Microbiology of the skin and the role of biofilms in infection

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ABSTRACT

The integrity of human skin is central to the prevention of infection. Acute and chronic wounds can develop when the integrity of skin as a barrier to infection is disrupted. As a multi-functional organ, skin possesses important biochemical and physical properties that influence its microbiology. These properties include a slightly acidic pH, a low moisture content, a high lipid content (which results in increased hydrophobicity) and the presence of antimicrobial peptides. Such factors have a role to play in preventing exogenous microbial colonisation and subsequent infection. In addition, the properties of skin both select for and enhance colonisation and biofilm formation by certain 'beneficial' micro-organisms. These beneficial micro-organisms can provide further protection against colonisation by potential pathogens, a process known as colonisation resistance. The aim of this paper is to summarise the microflora of skin and wounds, highlighting the role of certain micro-organisms and biofilms in associated infections.

Key words: Bacteria • Biofilms • Micro-organisms • Microflora • Skin

Key Points

- skin is an integral part of the innate immune system forming the first line of defense against infection by reducing microbial adherence and invasion

INTRODUCTION

The skin is the largest organ in the human body and in an adult, has on average, a total surface area of approximately 1.75 m^2 and a weight of 5 kg. Often considered only to be an outer covering of the body, skin is in fact a vital organ involved in regulating the body's internal environment (homeostasis), in particular its water content and temperature. Skin is an integral part of the innate immune system forming the first line of defense against infection by reducing microbial adherence and invasion (1,2).

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Microbiologically, the outer surface of adult skin is colonised by a small number of 'culturable' micro-organisms. These can regularly be detected when skin is analysed and represent a population referred to as the resident microflora, normal flora or indigenous microbiota (3,4). At any given location and over the lifetime of an individual, the indigenous microbiota is relatively stable, both in terms of composition and quantity.

In addition to the indigenous microbiota, skin also provides a supportive environment for other micro-organisms, which 'lie free' on its surface. These micro-organisms are the transient microflora and are not perpetual residents of skin (5). The role transient micro-organisms play in infection, and colonisation resistance of the skin surface remains largely unknown, although it is highly likely that they influence the infection life cycle.

The density and composition of the skin's indigenous microflora varies with anatomical site and it has been reported that a higher density of micro-organisms reside in moist

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regions such as the axillae, groin and between the toes. At other 'drier' regions, low moisture content and a neutral to slightly acidic pH enhances the adhesion of certain bacteria to the skin surface, whilst inhibiting others.

When skin is damaged the underlying tissue is exposed and this significantly increases the risk of infection (6). Indeed, the prevalence of skin and wound infections on a worldwide scale is high, with recent reports suggesting that for every million wound patients, at least 10 000 die from microbial infection (7–9). The most frequently encountered bacterial species in skin infections is *Staphylococcus aureus*, including meticillin resistant forms of this species (MRSA) (10–12). Other micro-organisms associated with skin infections are *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter* spp., and coagulase-negative staphylococci (CNS) including *Staphylococcus epidermidis* and *Staphylococcus lugdunensis* (11–13).

The aim of this paper is to summarise those micro-organisms commonly encountered on skin and wounds, and to highlight their roles in skin infection. Furthermore, the significance of biofilms in both skin and wound infections is considered.

DEFENSIVE MECHANISMS OF THE SKIN

Skin has many defensive mechanisms (Table 1) that protect the body from invasion by both opportunistic and strict pathogens (14–16). For example, the outermost layer of skin (the stratum corneum) consists of an upper region of dead keratinised cells which inhibit microbial adherence. The stratum corneum also contains low levels of nutrients and high levels of keratin and the latter can only be used as a nutrient source by a limited number of bacteria, so its presence serves to limit bacterial density.

Table 1 Properties of the skin associated with defence against microbial infection

Property	Skin association
Moisture content	Generally low (however some areas of the body such as the arm pits contain areas high moisture levels)
pH	Overall Acidic (pH 5.5)
Squamous cell shedding	Continuous
Salt content	High
Antimicrobial Peptides	Cathelicidins β-defensins, Bactericidal/permeability-increasing protein (BPI), Lactoferrin, Lysozyme, Dermcidin
Stratum corneum	Intact
Fatty acids and lipids	Present in high concentrations
Immunoglobulin	Present

In addition, the continuous shedding of squamous epithelial cells from the skin serves to remove attached micro-organisms from the skin's surface. The significance of this is highlighted by the fact that on average, most humans lose 9 g of skin (shedding of squames) per day, with each squame harbouring approximately 30 bacteria. As a result, micro-organisms that remain close to the skin surface have a greatly reduced potential to irreversible adhere, proliferate and form a biofilm.

