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## **Antimicrobials and the Skin Physiological and Pathological Flora**

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### **Abstract**

Healthy human skin is regularly colonized by nonpathogenic microorganisms. Bacterial genera isolated are coagulase-negative staphylococci and diphtheroid rods on the skin surface and propionibacteria in the infundibulum of the sebaceous glands. As for fungi, *Pityrosporum* (*Malassezia* spp.) is regularly present. The distribution and density of the flora is dependent on age and environmental factors such as sebum secretion, occlusion, temperature and humidity. Odor production in the axilla is related to the activity of aerobic diphtheroids. Antimicrobials may reduce the density of the skin resident flora, but they do not completely eliminate it. While antimicrobials may cause irritant and allergic contact dermatitis, no evidence exists that the use of antimicrobial substances may change the ecology of resident bacteria on the skin thereby leading to the overgrowth of pathogenic bacteria.

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Antimicrobials have been used in textiles originally to prevent rotting, especially under adverse environmental conditions such as tropical climate. This dates back to World War II when cotton fabrics used in the South Pacific by the US Forces were protected from microbial decay.

The application of antimicrobial substances in textiles is now extended to textiles for medical uses (e.g. in the treatment of atopic dermatitis) but also in sports and leisure. In order to understand the use of antimicrobials in textiles, textile engineers should have some basic knowledge of the anatomy and physiology of the skin, especially its microbiology. While textiles should support physiological functions, they should not pose a risk to human health under normal or reasonably foreseeable use.

## The Human Skin Flora

The skin is the largest organ of the human body. In the adult, it has a surface of 1.8 m<sup>2</sup> and a weight of 10 kg.

As the skin forms a barrier against harmful chemical and physical impact of the environment, it also protects the organism from infection by pathogens, parasites, fungi, bacteria and viruses.

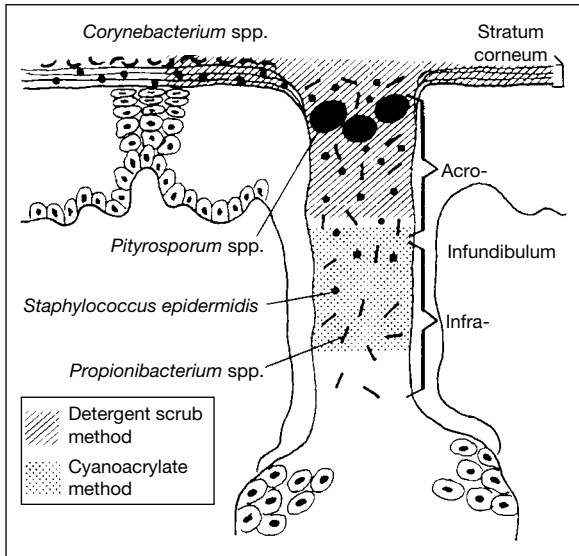
One important protective factor against infection by pathogenic organisms is skin surface pH that physiologically is between 5 and 6 at nonoccluded sites [1]. Intertriginous areas such as the axillae, the toeweb, the genital and the perianal regions have a higher pH and are more prone to infection. In addition to pH, certain stratum corneum lipids have been shown to have inhibitory effects on pathogenic bacteria [2]. In recent years, defensins, peptides with antibiotic properties, have been demonstrated in human skin [3]. Peptidoglycan recognition proteins 3 and 4 are bactericidal against several pathogenic and nonpathogenic Gram-positive bacteria of transient, but not normal flora [4].

Despite these antibacterial substances present on the skin surface, the skin is not sterile but harbors nonpathogenic bacteria. The human skin flora is defined as the microbes present in healthy skin on the skin surface, within the stratum corneum, in the infundibulum of the sebaceous glands and in the hair follicles (fig. 1) [6]. It may be differentiated: the resident flora that is continuously present on the skin and thus may be regularly sampled, the transient flora that is only sampled at low frequency or density and the temporary resident flora that transiently grows on the skin without leading to infection.

