The Microbial Ecology of Pilosebaceous Units Isolated from Human Skin

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A method allowing isolation and microbiological analysis of individual pilosebaceous units (follicles) was used to study biopsies of back skin obtained from volunteer acne vulgaris patients. The main microbial groups isolated were members of the genera *Propionibacterium*, *Staphylococcus* and *Pityrosporum*. The incidence (and mean density) of these organisms in 140 normal follicles was 12% (2.6×10^5 per follicle), 4% (5.5×10^3 per follicle) and 13% (10^2 per follicle) respectively. Colonized follicles were not distributed evenly amongst the subjects studied. The results are analysed and discussed from an ecological standpoint.

INTRODUCTION

It is generally acknowledged that the pilosebaceous unit (follicle) is the site of growth of the majority of saprophytic bacteria found on non-hydrated human skin (Noble, 1981). Unfortunately the great majority of sampling techniques used on the human skin fail both in sampling this environment efficiently and in differentiating the contents of individual follicles. Such methods are of limited value in the study of follicular ecology.

A technique for the isolation of pilosebaceous units from biopsies of skin after treatment with 1 M-CaCl₂ solution was described by Kellum (1966) and was first exploited for microbiological analysis by Puhvel *et al.* (1975). This procedure is unique in allowing the examination of the entire contents of a pilosebaceous unit in isolation from the skin surface and neighbouring follicles.

After confirming the observations of Puhvel *et al.* (1975) that Kellum's technique does not appreciably reduce the viability of cutaneous micro-organisms (Leeming, 1983), a study of acne vulgaris-affected skin was undertaken. It is the microbial ecology of normal follicles isolated from this skin which is discussed in this paper.

METHODS

Subjects. A total of 54 volunteer acne vulgaris patients attending the dermatology clinic at the Leeds General Infirmary were studied. Their average age was 22.0 years (range 14–37); all had mild to moderate acne and gave their informed consent before samples were taken. None had been receiving antibiotic treatment for at least six weeks previously. The biopsy procedure was approved by the Ethical Committee of the Leeds General Infirmary.

Biopsy processing. A 3 or 4 mm diameter punch biopsy was excised from an area of unblemished skin on the upper back. Local anaesthetic (lignocaine hydrochloride) was administered intradermally but the skin was otherwise unprepared. Drops of the anaesthetic applied to lawns of cutaneous micro-organisms did not inhibit their growth.

Each biopsy was soaked in 1 M-CaCl₂ at 4 °C for 2 h, which facilitated the peeling of the epidermis and attached follicles from the dermis under a dissecting microscope. Each follicle was cut from the epidermis using surgical microscissors and homogenized individually in a 0.2 ml capacity ground glass micro-homogenizer (Pierce & Warriner 20037; Pierce & Warriner, Chester, UK) containing phosphate-buffered 0.1% (v/v) Triton X-100 solution ('wash fluid'; Williamson & Kligman, 1965). Each biopsy yielded between one and eight follicles with an average density of approximately 50 follicles per cm² surface area (follicular density varied widely among patients). All follicles isolated intact were processed.

Microbiological assessment. Homogenates were diluted in half-strength wash fluid and viable counts were made on the surface of nutrient plates of the following compositions:

(i) RCMT plates: reinforced clostridial medium (Oxoid) supplemented with 0.2% (v/v) Tween 80 (Sigma); incubated anaerobically for 6 d at 37 °C.

(ii) Heated blood agar: blood agar base (Oxoid CM55) supplemented with 50 ml defibrinated horse blood (1^{-1} ; Oxoid); incubated aerobically for 48 h at 37 °C.

(iii) Aerobic coryneform medium: blood agar base (Oxoid CM55) supplemented with (1^{-1}) 2 g glucose, 3 g yeast extract (Oxoid L21), 2 ml Tween 80 (Sigma) and 50 ml defibrinated horse blood (Oxoid); incubated aerobically for 48 h at 37 °C.

(iv) *Pityrosporum* medium 1: aerobic coryneform medium supplemented with (1^{-1}) 10 g ox bile (Oxoid L50), 50 mg chloramphenicol and 50 mg cycloheximide; pH was adjusted to 6.0 before autoclaving. Cultures were incubated aerobically for 6 d at 37 °C in a chamber with 100% relative humidity.