If the barrier function of the skin is impaired, the cellular component of the innate immune system provides the next line of defence. Skin has its own lymphoid tissue, which is a source of Langerhans cells and dendritic cells (DCs). These antigen presenting cells (APCs) possess surface molecules that recognise specific markers associated with pathogens. Langerhans cells and DCs provide immune surveillance within the skin and upon detecting a pathogen, will communicate its presence through interaction with T-cells in local lymph nodes, thereby activating an immune response. In this way, APCs are involved in mediating both the humoral and cell-mediated responses of the immune system. Immunoglobulins A and G are found on the skin surface and assist in reducing microbial attachment.

Over 20 antimicrobial peptides (AMPs) have been reported on the surface of human skin (17) and these generally exhibit a broad spectrum antimicrobial activity. AMPs are produced by many types of skin cell, including mast cells and keratinocytes (18,19), and protect the skin from microbial invasion. The cationic nature of AMPs enables electrostatic interaction with negatively charged bacterial membranes, whilst the amphipathic properties of AMPs lead to microbial cell death through membrane disintegration and pore formation.

The first AMP reported in human skin was cathelicidin. Cathelicidin is also referred to as LL-37 or hCAP18 (20) and in healthy skin is produced at low levels by keratinocytes. The expression of cathelicidin is, however,

Key Points

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- other micro-organisms associated with skin infections are *Pseudomonas aeruginosa*, *Escherichia coli*, *Acinetobacter* spp., and coagulase-negative staphylococci (CNS) including *Staphylococcus epidermidis* and *Staphylococcus lugdunensis*
- if the barrier function of the skin is impaired, the cellular component of the innate immune system provides the next line of defence
- over 20 antimicrobial peptides (AMPs) have been reported on the surface of human skin and these generally exhibit a broad spectrum antimicrobial activity

up-regulated during episodes of infection, inflammation or when the skin's integrity is disrupted (21). Cathelicidin is present in the skin of newborn babies where it has been shown to significantly inhibit the growth of *S. epidermidis*, highlighting its importance to the ecological stability of the skin microbiota (22).

Human β-defensin-2 is another AMP expressed by normal keratinised skin (23). This cationic AMP has been shown to have antimicrobial activity against Group A streptococci (GAS), *S. aureus*, *E. coli*, *P. aeruginosa* and the yeast *Candida albicans* (24–26).

The primary function of sweat is to mediate thermoregulation of the body. However, in recent years, a constitutively expressed AMP called dermcidin has been detected within human sweat glands, indicating an additional defensive function of sweat (27).

Eccrine sweat glands produce the enzyme lysozyme, which cleaves β1–4 glycosidic bonds. These bonds link components of the cell walls of Gram-positive (*N*-acetylglucosamine and *N*-acetylmuramic acid) and Gram-negative bacteria (peptidoglycan) and cleavage of these structures is bactericidal. Skin lysozyme has also been found to have a synergistic effect on the antimicrobial activity of AMPs produced by keratinocytes (28). Finally, the high salt content on skin is also antimicrobial and this occurs partly due to sweat evaporation.

MICROFLORA OF THE SKIN

Skin microbiology studies accrued over the past 50 years have relied largely on a variety of sampling and culture techniques to detect and quantify micro-organisms from different anatomical sites. Whilst previous studies have undoubtedly made significant contributions to the subject of skin microbiology, their reliance on cultural approaches has been a limitation (29–32). Culture methods generally result in a gross underestimation of the skin microbiota in both qualitative and quantitative terms (33). Thus, there is a need for a more accurate understanding of the density and diversity of bacteria at different skin regions (29,30) together with an understanding of the roles specific micro-organisms have in infection.

The Human Microbiome Project (HMP) was initiated in 2007 (International Human Microbiome Project – NIH) to analyse the human microbial flora using molecular methods. The purpose of the project was to better define molecular tools, indicate the limitations of standard culture techniques and redefine the microbiome at various body sites including sebaceous, moist and dry skin locations. A key outcome from the study has been the recognition that the majority of micro-organisms inhabiting the skin are viable, but non-culturable (VBNC).

