Methods for Determining the Distribution of Bacteria in the Skin

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Abstract

Five methods used to study bacteria in the skin are reviewed: swabbing, scrubbing, or scraping; biopsy; impression plate; adhesive stripping; and air-sampling for bacteria-shedders.

Scraping and swabbing methods give only a rough idea of the numbers and kinds of bacteria on a given area of skin. More recent adaptations of these techniques have introduced the use of soaps or surfactants to disperse the bacteria, followed by quantitative plate counts on serial dilutions. Biopsy methods are little used because they give only qualitative results and are traumatic to the experimental subject. Impression plates give low counts in most cases and enumerate only those bacteria which are lying on the surface layer of the *stratum corneum*. Adhesive stripping methods are the only ones which give a precise determination of the distribution of colonies of bacteria with regard to both area and depth in the *stratum corneum*. Counts run much higher than impression plates.

Introduction

TO THOSE WHO HAVE NOT BEEN INVOLVED in experimental work intended to evaluate the importance of the skin as a reservoir for bacteria and as a potential source of infection, the methods of enumerating bacteria in the skin may seem a rather dry and narrow subject. Those who have worked in this field however are painfully aware of the lack of precision and of the confusion and differences of opinion. This situation generates much frustration, heat, and controversy, but it does help to keep things interesting. The practical applications of methods of studying and enumerating micro-organisms in the skin lie in three general areas.

In dermatology, many diseased conditions of the skin are brought about by bacteria and fungi. Thus it should come as no surprise that many of the most fundamental studies on skin bacteria and fungi have been carried out by dermatologists.

A second major area of application is in surgery since it is vitally important for the surgeon to reduce bacterial contamination of the area of the patient to be incised, as well as his own hands, to an absolute minimum. Thus surgeons make important contributions.

A third, more heterogenous group which has contributed to the subject is composed of those who are involved in attempts to develop antiseptics, germicides, surgical scrubs, and antibacterial soaps for use on the skin. Most of these investigators are interested primarily in developing commercial products such as antiseptic soaps and lotions, and most are employed in industry.

Discussion

Five general methods have been employed to study the numbers and kinds of bacteria in the skin. As has been pointed out by J. Ulrich of the Mayo Clinic (1), each of these methods determines a different aspect of microbial skin populations, and they supplement one another. No single method by itself can yield a rounded view of the microbiology of skin, and most applied problems require the use of several methods before valid and useful conclusions can be drawn.

The five general methods are swabbing, scrubbing, or scraping; biopsy; impression plate; adhesive stripping; and air-sampling for bacteria shedders. To be discussed briefly are each method, its advantages and limitations, and a few examples to indicate the kind of information to be obtained.

Swabbing, Scrubbing, and Scraping Methods

The earliest methods, dating back to the dawn of bacteriology, were various swabbing, scraping, and scrubbing methods. The swab method has been used in recent studies as well as the oldest. A sterile cotton or alginate swab is removed from a sterile container, dipped in sterile water or saline, then rubbed over the surface of the skin. The swab is next used to inoculate agar plates of suitable culture media, after which the plates are incubated and colonies of bacteria are counted. The method has the great advantages of simplicity and rapidity but is not quantitative and thus can be used only for rough comparisons.

MacPherson, Sparkman, and Whitney (2) used the method to study the effectiveness of hard bar soaps containing 0.5 and 2.0% hexachlorophene under hospital conditions. The lack of quantitativeness of the method was compensated for by making a large number of tests on a large number of nurses over a long period of time. The authors concluded that the soaps did not reduce the number of bacteria per unit area of skin appreciably. This conclusion is at variance with that expressed by Quinn, Voss, and Whitehouse (3). Their studies were carried out with the more accurate split-use serial basin scrub method and indicated a reduction of 85% in the bacterial count by the use of 2% hexachlorophene soap. Further research is needed to clarify this apparent contradiction.

