Uneven Distribution of Aerobic Mesophilic Bacteria on Human Skin

WILLIAM A. KEITH, JR.,* ROKO J. SMILJANIC,† WILLIAM A. AKERS,‡ AND LONNIE W. KEITH§

Department of Dermatology Research, Letterman Army Institute of Research, Presidio of San Francisco, California 94129, and Mathematics Component, Student Learning Center, University of California, Berkeley, California 94704

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Viable aerobic mesophilic bacteria are not evenly distributed on the skin of the volar forearm. An increase in the size of the area sampled did not result in a proportional increase in the number of the viable aerobic mesophilic bacteria recovered.

The presence of aerobic mesophilic bacteria on the surface of human skin is extensively documented (4, 6, 8, 9). The composition and the mean number of these bacteria vary from subject to subject, site to site, and day to day (3, 5, 11, 12). Although the volar forearm has been used for testing the efficacy of various antibiotics on the cutaneous microflora, none of the investigators has provided adequate evidence concerning the distribution of the bacteria on those skin sites (7, 12). Consequently, in this report, we sampled three sites on the volar forearm over a 5-month period on the same subject to determine whether the number of viable aerobic mesophilic bacteria are evenly distributed on the volar forearm. Our results indicated that these bacteria are not evenly distributed on the surface of the skin, and that the results obtained by sampling a smaller site (2.25 cm²) may not be predictably related to a distant site on the volar forearm.

To eliminate the variation in the mean bacterial count per square centimeter among different volunteers, we chose one freely consenting 36-year-old healthy volunteer. During the entire sampling period, the volunteer abstained from the usage of deodorant soaps. Bathing and showering were permitted except on the day of sampling. The skin surface on the left volar forearm was separated into three areas for sampling purposes, near-wrist, mid-volar forearm, and near-antecubital fossa. The area sampled on the near-wrist was 2.25 cm², the site for the mid-volar forearm was 4.5 cm², and the area on the near-antecubital fossa was 9.0 cm². Sampling was accomplished by placing a sterile stainless steel template (1.5 by 1.5 cm) on the skin surface (10). The template was filled with 1 ml of phosphate-buffered Triton X-100 (pH 7.9), and the skin surface was scrubbed horizontally in a back-and-forth motion 50 times with a sterile stainless steel policeman. The stainless steel policeman was attached to a mechanical linear sampler (developed at Letterman Army Institute of Research), which scrubbed at a constant pressure (255 g) and stroke speed. The mechanical linear sampler (Fig. 1) consists of a clock electric motor, an electric impulse counter, a 58-cm-length aluminum arm, a stainless steel policeman, a stainless steel template, an on/off switch, two Boston gears, and a 255-g weight. The aluminum arm holding a stainless steel policeman is pushed and pulled through a 1.5-cm distance at approximately 57 to 60 times per min by the clock electric motor.

After the first 1 ml was removed and saved, another 1 ml was pipetted into the template. Again the surface of the skin was scrubbed for 50 times. The second 1 ml of scrub fluid was added to the first, and a final 1 ml was pipetted into the template to rinse the surface. The rinse fluid was removed and added to the previous two scrub fluids. The pooled sample contained that quantity of aerobic mesophilic bacteria recovered from a 2.25-cm² area of the skin. To sample an area of 4.5 cm², two 2.25-cm² areas of skin were scrubbed, and the sample samples were pooled. A 9.0-cm² area of skin was sampled by scrubbing four 2.25-cm² areas of skin and pooling the resulting scrub fluid.

After approximate decimal dilutions in Trypticase soy broth, bacterial counts were made by spread inoculation of Trypticase soy agar plates in triplicate. Plates were incubated for 3 days at 37°C, and colonies were identified with the use of routine diagnostic media and procedures (11).

The cumulative bacterial count data were tested by using the chi-square goodness-of-fit test (10); the probability for the difference in the median bacterial count among sites was calculated by using the Kruskal-Wallis test (2).