Many factors affect the microbiology of skin. These include patient age (31), sex (32,33), skin site, level of hygiene and type of cleansers used, climate, occlusion, race, occupation and whether an individual is hospitalised (34). In addition, external and internal temperatures and humidity can significantly affect microbial numbers and composition. For example, bacteria tend to survive for extended periods on wet skin surfaces compared with drier areas (35).

SKIN MICROBIOLOGY OF THE NEWBORN

Vernix caseosa (a white, creamy film) is a skin coating covering newborns and provides the skin with a neutral physiological pH (36). A short time after birth, this vernix disseminates, resulting in a lowering of the skin's pH (ranges of pH 3.0–5.9 have been reported) (37). The acidic environment generated aids in the selection of particular types of colonising micro-organisms.

Vernix caseosa is considered to have multiple protecting and barrier-supporting properties before and after birth, which have been confirmed using synthetic vernix caseosa (38). Natural vernix caseosa possesses multiple and diverse AMPs, and combined with its barrier properties and ability to suppress bacterial adhesion, offers an excellent defence mechanism against infection (39–42).

Coagulase-negative staphylococci (CNS) are frequent isolates from blood cultures of preterm and term neonates, often associated with the use of intravenous catheters (43,44). Following a study by Keyworth *et al.* (43) it was found that the skin's microflora development was the same for babies born by surgical and normal births, with bacteria being found on the skin within 6 h post-natal. In the study, CNS were found in 92% of cases, with bacterial counts increasing rapidly over the first 7 days. Of the CNS, 82% were *S. epidermidis*, and these were isolated from all sites. Other

micro-organisms such as *Propionibacterium* sp, α-haemolytic streptococci, aerobic spore bearing bacilli, aerobic coryneforms, *C. albicans*, *Klebsiella oxytoca*, *Pityrosporum* sp, *Klebsiella pneumoniae* and *E. coli* were also cultured, although none were found to predominate.

A study by Kitajima (45) found that the causative agents of infections in neonates were MRSA (34%), *P. aeruginosa* (9.4%) and *Candida* sp. (3.8%). Sarkany and Gaylarde (46) performed a contact plate method to identify the bacterial flora of 33 newborn and 410 babies over a 6 day period and found that staphylococci and diphtheroid bacilli were mainly cultured from the skin of a newborn. Coliforms were found in 10% of cases and 4.5% had streptococci, with the axilla most heavily colonised. It was evident that the skin of babies born by caesarian section whilst initially sterile, followed the same colonisation pattern as babies born normally.

The skin microbiology of neonates becomes a significant infection problem during hospital stays where colonisation by bacteria from the hospital environment can occur (47). In addition, the transfer of potential pathogens from a mother's skin to that of the neonate may also be a significant source of infection (48).

A study by Cutland *et al*. (49) assessing the value of chlorhexidine wipes to prevent vertical transmission of pathogenic bacteria not only showed that the wipes were inadequate but also that transfer of pathogenic bacteria, for example, β-haemolytic streptococci from mothers during birth may have posed a significant risk of neonatal sepsis.

The effect of routine bathing on environmentally acquired pre-term neonatal skin bacterial populations was examined by da Cunha and Procianoy (50). This study found a predominance of CNS on the skin and that bathing with water, or soap and water was effective in reducing Gram-positive and Gram-negative bacterial colonisation. Specific microbial species identified from axillary cultures included *S. aureus*, *K. pneumoniae*, *Enterobacter* sp., *E. coli*, *K. oxytoca*, *Stenotrophomonas maltophilia*, *Acinetobacter* sp., *Serratia* sp. and *Candida* sp.

In a cross sectional study of 300 pregnant women who attended a hospital antenatal clinic with their newborns in Dar es Salaam, Tanzania, cultures were obtained from swabs from high vaginal, rectal, nasal, ear and umbilical sites (51). Group B streptococcal (GBS) colonisation was confirmed for 23% of the pregnant women and 8.9% of neonates. Prolonged labour (>12 h) was also shown to influence the GBS colonisation rates in neonates ($P < 0.05$). The findings indicated that approximately 10% of newborns from women colonised with GBS were also colonised by these bacteria.