A more quantitative scrubbing or scraping procedure is that of Pachtman, Vicher, and Brunner (4). A sterile glass cylinder 23 mm in diameter and two or three mm in length is pressed against the skin area to be sampled. Then 2–3 ml of brain heart infusion broth are placed in the glass cylinder, and the skin surface within the cylinder is rubbed briskly with a sterile wooden applicator for a few seconds. A 1-ml sample of the broth is removed from the glass cylinder and serially diluted in sterile water; the dilutions are plated out in brain heart infusion agar by using a standard pour-plate technique. The authors showed that higher skin counts were obtained by alkaline treatment of the skin prior to sampling. (A gauze pad soaked in 2% sodium carbonate was allowed to remain on the skin for two minutes.) Presumably this resulted in the liberation of more keratinized cells and bacteria from the deeper layers of the skin. Large differences in counts were observed

between different individuals and between different skin areas on the same individual. Counts ranged from 18,000 to 5,000,000 per 4.15 cm^2.

The method of Williamson (5) is the result of a careful study of the Pachtman method, in which every effort was made to obtain maximum counts of the highest accuracy and precision. The sterile glass cylinder of 3.8 cm^2 cross-sectional area is held against the skin as before, and 1 ml of 0.1% Triton X-100 in 0.075 M phosphate buffer solution (pH 7.9) is poured in, and the skin is rubbed with a Teflon spatula for one minute. An aliquot of the sample is diluted in ten-fold steps with 0.05% Triton X-100 in 0.037 M phosphate buffer, and the dilutions are plated in Tryptic soy agar, incubated for 48 hours at 37C, and plates are counted. This method does not sample the organisms which may be deep within the follicles, where the bacteria are largely anaerobic. Williamson has worked with A. M. Kligman, a dermatologist at the University of Pennsylvania, and their findings are summarized in a recent paper (6). Kligman points out some rather serious errors in earlier publications and concludes that most areas of the skin have a relatively sparse bacterial flora of a few thousand or less cells per cm^2, restricted to a few species. The dominant organisms almost everywhere are aerobic or facultative staphylococci (*S. albus* and *S. epidermidis*) and diphtheroids (several species of *Corynebacterium*).

The enlarged sebaceous follicles of acne ordinarily contain enormous numbers of the anaerobic *Corynebacterium acnes,* but such follicles are found in only a few areas of the body (back and face, particularly).

The surgeon, P. B. Price, is one of the pioneers of skin bacteriology who has done a great deal of excellent work on the evaluation of skin antiseptics and surgical scrubs as well as in the development of the serial-basin surgical scrub method. Price published his serial-basin surgical scrub method in 1938 (7), and this method, as well as many published variations, has been used more widely than any other to evaluate surgical scrubs and antibacterial toilet soaps. A row of sterile basins, each containing a measured volume of sterile water and a sterile nail brush, is set up on the laboratory bench. The subject scrubs both hands and forearms for 35 seconds, rinses in the basin, moves on to the next basin, repeats the procedure, and so on for eight basins. Aliquots of 0.1 and 1 ml from each basin are transferred to sterile Petri dishes, and nutrient agar, melted and cooled to 45C, is poured into each dish. The dishes are incubated at 37C for 48 hours, and the bacterial colonies are counted. When the logarithm of the number of bacteria is plotted against the scrub time, a straight-line function results. The number of bacteria declines rapidly with time of scrubbing, that is, from the first basin to the last. After obtaining base counts in this fashion, the individual may use an antibacterial soap for a time, then repeat the test to determine the effectiveness of the antibacterial soap.

The papers of Pohle and Stuart (8), Cade (9), and Quinn, Voss, and Whitehouse (3) come to mind in this connection. The last method, called the split-use method, was particularly ingenious. Earlier studies showed a great variability in bacterial count and in the resistance of the bacteria to antiseptics from person to person. In the split-use method each individual serves as his own control. One hand is scrubbed with conventional soap as a control, and the other with the antiseptic soap. First, the control hand is protected by a rubber glove, then the other hand. In this manner, two of the largest variables, the difference in bacterial flora between individuals and the variation in a given individual with time, can be compensated for.

This paper (3) shows the comparative degerming effectiveness of soaps prepared with several different antibacterial agents. Soaps containing hexachlorophene, dichlorophene, bithionol, sodium pentachlorophenate, and zinc dimethyl dithiocarbamate gave from 78 to 92% reduction in the number of skin bacteria. The standard errors ranged from 5% to 23%; thus there are no significant differences between the five different test soaps. The hand-scrubbing methods are the most accurate yet developed for the evaluation of surgical scrubs and antibacterial soaps. Yet they have been severely criticized for giving misleading results. Kligman (6) states that the logarithmic rate of removal of bacteria from hands is a consequence of the mechanical problem of removing debris accumulated under the nails and in the nail folds. Over most of the body surface it is possible to remove more than 95% of the aerobic organisms with a one-minute scrub with nonantiseptic soap.