Using a single subject while sampling over a
128-day period, we determined the number of aerobic mesophiles on selected sites of the volar forearm (Fig. 2). The bacterial counts varied from 0 to $1.109 \times 10^3$ colony-forming units per cm$^2$ on the left volar forearm. When the near-wrist site (2.25 cm$^2$) was assayed for the number of aerobic mesophilic bacteria, the mean number over a 128-day period was $1.413 \pm 845 \times 10^3$ (mean and standard error). However, when the mid-volar-forearm area, which was twice as large as the near-wrist site, was assayed, the mean number was nearly the same as the first site, $1.554 \pm 424 \times 10^3$. On sampling the near-antecubital-fossa site, four times the size of the first site, the mean number of aerobic mesophiles was $3.320 \pm 978 \times 10^3$.

The cumulative number of aerobic mesophiles over the 128-day period did not fit the expected hypothesized ratio of 1:2:4 when tested by the chi-square goodness-of-fit test; $\chi^2 = 2.729, P < 0.005$.

When the total number of aerobic mesophilic bacteria per square centimeter was calculated for each site, the near-wrist site bacterial count was $628 \pm 75$, the mid-volar-forearm bacterial count was $345 \pm 94$, and the near-antecubital-fossa count was $369 \pm 108$.

Our results confirm the previous findings and

![Image of mechanical linear sampler](image_url)

**Fig. 1.** Mechanical linear sampler. (1) Stainless steel template; (2) stainless steel policeman; (3) aluminum arm; (4) 255-g weight; (5) leveling device; (6) vertical/horizontal joint; (7) on/off switch; (8) electric motor; (9) support stand; (10) electrical impulse counter; (11) spare gear; (12) wooden base; (13) line cord.

![Graph of viable aerobic bacteria](image_url)

**Fig. 2.** Comparison of the number of viable aerobic mesophilic bacteria recovered from three sites on the left volar forearm during a 128-day period. Each skin site was moistened with a detergent containing 0.1% Triton X-100 in 0.075 M phosphate buffer (pH 7.9) and scrubbed with a stainless steel policeman. The resulting scrub fluid was diluted in Trypticase soy broth and plated on Trypticase soy agar. Data are expressed as colony-forming units per area: near-wrist, 2.25 cm$^2$ (●); mid-volar forearm, 4.5 cm$^2$ (○); near-antecubital fossa, 9 cm$^2$ (■).
suggest that aerobic mesophilic bacteria may not be evenly distributed on the volar forearm. We found that an increase in the size of the area sampled did not result in a proportional increase in the number of aerobic mesophiles (Fig. 2). Moreover, an analysis of the median number of bacterial counts by using the Kruskal-Wallis test revealed no significant difference between the three sites; therefore, we are entitled to believe that the median bacterial counts of the three sites are the same.

The failure to realize a proportional increase could be attributed to either (i) the sites being occupied by anaerobes or (ii) the possibility that the aerobic mesophilic bacteria are distributed on the skin unevenly. The first interpretation does not appear to be correct, since most reports offer evidence for a paucity of anaerobes on the volar forearm (8). Although our plate count assay did not distinguish between the effect of the single cell and the effect of the colony-forming unit on the viable count, the second interpretation provides the simplest explanation for our data.

These results are significant because they raise two important questions. The first question concerns the validity of using bacterial counts obtained from sampling a skin site to estimate the bacterial counts on an adjacent skin site; and the second question, probably the more important, is whether the uneven distribution of bacteria on the forearm of a single individual is a universal phenomenon or limited to the volunteer tested. Our results strongly suggest that bacterial counts determined from a skin site cannot reliably predict the number of bacteria inhabiting an adjacent skin site. Although the question concerning the universality of the observed phenomenon, i.e., the uneven distribution of bacteria on the human forearm, cannot be answered definitively without sampling a large number of volunteers, we would expect similar findings on other volunteers.

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