Staphylococcus aureus and less predominant organisms such as *S. epidermidis*, coliforms, *Pseudomonas* sp. and yeast, are known to colonise the skin of babies to no obvious detrimental effect (52). As children get older, micro-organisms such as *Propionibacterium* and the yeast, *Malassezia folliculitis* can be found in abundance and particularly so during and after puberty. These micro-organisms also colonise infant (3–6 months) skin (53), with *Malassezia* also reported as a cause of neonatal sepsis (54).

NORMAL ADULT SKIN MICROFLORA

The levels of bacteria on adult skin have been estimated at between 6×10^2 and 2×10^6 bacteria/cm^2. The micro-organisms identified from adult skin surfaces have included *Staphylococcus*, *Micrococcus*, *Corynebacterium*, *Propionibacterium*, *Malassezi*, *Brevibacterium*, *Acinetobacter* and *Dermabacter*. The frequency of isolation of these organisms is dependent on the culture methods employed, and as mentioned previously, such techniques tend to greatly underestimate the true microbial richness and diversity of the skin. To overcome this problem and to enable identification of VBNC bacteria, modern molecular techniques are being employed to further investigate the microbiology of skin (55,56).

Bacteria frequently isolated from adult skin include CNS, with 50% of these identified as *S. epidermidis* which are particularly abundant from upper regions of hair follicles (57,58). Other CNS isolated included *S. saprophyticus*, *S. hominis*, *S. warneri*, *S. haemolyticus* and *S. capitis*. In addition to CNS, coagulase positive staphylococci, such as *S. aureus* are frequently isolated being particularly prevalent in the anterior nares of humans (59–61). Coryneforms, micrococci, and *Bacillus* spp. have been reported to be the most predominant

Key Points

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- the skin microbiology of neonates becomes a significant infection problem during hospital stays where colonisation by bacteria from the hospital environment can occur
- in addition, the transfer of potential pathogens from a mother's skin to that of the neonate may also be a significant source of infection
- the levels of bacteria on adult skin have been estimated at between 6×10^2 and 2×10^6 bacteria/cm^2

Key Points

- skin can be divided into three distinct regions and these differ in their microbiology
- the regions include moist areas such as the groin, toe web areas, and the armpits (these sites provide highly favourable conditions for bacteria to proliferate), oily areas, that is, the forehead and nose, and dry areas

species isolated from the head, legs and arms (61).

Nagase *et al*. (60) reported the distribution of *Staphylococcus* species on the skin of animals and humans. The research showed that the predominant staphylococci from a variety of animal species were novobiocin-resistant *S. xylosus* and *S. sciuri*. On human skin however, the most frequently isolated staphylococci were novobiocin-sensitive species including *S. epidermidis* (63.8%), followed by *S. warneri* (28.8%) and *S. hominis* (13.8%).

Micrococcus luteus is commonly isolated from human skin, together with the less frequently recovered *M. varians M. lylae*, *M. sedentarius*, *M. roseus*, *M. kristinae* and *M. nishinomiyaensis* (61–63). Aerobic bacteria such as *Propionibacterium*, and in particular *P. acnes,* are also commonly recovered from human skin and these are particularly prevalent at hair follicle sites and in sebaceous glands (64,65).

Gram-negative bacteria isolated from human skin include *Acinetobacter* spp and *Pseudomonas* spp. with the former constituting up to 25% of the adult skin microflora particularly during the warmer months of the year (66–68).

Fungi and yeast are recognised as being significant in skin infections. Frequently isolated yeast include *Malassezia* which are found in 75–80% of healthy adults readily colonising hair follicles (69,70). Seven species of *Malassezia* have been isolated from human skin including *M. furfur, M. sympodialis, M. globosa, M. slooffiae, M. restricta, M. obusta* and *M. pachydermatis.* The prevalence of *Malassezia* species at various body sites in humans does vary with age (71,72). *Malassezia* species have been implicated in numerous diseases including pityriasis versicolor, seborrheic dermatitis, *M. folliculitis* and atopic dermatitis (73).

Arzumanyan *et al*. (74) studied the yeast microflora on the skin of 91 patients with atopic dermatitis, in bronchial secretions of 13 patients with bronchial asthma and 8 patients with allergic bronchopulmonary mycosis. Of the 48 isolates recovered from the skin *Candida* (48%) and *Rhodotorula* (29%) species were most prevalent. In other studies of skin and nail mycology *Trichosporon mucoides*, *Candida guilliermondii*, *C. parapsilosis*, *C. famata* and *M. furfur* were predominant (74).

