surface environment

Y_d = density of the organisms deep in sebaceous duct

k_s = the fraction of Y_s removed per wash

k_d = the fraction of Y_d removed per wash.

This model assumes $k_s \gg k_d$. Equation V becomes equation I if any of the following are true within experimental error:

$$k_s \cong k_d \quad \text{(VI)}$$

$$Y_s \gg Y_d \quad \text{(VII)}$$

$$Y_d \gg Y_s \quad \text{(VIII)}$$

The proposed model predicts an initial rapid decay in yields followed by removal at a nearly constant rate as organisms deep within the follicle are washed out.

Equation V is less useful for several reasons. On most subjects we did not observe an initial rapid decay followed by a slower rate, as predicted by this model. This suggests that one of assumptions VI, VII, or VIII is true. Possibly the surface population is small (assumption VIII) due to its exposure to the atmosphere.

On some subjects, instead of an initial decay in yields, we saw an increase in yields over the first five washes. Subject 7 had a slight increase in yields over the first five washes. When he was followed for 21 washes, he showed a greater rate of decay over the last 10 washes than over the first 10. In addition to possible effects of oxygen, we speculate that a part of the error on a subject like this is that the skin may change its characteristics in response to the sampling technique.

DISCUSSION

Our data generally agree with the data gathered by other investigators using the Williamson & Kligman technique (6). Most investigators use two 1-min washes (pooled together) to quantitate bacteria. According to our model, two 1-min washes remove approximately 19.5% of the resident anaerobes: 19.5% of our mean computed total for these subjects is 8.8×10^5 organisms/cm^2. Marples & Kligman (2) found a mean of 1.7×10^6 organism/cm^2 ($n=57$ samples) on the forehead, twice what we estimate from our subjects. On the scalp they found a mean of 1.9×10^5 anaerobes/cm^2 ($n=17$) (1). Martin et al. (3) observed a mean of 6.9×10^5 anaerobes/cm^2 on the forehead ($n=10$). Somerville & Murphy (5) noted a mean of 4.0×10^4 anaerobes/cm^2 ($n=22$) on the forehead, using only one 1-min scrub. Possibly they had some subjects who greatly lowered their mean; their average was 5.5×10^5 organisms/cm^2. For our first 1-min scrub we removed a mean of 1.8×10^5 organisms/cm^2 and an average of 3.4×10^5 organisms/cm^2. Our data fall in the middle of this range of values.

There are few reports in the literature concerning the quantification of the total population of organisms in the sebaceous duct. Puhvel et al. (4) developed an intrafollicular sampling technique. They estimate that the average anaerobic population of a follicle from the upper back is 3.37×10^5 organisms per follicle (average of 28 biopsies). By means of calculation, this data corresponds to a density of approximately 1.7×10^7 organisms/cm^2. Our estimated total for the forehead is less than half this, 6.3×10^6 organisms/cm^2. One must be cautious in such an extrapolation. Somerville & Murphy (5) found a mean of more than twice as many anaerobic diphtheroids on the back as on the forehead, in the first 1-min scrub ($n=22$ subjects). Possibly the back supports a population twice that of the forehead. We find the correlation between the two methods of interest, but not conclusive.

We interpret the data on serial washings of the anaerobic microflora with caution. By this method of extrapolation we estimate that the total anaerobic population on the forehead is approximately 6×10^6 organisms/cm^2. Two 1-minute washes on the forehead remove roughly one-fifth of the total population of anaerobes present. We suspect this rate of removal to be so slow as to present significant obstacles in interpreting quantitative antimicrobial studies.

REFERENCES

1. Marples, R. R., Downing, D. T. & Kligman, A. M.: Control of free fatty acids in human surface lipids by

Corynebacterium acnes. J Invest Dermatol *56:* 127, 1971.
2. Marples, R. R. & Kligman, A. M.: Ecological effects of oral antibiotics on the microflora of human skin. Arch Dermatol *103*: 148, 1971.
3. Martin, R. J., Kahn, G., Goodling, J. W. & Brown, G.: Cutaneous porphyrin fluorescence as an indicator of antibiotic absorption and effectiveness. Cutis, 758, November 1973.
4. Puhvel, S. M., Reisner, R. M. & Amirian, D.: Quantitation of bacteria flora in isolated pilosebaceous follicles in normal skin. J Invest Dermatol *65*: 525, 1975.
5. Somerville, D. A. & Murphy, C. T.: Quantitation of *Corynebacterium acnes* on healthy human skin. J Invest Dermatol *60*: 231, 1973.
6. Williamson, P. & Kligman, A. M.: A new method for the quantitative investigation of cutaneous bacteria. J Invest Dermatol *45*: 498, 1965.

Received March 7, 1978

R. Aly, Ph.D.
Department of Dermatology, 342 A
University of California
San Francisco, CA 94143
USA