Biopsy Methods

The biopsy methods were employed by surgeons and dermatologists in early studies to determine the origin of skin bacteria and to study the effectiveness of skin antiseptics used to prepare the patient's skin for surgery. The methods have yielded valuable results but are now rarely used because they are traumatic and do not yield quantitatively accurate data.

Impression Plate Methods

Impression plate methods, such as the Rodac plate, have been used to evaluate pre-surgical antiseptics. They give very low counts since they enumerate colonies of bacteria, not individual cells, and only those colonies which are lying loose on the surface of the skin. In this method a film of nutrient agar or blood agar is pressed directly against the skin area to be counted, the plate is incubated for two or three days at 30 to 37C, and the colonies are counted.

Adhesive Stripping Methods

Adhesive stripping methods have been used by the dermatologists Röckl and Müller (10) in Germany,

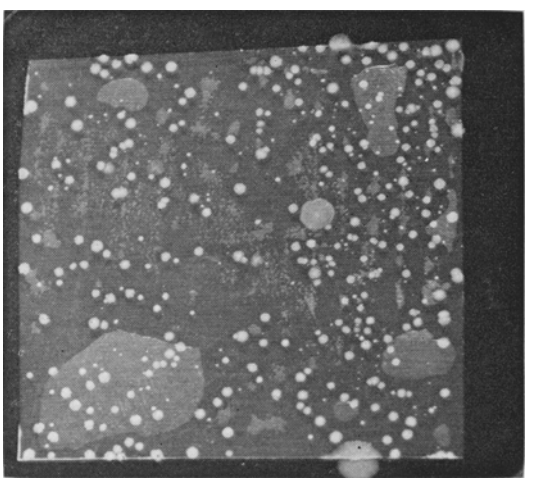

FIG. 1. Agar plate showing bacterial colonies obtained from skin stripping with plastic tape.

TABLE I

Variation of Bacterial Count per 4 cm² of Skin Area with Depth in the Skin

Skin-Stripping Number	Plates Incubated Four Days (skin of back)		
	Series 1	Series 2	Series 3[1]
1	300	540	3
2	210	540	6
3	160	320	13
5	52	220	5
7	82	190	3
9	77	240	11
11	35	128	8
13	98	89	9
14	38	92	8

[1] The skin was first scrubbed with 70% ethyl alcohol for one minute.

by Updegraff (11) and Kooyman and Simons (12) in this country. Röckl and Müller and Updegraff used transparent plastic tape, coated with a pressure-sensitive adhesive, whereas Kooyman and Simons used plastic discs approximately one-half inch in diameter and coated with a pressure-sensitive adhesive. The tape or disc is pressed to the skin and stripped off, carrying with it a layer of the dead cells of the *stratum corneum*. The plastic disc or tape is then laid on an agar plate, with the adhesive side, carrying the skin cells, against the agar. Alternatively the tape may be placed, adhesive side up, in the bottom of a sterile Petri dish, and melted and cooled agar may be poured over it. The plates are then incubated for two to four days at 37C, and the bacterial colonies which develop are counted. Figure I is a photograph of a typical plate prepared in this way. Repeated strippings from the same area give an indication of the variation of the number of colonies of bacteria with depth in the skin. As an example of this effect, Table I presents data obtained on successive skin strippings from a man selected for his unusually high skin count. The good agreement from Series 1 and 2, taken at different times on the same general area of the same person, may be noted, also the sharp reduction in bacteria accomplished by a one-minute scrub with 70% alcohol at the same time as Series 2. An approximate total count can be obtained by summation. This summation is compared in Table II with bacterial counts on adjacent areas obtained by a modified Price scrub method, by the scalpel scraping method of Evans et al., and by the wooden-applicator scraping method of Pachtman et al. Counts by the scrubbing and scraping methods run from three to 23 times as high as those done by tape stripping. This is as expected since the tape-stripping method enumerates the number of colonies of bacteria in the skin whereas the other methods enumerate individual cells. As mentioned earlier, impression plate methods give low counts since they enumerate only those colonies of bacteria which are right on the