FACTORS EFFECTING DISTRIBUTION AND ABUNDANCE OF MICRO-ORGANISMS

Skin can be divided into three distinct regions and these differ in their microbiology (75,76). The regions include moist areas such as the groin, toe web areas, and the armpits (these sites provide highly favourable conditions for bacteria to proliferate), oily areas, that is, the forehead and nose, and dry areas (77).

Leyden *et al*. (77) have shown that the skin between the toes and axillae is heavily colonised by coryneforms and bacteria belonging to the *Micrococcaceae* group. This study also found that on the skin around the perineum region, large numbers of *Micrococcaceae* could be isolated compared to the axilla. As would be expected in the perineum region, large numbers of both Gram-positive and Gram-negative rods of faecal origin are encountered (77,78).

At oily areas of skin, relatively low levels of *Micrococcaceae* and coryneform bacteria are isolated, compared with high levels of *Propionibacterium* species (79). In addition, *S. hominis*, *S. epidermidis*, *Malassezia* sp. and coryneforms are also encountered (80). The skin of the scalp contains an abundance of sebaceous glands which enhance the moisture content of the scalp. Further variation in terms of temperature, pH, and a high concentration of eccrine glands affects colonisation and species diversity at these regions.

High numbers of micro-organisms, and in particular yeast, can be recovered from the skin of elderly individuals. The reason for this is possibly due to decreased sweat production and the development of dry skin (81) where staphylococci are found in abundance (67,77,82).

Staphylococcus aureus is frequently carried by sufferers of atopic dermatitis and eczema, and is implicated with common complications of these conditions (83). The antigenic toxins associated with *S. aureus* are also thought to play a role in the exacerbation of the skin disorder (84). Eczematous lesions are thought to be a source of transmission of *S. aureus* (85).

There have been many reports on skin microbiology in relation to the effects of hospitalisation. In general, the majority of these have concluded that a higher proportion of Gram-positive bacteria colonise the skin of hospitalised patients compared with 'normal' healthy individuals (66,86,87).

KEY MICRO-ORGANISMS AND SKIN INFECTION

The indigenous microbiota of healthy adult skin is important in maintaining human health as these organisms can resist colonisation of the skin from invading pathogens, a process known as 'colonisation resistance' (88). In addition, the skin's indigenous microbiota also has the ability to effect reactions derived from the body as well as any xenobiotic agents (89). However, the indigenous microbiota of the skin is also considered a potential source of infection (90), particularly when there is disruption to the skin's normal microbiological balance (91–94).

Complicated skin and skin structure infections (cSSSIs) represent a significant clinical challenge with *S. aureus*, *S. pyogenes* and *Enterobacteriaceae* often being implicated. Treatment concerns are raised further with the increase in meticillin resistance among *S. aureus* (95), with such resistance thought to be acquired via genes acquired from commensal organisms present on the skin (96–98).

CNS are important bacteria in skin and wound infections and major causes of device-related infections, and adept at forming biofilms (99). Treatment is further complicated as CNS often exhibit resistance to an array of different antimicrobial agents (100).

In the human microbiome (the full spectrum of microbial species residing in humans), *Propionibacterium acnes* is ubiquitous, whilst *S. aureus* is present in ~25% of individuals (101). It has also been demonstrated that *P. acnes* enhances the hemolytic activity of *S. aureus*, suggesting that a specific interaction of the bacteria occurs in the human microbiome (102,103).

Staphylococci

Staphylococcus epidermidis

Staphylococcus epidermidis is the most prevalent bacterium of skin, representing over 90% of the aerobic resident microflora and is therefore often deemed a skin contaminant when isolated during infection (104). Considered to be a normal commensal of skin, *S. epidermidis* is thought to have evolved mechanisms to help maintain a benign relationship with its host (104). Interestingly, AMPs produced by *S. epidermidis* have recently been identified on the surface of skin and these peptides are considered to be significant in preventing the growth and proliferation of potentially pathogenic micro-organisms, that is, colonisation resistance (105).