(v) *Pityrosporum* medium 2 (after Faergemann & Bernander, 1979): comprising (1^{-1}) 10 g bacteriological peptone (Oxoid L37), 40 g glucose, 10 g ox bile (Oxoid L50), 0.1 g yeast extract (Oxoid L21), 2.5 g glycerol monostearate, 2 ml Tween 80 (Sigma), 20 ml olive oil, 10 g agar (Oxoid L11), 50 mg chloramphenicol, 50 mg cycloheximide. Cultures were incubated as for *Pityrosporum* medium 1.

After incubation differential counts were made according to colony type, Gram reaction and cell morphology. Micrococci were separated from staphylococci on the basis of furazolidone sensitivity (von Rheinbaben & Hadlok, 1981). Isolates of the genera *Propionibacterium* and *Staphylococcus* were identified to species level according to the schemes of Marples & McGinley (1974) and Kloos & Schleifer (1975). *Propionibacterium acnes* isolates were further subdivided into types I and II according to their ability to produce acid from D-sorbitol (Johnson & Cummins, 1972).

Follicular homogenates were also assessed for the presence of *Pityrosporum* by making total counts of cells deposited on membrane filter discs and stained with oxalate crystal violet (Mulvany, 1969).

Skin surface scrub. For comparative purposes a site adjacent to each of 24 biopsies was sampled by the more conventional surface scrub method of Williamson & Kligman (1965) which removes bacteria from the surface of the skin by agitation with detergent solution. These samples were processed in the same manner as the follicular homogenates.

Statistical tests. Statistical analysis was done as recommended by Sokal & Rohlf (1981).

RESULTS AND DISCUSSION

The most commonly isolated bacteria were staphylococci and propionibacteria. Fig. 1 shows the distribution of densities of these two groups amongst 140 normal follicles. Both plots are bimodal, being divisible into Poisson-like distributions of low population densities and lognormal distributions of high population densities. The Poisson-like distributions are attributable to contaminating (allochthonous) organisms which become associated with a follicle either naturally, due to the exposure of the follicular orifice to the environment, or experimentally during the microdissection processes. The lognormal distributions are consistent with parameters expected of resident (autochthonous) populations. For meaningful analysis of an ecosystem it is necessary to differentiate autochthonous and allochthonous populations. In this case such differentiation was possible on a simple numerical basis because, when the data were considered without logarithmic transformation, clear gaps emerged between the high and low density distributions. In subsequent analyses only those follicles containing the higher densities of bacteria were considered colonized.

Staphylococcus epidermidis accounted for approximately 50% of staphylococcal colonists, the rest were other coagulase-negative species. All propionibacterial colonists were *Propionibacterium acnes* strains; *P. acnes* type I outnumbered *P. acnes* type II by 3:1.

The third major microbial group isolated from follicles was *Pityrosporum* spp. Frequency distributions could not be deduced for this yeast because many strains have a very low plating efficiency on currently available media (Sloof, 1970) and the total count method used had a high threshold of sensitivity. Any follicle from which *Pityrosporum* was either cultured or visualized was therefore considered to be colonized for the purposes of data analysis.

Table 1 shows the results obtained by microdissection and by surface scrubbing. The incidence of each microbial group was considerably lower intrafollicularly than at the skin surface. However, the scrub technique samples 5 cm² of skin and therefore at least 100 follicles on the backs of most individuals. The observed follicular populations are therefore more than adequate to explain the surface densities of propionibacteria, staphylococci and *Pityrosporum* spp., supporting the view that the pilosebaceous unit is the primary site of growth of these organisms. These results also emphasize the inefficiency of the Williamson & Kligman (1965) sampling technique.

Fig. 1. Distribution of bacterial population densities amongst 140 normal follicles isolated from the upper backs of 54 acne vulgaris patients. One to eight follicles were isolated at random from each individual by biopsy (see Methods). The theoretical lower limit of sensitivity was 12.5 bacteria per follicle.

Table 1. *Comparison of follicular and surface scrub methods*

Microdissection results are from 140 follicles isolated from 45 patients; surface scrubs were done at an adjacent site.