TABLE II

Comparison of Bacterial Counts by Four Different Methods on Skin from the Flexor Surface of the Forearm

Individual	Bacteria per Square CM			
	Tape stripping	Price scrub	Evans	Pachtman
D.M.U.	4.6	106	13.7	.....
J.L.S.	677	.....	2,320	5,560

TABLE III

Comparison of Bacterial Counts on Skin of the Flexor Surface of the Forearm by the Rodac Impression Method and the Tape-Stripping Method

Individual	Number of Colonies per 16 CM² of Skin						
	Area 1		Area 2			Area 3	
	Tape 1 (Down)	Tape 2 (Down)	Rodac 1	Rodac 2	Tape 1 (Down)	Tape 1 (Up)	Tape 2 (Up)
L.D.	488	278	8	9	177	948	708
D.M.U.	76	35	2	2	72	59	31

surface of the *stratum corneum*. Table III gives the data on comparing the Rodac impression plate with tape strippings on two individuals. The tape-stripping method gives counts from 15 to 88 times as high as the Rodac impression method.

Air-Sampling Methods

The last method to be mentioned, air sampling to detect shedders, has been of great value in controlling infection in operating rooms and hospitals wards. The concern is not with the bacteria which remain on the individual and hence cannot infect anyone else in the vicinity but with bacteria which are shed by an individual into the air. Certain individuals have been shown to shed billions of pathogenic *Staphylococcus aureus* where they go. The potential hazard to hospital patients from such individuals is obvious. Sophisticated air-sampling devices, such as the Anderson sampler, have been developed to draw in air at a measured rate and impinge it in a suitable pattern onto a series of agar plates. By suitable statistical methods, good quantitative counts of the number of bacteria per unit of volume of air may be obtained. Shooter (13) has shown that most of the *Staphylococcus aureus* shed into the air come from the perineum and that shedding is increased after bathing. Shedding can be greatly reduced by the application of lanolin to the perineum. This does not harm the bacteria but merely glues them in place. Shooter has expressed pessimism however about his chances of persuading the surgeons and surgical nurses of Great Britain to apply lanolin to their perinea before going into the operating room.

REFERENCES

1. Ulrich, J. A., Health Laboratory Science *1*, 133–136 (1964).
2. MacPherson, C. R., M. Frances Sparkmen and D. R. Whitney, Am. J. Surgery *109*, 699–704 (1965).
3. Quinn, H., J. G. Voss and H. S. Whitehouse, Appl. Microbiol. *2*, 202–204 (1954).
4. Pachtman, E. A., E. E. Vicher and J. J. Brunner, J. Invest. Derm. *22*, 389–396 (1954).
5. Williamson, P., Quantitative Estimation of Cutaneous Bacteria in "Skin Bacteria and Their Role in Infection," ed. H. I. Maibach and G. Hildick-Smith, McGraw-Hill Book Company Inc., New York, N. Y. 1965.
6. Kligman, A. M., The Bacteriology of Normal Skin in "Skin Bacteria and Their Role in Infection," ed. H. I. Maibach and G. Hildick-Smith, McGraw-Hill Book Company Inc., New York, N. Y. 1965.
7. Price, P. B., J. Infect. Dis. *63*, 301–318 (1938).
8. Pohle, W. D., and L. S. Stuart, J. Infect. Dis. *67*, 275–281 (1940).
9. Cade, A. R., Special Technical Publication No. 115, American Society for Testing Materials, pp. 33–39 (1952).
10. Röckl, H., and E. Müller, Archiv für Klinische u. Experimentelle Dermatologie *209*, 13–29 (1959).
11. Updegraff, D. M., J. Invest. Derm. *43*, 129–137 (1964).
12. Kooyman, D. J., and R. W. Simons, Arch. Dermat. *92*, 581–584 (1965).
13. Shooter, R. A., The Environment in "Skin Bacteria and Their Role in Infection," ed. H. J. Maibach and G. Hildick-Smith, McGraw-Hill Book Company Inc., New York, N. Y. (1965).