It has further been suggested that whilst *S. epidermidis* is vital in maintaining the balance of the skin microflora, it also is a source of antibiotic resistance genes, as well as being responsible for a number of nosocomial infections (104). Whilst this species is historically considered innocuous or, rarely opportunistic, its role with other CNS in human infection is now increasingly being appreciated. In respect of dissemination of resistance genes, *S. epidermidis* has been implicated in promoting the development of MRSA as mentioned previously (106–108). Consequently, *S. epidermidis* should not merely be regarded as a contaminant of infections and appropriate medical treatment and preventive guidelines should be applied when this species is isolated (109).

Staphylococcus epidermidis is adept at forming biofilms and the clinical importance of this is evident from the studies of Hajdu *et al*. (110) who investigated the effects of vancomycin, daptomycin, fosfomycin, tigecycline and ceftriaxone on *S. epidermidis* biofilms. From this study, biofilm eradication required additional measures such as debridement, in conjunction with antibiotics.

Staphylococcus aureus

Staphylococcus aureus is a 'transient' coloniser of the skin, with 35–60% of the human population intermittently carrying this organism (111). *Staphylococcus aureus* is also reported to be a normal constituent of the nasal flora (112,113). Certain patient groups such as diabetics, intravenous drug users and immunocompromised individuals, tend to have higher carriage rates. Risk factors for colonisation include hospitalisation or residence in long-term care facilities, as well as close contact within groups such as in playgrounds, sports teams and prisons (114–119).

Staphylococcus aureus is a causative agent of minor or self-limited skin infections including impetigo, folliculitis, furuncles, subcutaneous abscesses and scalded skin syndrome (120,121). However, the bacterium is also implicated with high mortality rates following incidences of bacteraemia, toxic shock syndrome, pneumonia, osteomylitis and endocarditis (119,122–124). The most common sites

Key Points

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of *S. aureus* infection are the skin and soft tissue, with over 75% of these due to MRSA (125).

Staphylococcus aureus possesses a wide range of virulence factors (e.g. enterotoxins and cytotoxins) that allow it to colonise and overcome the host resulting in major illnesses. Panton-Valentine Leucocidin (PVL) is a cytotoxin that lyses lymphocytes and has been implicated in the development of furunculosus and the high mortality necrotising pneumonia (119,126,127). MRSA causes epidemic outbreaks of community-associated (CA) skin infections, with the strains involved frequently containing the genes for the PVL toxin (128,129). PVL-positive *S. aureus* are also associated with follicular skin infections (130). MRSA skin and soft-tissue infections have been reported to account for more than 50% of soft-tissue infections in the United States (131,132) including myositis, pyomyositis, and necrotising fasciitis (133).

Indeed, skin and soft tissue infections make up most of the cases of MRSA infections (134) although community-associated MRSA infections may cause more severe necrotising pneumonia (135) and bacteremia (136). Importantly, skin can be the primary site of colonisation through which *S. aureus* enters a human host.

Whilst producing a number of virulence factors, *S. aureus* is also a prolific biofilm former, facilitated through the expression of intracellular adhesion molecules (coded by the *ica* operon). The expression of the *ica* operon has been implicated in the failure of implanted medical devices, for example, hip and dental implants (137–142) and subsequent infection. Therefore, colonisation of the skin by *S. aureus* represents a significant threat to both health and morbidity.

Staphylococcus lugdunensis

Staphylococcus lugdunensis was first described in 1988 and is an infrequent pathogen when compared with *S. aureus* and *S. epidermidis* (143). However, a number of *S. lugdunensis* infections, including those of the skin, resemble those caused by *S. aureus* (144). *Staphylococcus lugdunensis* is associated with a range of clinical conditions including abscesses and wound infections, urinary tract infection, and infection of intravascular catheters as well as other implanted medical devices. Such infections are largely because of the ability of *S. lugdunensis* to bind to, and interact with host cells to form biofilms (145). *Staphylococcus lugdunensis* can exhibit an elevated degree of virulence when compared with other CNS. Unlike other CNS, *S. lugdunensis* has the propensity to cause native valve endocarditis, mimicking many of the characteristics of *S. aureus* (146).

A recent study by Tena *et al*. (147) investigated the clinical and microbiological characteristics of 20 cases of skin and soft tissue infections (SSTIs) due to *S. lugdunensis*. Abscesses (seven cases), surgical wound infections (six cases) and cellulitis (three cases) were the most common clinical conditions associated with this organism and the authors concluded that *S. lugdunensis* should be considered a potential pathogen when isolated from SSTIs, especially in patients with skin diseases or after trauma or surgery (148). Presently this species is underrated by many laboratories and there is an opinion that *S. lugdunensis* should now be accepted as a significant pathogen in SSTI and examined for and fully identified in all routine bacteriological examinations (78,148–150).