Colonizing organism	Microdissection		Surface scrub	
	Proportion of follicles colonized (%)	Geometric mean bacterial count per colonized follicle	Incidence (%)	Geometric mean per cm ⁻² (amongst colonized individuals)
Propionibacteria	12	2.6×10^5	100	1.6×10^4
Staphylococci	4	5.5×10^3	96	9.5×10^2
<i>Pityrosporum</i> spp.	13	$\sim 10^2$	100	1.4×10^3
Aerobic coryneforms	<0.7	—	67	5.5×10^2
Micrococci	<0.7	—	42	4.0×10^1

The ecological status of aerobic coryneform bacteria and micrococci is not clear. When present on skin they constitute only a small proportion of the total microbial population of most individuals. They may never form stable follicular populations but occupy niches on the skin surface – Noble & Pitcher (1976) observed surface populations of aerobic coryneforms by scanning electron microscopy. However, the populations of these organisms detected at the skin surface in this study may have been produced by occasional follicular colonization, which would be detected only if a very large sample of follicles was studied.

The proportion of follicles colonized by micro-organisms varied widely amongst patients. Of the follicles colonized by propionibacteria, 82% (14/17) were isolated from only 7.4% (4/54) of the patients sampled. This inequality probably extends to the other microbial groups growing on skin (insufficient follicles were isolated from each patient to check this) and probably explains the large and persistent individual differences in microbial densities found at the skin surface (Evans, 1975; Evans & Strom, 1982).

Table 2 shows an analysis of the degree of association between cutaneous micro-organisms.

Table 2. *Contingency tables showing associations of propionibacteria, staphylococci and Pityrosporum spp. in normal follicles*

The figures in each table identify the numbers of isolated follicles which were either colonized (+) or not colonized (–) by the genera specified. Probabilities refer to the 2-tailed null hypothesis predicting independent assortment (Fisher's exact test).

Staphylococcal colonization					Propionibacterial colonization					Staphylococcal colonization				
- +					- +					- +				
Propionibacterial colonization	-	117	4	121	Pityrosporal colonization	-	101	9	110	Pityrosporal colonization	-	106	4	110
	+	16	1	17		+	14	2	16		+	15	1	16
<hr/>					<hr/>					<hr/>				
133 5 138					115 11 126					121 5 126				
<i>P</i> = 0.49					<i>P</i> = 0.63					<i>P</i> = 0.50				

There was no evidence of interaction, either antagonistic or cooperative, between any two microbial groups at the follicular level. This observation indicates that they probably occupy distinct niches within the follicular environment and therefore do not normally compete intergenerically. It also follows that a follicle favourable for the growth of one micro-organism is not necessarily favourable to the growth of another cutaneous group, although there is considerable evidence that when cutaneous genera are displaced from follicles by antimicrobial therapy, other groups (particularly enterobacteria) will sometimes fill the niches left unoccupied (Fulton *et al.*, 1968).

The physical and chemical parameters of pilosebaceous units are poorly defined because this environment is inaccessible to most investigative probes. Therefore the precise nature of microbial niches and the variables determining the susceptibility of a given follicle to microbial colonization remain to be elucidated. Some factors which may preclude follicular colonization are nutritional deficiency, low water activity (known to be important at the skin surface; Rebell *et al.*, 1950), extremes of pH, E_h (in the case of propionibacteria which are anaerobic or micro-aerophilic on primary isolation), immunological or non-specific host antimicrobial activity and washout conditions, which may prevail in follicles with a high turnover of sebum and keratinocytes. It is most likely that it is a combination of these factors rather than one alone which determines the probability of a follicle becoming colonized after micro-organisms randomly gain access to the follicular orifice.

Gross morphological changes of follicles are observed during maturation and hair cycles (Montagna & Parakkal, 1974) and there are likely to be corresponding microbiologically significant changes in the intrafollicular environment. Hence, the great increase in numbers of micro-organisms harboured on the skin at puberty (Matta, 1974; Leyden *et al.*, 1975; Faergemann & Fredriksson, 1980) is probably due to a great increase in the proportion of follicles suitable for microbial colonization following follicular maturation.

This study has emphasized the heterogeneity of human skin as an environment; not only are there great microbiological differences among individuals but also among follicles of the same individual and probably among niches present in a single follicle. Analysis of isolated follicles is essential to define this environment with precision; information gained from such analyses would be of great significance to studies of microbial ecology of skin, skin disinfection and cutaneous disorders such as acne vulgaris in which skin micro-organisms have been implicated.