The following genera make up the skin resident flora: micrococci with coagulase-negative staphylococci, *Peptococcus* spp., *Micrococcus* spp., diphtheroids with corynebacteria and *Brevibacterium* spp., propionibacteria and gram-negative rods.

The bacteria of the resident flora of the normal human skin live as microcolonies [7] on and between the layers of the stratum corneum [8] and in the follicles of the sebaceous glands [9]. In the uppermost layer of the stratum corneum, *Corynebacterium* spp. and sometimes microorganisms of the transient skin flora are present. In the infundibulum of the sebaceous glands, there is a topographic distribution of *Propionibacterium* spp. from the deepest part of the infundibulum up to the entrance of the acroinfundibulum and of coagulase-negative staphylococci from the middle part of the infundibulum to its entrance.

*Pityrosporum* spp. are located near the ostium in the acroinfundibulum [9]. Skin bacteria are distributed within a three-dimensional space and not on a surface. Penetration of antimicrobial agents, especially into the infundibulum, is particularly difficult, because of the sebum/bacteria/corneal cell mass. Reduction



**Fig. 1.** Three-dimensional distribution of human physiological skin flora (based on Hartmann et al. [5]).

of the CFU counts per square centimeter of the bacteria of the resident flora to zero in vivo is impossible even after repeated applications of a potent skin disinfectant [10].

Korting et al. [11] have pointed out that the composition of the skin flora is subject to age: the streptococci which are found in infants disappear, and coryneform bacteria occur, which are mainly responsible for odor production. Anaerobic propionibacteria are more numerous in juveniles and young adults, a fact that may be explained by increased sebum production [11].

The relevance of skin resident flora for the healthy skin lies in the fact that it generates an ecological system protecting from pathogens. Thus *Staphylococcus epidermidis*, *Propionibacterium acnes*, corynebacteria and *Pityrosporum ovale* produce lipases and esterases that break triglycerides to free fatty acids leading to a lower skin surface pH and thereby unfavorable growing conditions for skin pathogens. *S. epidermidis* and *P. acnes* are known to produce antibiotics that may interfere with pathogenic organisms.

Endogenous and exogenous factors may influence the skin flora, thus facilitating skin infections. These factors include cellular and humoral immunity (e.g. diabetes, HIV infection), medication (e.g. Gram-negative folliculitis induced by long-term antibiotic treatment), environmental factors (e.g. humidity,

temperature), and cosmetics and hygiene (e.g. antibacterial washes). Occlusive garments may be a risk factor for skin infections, and it is a well-known clinical experience that contaminated clothing may lead to a spread of infection, e.g. in staphylococcal folliculitis in hot, humid climates.

### **Skin Odor and Bacterial Flora**

Already in the 1980s, several groups studied the relationship between skin odor and bacterial flora. Leyden et al. [12] showed that the axillary flora is a stable mixture of Micrococcaceae, aerobic diphtheroids and propionibacteria. Higher numbers of bacteria can be recovered from the axilla of persons with pungent axillary odor. This is related to the activity of aerobic diphtheroids, which could be ascertained in experiments with droplets of apocrine sweat placed on the forearm and inoculated with various bacteria: only diphtheroids generated the typical body odor. Cocci produced a sweaty odor attributable to isovaleric acid [12]. No ecological interactions between the members of the resident flora were found in the axilla [13]. Similar results were reported for foot odor, where high population densities of staphylococci and aerobic coryneform bacteria predispose to odor [14].

Malodorous androstenes ( $5\alpha$ -androst-16-en-3-one, 4,16-androstadien-3-one, 5,16-androstadien- $3\beta$ -ol,  $5\alpha$ -androst-16-en- $3\alpha$ -ol and  $5\alpha$ -androst-16-en- $3\beta$ -ol) seem to be produced by bacteria in the axilla [15]. Recently, the biochemical pathways for the production of these steroid substances have been described and a metabolic map for axillary corynebacterial 16-androstene biotransformations was proposed, detailing potential enzyme activities [16].