Biofilms play a role in the pathogenesis of many *S. lugdunensis* infections, but studies are limited. As biofilm formation perturbs the efficacy of antimicrobial agents, this is an important consideration in determining the clinical course of treatment (151).

β-haemolytic Streptococcus

The type of haemolytic reaction observed on blood agar is often used as a method to classify *Streptococcus* species. β-haemolysis is defined as the complete lysis of red blood cells around colonies, whilst α-haemolysis is seen as partial haemolysis. The term γ-haemolytic is used to describe non haemolytic streptococci. Most group A streptococci (GAS) are β-haemolytic, and perhaps best represented on the skin by *Streptococcus pyogenes*. The majority of infections caused by these bacteria occur in elderly individuals, often with an underlying medical condition such as diabetes or immunodeficiency.

β-haemolytic streptococci are significant pathogens, which whilst being resident in the normal microflora of the skin, can cause bacteraemia, necrotising fasciitis and other skin conditions (152–155). Many studies show that group A β-haemolytic streptococci are

frequently associated with streptococcal pyoderma. Children colonised with these bacteria are thought to be at greater risk of developing impetigo lesions (154). Burn wounds colonised and infected with streptococci often show impaired healing and progressive wound depth (156). A study on burn victims showed that 1.1% harboured β-haemolytic streptococci and a third of these were associated with GAS, 43% group D streptococci and 21% group C streptococci (156).

Corynebacterium sp

Corynebacteria are a group of bacteria known to colonise the skin of many animals including humans. In the case of humans, *Corynebacterium jeikeium* is the most prevalent species and often regarded to be part of the normal skin flora (157). *Corynebacterium jeikeium* is abundantly found on the skin of hospitalised patients (158) and in such individuals is considered an important opportunistic pathogen (158,159). Treatment of infections caused by this species can be problematic because of its ability to resist many antibiotics.

Propionibacterium sp

Propionibacteria are prevalent skin colonising bacteria with *P. acnes* accounting for approximately half of the total skin microbiome with an estimated density of 10^2–10^6 colony forming units/cm^2 (160–162). Although *P. acnes* has been found to predominate on facial skin (163), it can be found almost everywhere on the body (164,165).

Acne vulgaris is perhaps the most well known skin condition caused by *P. acnes*, affecting up to 80% of adolescents (166). Acne is a chronic genetic disease of the sebaceous follicles. *Propionibacterium acnes* metabolises free fatty acids within the sebaceous gland and this can lead to both the initiation, as well as the promotion of inflammation during acne episodes. *Propionibacterium acnes* has also been associated with foreign device infections and should not be dismissed as merely a contaminant (167).

There is growing evidence that *P. acnes* and *S. aureus* coexist in many human diseases, including acne lesions (168), implant infections (169,170) and sepsis (171). It has also been suggested that *P. acnes* residing within the hair follicles of the skin grows in the form of a biofilm (172).

Acinetobacter sp

Acinetobacter baumannii is associated with a wide range of diseases ranging from nosocomial, community-acquired infections to those obtained during war, especially in wounded military personnel. *Acinetobacter* are generally regarded as non-pathogenic, however, where host immunity is compromised, infections can occur and indeed be life-threatening.

Acinetobacter baumannii is an aerobic, Gram-negative coccobacillus and a known cause of skin infections particularly those of wounds and burns. In such patients, the organism is also associated with endocarditis, septicaemia and respiratory tract infection (in particular ventilator-associated pneumonia) and meningitis (173). *Acinetobacter baumannii* is a significant cause of health care associated infection as it can cross infect between human reservoirs (174) and survive locally in the environment (175,176). In addition, *A. baumannii* can be resistant to multiple antimicrobial agents, including carbapenems, and in such circumstances colistin and tigicycline are often the only treatment options (177). The Health Protection Agency (HPA) working party (178) defined multi-resistant *Acinetobacter* spp (MRAB) as *Acinetobacter* spp isolates that are resistant to any aminoglycoside (e.g. gentamicin) and to any third generation cephalosporin (e.g. ceftazidime, cefotaxime). There have now been several outbreaks of MRAB in ICUs across the world (179–182).