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REFERENCES

- EVANS, C. A. (1975). Persistent individual differences in the bacterial flora of the skin of the forehead: numbers of propionibacteria. *Journal of Investigative Dermatology* **64**, 42–46.
- EVANS, C. A. & STROM, M. S. (1982). Eight year persistence of individual differences in the bacterial flora of the forehead. *Journal of Investigative Dermatology* **79**, 51–52.

- FAERGEMANN, J. & BERNANDER, S. (1979). *Tinea versicolor* and *Pityrosporum orbiculare*: a mycological investigation. *Sabouraudia* **17**, 171–179.
- FAERGEMANN, J. & FREDRIKSSON, T. (1980). Age incidence of *Pityrosporum orbiculare* on human skin. *Acta dermatovenereologica* **60**, 531–533.
- FULTON, J. E., MCGINLEY, K., LEYDEN, J. & MARPLES, R. R. (1968). Gram negative folliculitis in acne vulgaris. *Archives of Dermatology* **98**, 349–353.
- JOHNSON, J. L. & CUMMINS, C. S. (1972). Cell wall composition and deoxyribonucleic acid similarities among the anaerobic coryneforms, classical propionibacteria and strains of *Arachnia propionica*. *Journal of Bacteriology* **109**, 1047–1066.
- KELLUM, R. E. (1966). Isolation of human sebaceous glands. *Archives of Dermatology* **93**, 610–612.
- KLOOS, W. E. & SCHLEIFER, K. H. (1975). Simplified scheme for routine identification of human *Staphylococcus* species. *Journal of Clinical Microbiology* **1**, 82–88.
- LEEMING, J. P. (1983). *The microbial colonisation of individual pilosebaceous follicles in acne vulgaris affected skin*. PhD thesis, University of Leeds.
- LEYDEN, J. J., MCGINLEY, K. J., MILLS, O. H. & KLIGMAN, A. M. (1975). Age-related changes in the resident bacterial flora of the human face. *Journal of Investigative Dermatology* **65**, 379–381.
- MARPLES, R. R. & MCGINLEY, K. J. (1974). *Corynebacterium acnes* and other anaerobic diphtheroids from human skin. *Journal of Medical Microbiology* **7**, 349–357.
- MATTA, M. (1974). Carriage of *Corynebacterium acnes* in school children in relation to age and race. *British Journal of Dermatology* **91**, 557–561.
- MONTAGNA, W. & PARAKKAL, P. F. (1974). *The Structure and Function of Skin*, 3rd edn. New York: Academic Press.
- MULVANY, J. G. (1969). Membrane filter techniques in microbiology. In *Methods in Microbiology*, vol. 1, pp. 205–253. Edited by J. R. Norris & D. W. Ribbons. London: Academic Press.
- NOBLE, W. C. (1981). *Microbiology of Human Skin*, 2nd edn. London: Lloyd-Luke.
- NOBLE, W. C. & PITCHER, D. G. (1976). Microbial ecology of the human skin. In *Advances in Microbial Ecology* **2**, 245–289.
- PUHVEL, S. M., REISNER, R. M. & AMIRIAN, D. A. (1975). Quantification of bacteria in isolated pilosebaceous follicles in normal skin. *Journal of Investigative Dermatology* **65**, 525–531.
- REBELL, G., PILLSBURY, D. M., PHALLE, G., DE SAINT, M. & GINSBERG, D. (1950). Factors affecting the rapid disappearance of bacteria placed on the normal skin. *Journal of Investigative Dermatology* **14**, 247–263.
- SLOOF, W. CH. (1970). *Pityrosporum* Sabouraud. In *The Yeasts*, 2nd edn, pp. 1167–1186. Edited by J. Lodder. Amsterdam: North Holland Publishing Co.
- SOKAL, R. R. & ROHLF, F. J. (1981). *Biometry*, 2nd edn. San Francisco: W. H. Freeman & Co.
- VON RHEINBACH, K. E. & HADLOK, R. M. (1981). Rapid distinction between micrococci and staphylococci with furazolidone agars. *Antonie van Leeuwenhoek* **47**, 41–51.
- WILLIAMSON, P. & KLIGMAN, A. M. (1965). A new method for the quantitative investigation of cutaneous bacteria. *Journal of Investigative Dermatology* **45**, 498–503.