Skin odor may be reduced by antibacterial agents without modifying the amount of sweat secreted. A clinical study has shown that the improvement of malodor correlates with a reduction of both micrococci (70%) or diphtheroids (73%) [17]. In persons presenting with persistent bromidrosis, the bacterial count per square centimeter did not significantly decrease and remained above  $10^4$  diphtheroids/cm<sup>2</sup> suggesting that body odor may be at least indirectly correlated to microbial counts with a bacteria threshold ranging around and above  $10^4$  [17].

### **Effects of Antimicrobials on the Bacterial Flora of the Skin**

Antimicrobial substances are used to reduce the skin resident and transient flora, especially to eliminate pathogens in hygienic and surgical disinfection.

**Table 1.** Antimicrobial spectrum and characteristics of hand hygiene antiseptic agents (from Boyce and Pittet [19])

Group	Gram-positive bacteria	Gram-negative bacteria	Mycobacteria	Fungi	Viruses	Speed of action	Comments
Alcohols	+++	+++	+++	+++	+++	fast	optimum 60–95%; no concentration persistent activity
Chlorhexidine (2% and 4% aqueous)	+++	++	+	+	+++	intermediate	persistent activity; rare allergic reactions
Iodine compounds	+++	+++	+++	++	+++	intermediate	causes skin burns; usually too irritating for hand hygiene
Iodophors	+++	+++	+	++	++	intermediate	less irritating than iodine; acceptance varies
Phenol derivatives	+++	+	+	+	+	intermediate	activity neutralized by nonionic surfactants
Triclosan	+++	++	+	–	+++	intermediate	acceptability on hands varies
Quaternary ammonium compounds	+	++	–	–	+	slow	used only in combination with alcohols; ecological concerns

+++ = Excellent; ++ = good, but does not include the entire bacterial spectrum; + = fair; – = no activity or not sufficient. Hexachlorophene is not included because it is no longer an accepted ingredient of hand disinfectants.

In Germany, the German Society for Hygiene and Microbiology has been publishing standards for the testing of disinfecting substances since 1959, and it publishes a list of tested and approved disinfectants including methods of hand decontamination and hygienic hand wash. The most widely used method for testing the efficacy of antiseptics in Europe is European standard EN 1500. In the USA, requirements for in vitro and in vivo testing of health care worker handwash products and surgical hand scrubs are outlined in the Food and Drug Administration’s ‘Tentative final monograph for healthcare antiseptic drug products’ [18]. A list of the most frequently used approved topical antimicrobials with their spectrum of activity is given in table 1.

The main concern with the regular use of topical antimicrobial substances is the development of irritant and allergic contact dermatitis [19]. As a consequence of contact dermatitis in health care workers, the bacterial flora will change. Eczematous skin is characterized by papules, vesicles and, in irritant dermatitis, fissures that will lead to the exudation of intercellular fluid, thus promoting the growth of pathogens. In a prospective observational study of 40 nurses (20 with diagnosed hand irritation and 20 without), nurses with damaged hands did not have higher microbial counts ( $p = 0.63$ ) but did have a greater number of colonizing species (means: 3.35 and 2.63,  $p = 0.03$ ) [20]. Twenty percent of nurses with damaged hands were colonized with *Staphylococcus aureus* compared with none of the nurses with normal hands ( $p = 0.11$ ). Nurses with damaged hands were also twice as likely to have Gram-negative bacteria ( $p = 0.20$ ), enterococci ( $p = 0.13$ ) and *Candida* ( $p = 0.30$ ) present on the hands [20]. In using topical antimicrobial products, it is therefore important to select those with the lowest irritant and sensitizing potential under the given situation. When considering antimicrobials for the use in textiles with a possible transmission of the substances onto the skin, safety regarding irritation and sensitization should be shown by appropriate use tests employing noninvasive bioengineering technology [21]. A safety assessment as required by the European Cosmetics Directive is mandatory in these applications.

While evidence exists that the use of antimicrobial substances may change the ecology of resident bacteria of the gut thereby leading to the overgrowth of pathogenic bacteria, there are no such data for the skin [22]. As Jones [23] stated in 1999, based on current knowledge, the benefit from the use of topical antimicrobial wash products in combination with standard infection control and personal hygiene practices far outweighs the risk of increased antibiotic resistance.

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