The significance of *A.baumannii* infections has grown and as mentioned above, particularly so in military personnel with war wounds. Treatment of these wounds has become more difficult, not just because this bacterium can exhibit extensive antimicrobial resistance (183) but also because they readily form biofilms, which in turn resist host defenses and antimicrobial intervention (184). Consequently, these bacteria have an effect on non-healing in wounds, particularly those in burns patients (184).

Pseudomonas sp

Pseudomonas aeruginosa is a Gram-negative opportunistic pathogen that frequently persists in an innocuous state on human skin. As this species has a high affinity for water, it tends to thrive on moist surfaces. *Pseudomonas aeruginosa* has been implicated in cases of cross infection in medical settings and has

Key Points

- whilst the skin of a foetus is historically reported to be microbiologically sterile, recent findings have shown that prior to birth, babies in the womb may be exposed to micro-organisms in an environment that was once considered to be sterile
- Romero *et al*. established that within amniotic fluid, bacteria can be embedded within an amorphous biofilm
- biofilms protect micro-organisms from outside perturbations, allowing for microbial communication, enhanced virulence and breakdown of nutrients aiding microbial succession and development

been found on catheters and other medical devices which allow their entry from colonised skin surfaces into the body (185). *Pseudomonas aeruginosa* has the capacity to infect a wide range of tissues and its infections are primarily associated with compromised patients (186).

Pseudomonas aeruginosa causes mild episodes of dermatitis, which often manifest in community settings where dissemination via contaminated water occurs, and the communal sharing of hot tubs is a notorious factor for this (187–189). Infections in immunocompromised patients are generally more serious, with respiratory infection particularly evident in patients with cystic fibrosis or those who are mechanically ventilated (190). *Pseudomonas* species are very good at forming biofilms and treatment is complicated further by their ability to rapidly acquire antibacterial resistance (191).

BIOFILMS AND SKIN

It was following birth, that initial contact with micro-organisms was originally deemed to occur. However, recent studies have shown this may now not be the case. The skin of a foetus is reported to be microbiologically sterile. Recent findings have however shown that prior to birth, babies in the womb may be exposed to micro-organisms (192). Romero *et al*. (192) established that within amniotic fluid, bacteria can be embedded within an amorphous biofilm.

A biofilm is best described as a microbially derived, sessile community characterised by cells attached to a substratum, interface or to each other, and are embedded in a matrix of extracellular polymeric substances (EPS) that they have produced. Biofilm cells exhibit an altered phenotype with respect to growth rate and gene transcription when compared with their free living counterparts (193).

Biofilms protect micro-organisms from outside perturbations, allowing for microbial communication, enhanced virulence and breakdown of nutrients aiding microbial succession and development. Exposure to a biofilm prior to birth may aid in 'conditioning' of the skin surface and enhance the microbial colonisation by 'beneficial' bacteria, which are themselves protective to the host.

Biofilm formation by the skin's own indigenous microbiota can also be significant in the prevention of skin infection. However, the ability of bacteria to form biofilms also has significance to infections elsewhere in the body. The protection afforded by the indigenous microbiota, as mentioned previously, is referred to as colonisation resistance and is a significant skin defensive mechanism in preventing exogenous bacteria and fungi from attaching to the skin surface.

The first reported evidence of skin biofilms followed work by Mowad *et al*. (194), where CNS (*S. epidermidis*) were shown to produce EPS. Other studies have reported on the ability of *S. epidermidis* to form biofilms (195). A study by Suzuki *et al*. compared the prevalence of biofilm-forming strains of *S. epidermidis* in the conjunctival and facial skin microflora (196). The research examined the biofilm-forming ability of 10 *S. epidermidis* strains from the conjunctival sac of healthy volunteers and 40 strains obtained from the facial skin of healthy volunteers. Additionally, the ability of 36 *S. epidermidis* strains from the conjunctival sac of pre-cataract patients to form biofilms was investigated. The authors concluded that the biofilm-forming ability of *S. epidermidis* isolates from the conjunctival sac was higher than isolates from the facial skin.

Schierle *et al*. (197) presented a novel murine cutaneous wound system that directly demonstrated delayed reepithelialisation caused by the presence of a *S. aureus* or *S. epidermidis* biofilm.

In a recent study by Frank *et al*. (198), planktonic minimum inhibitory concentrations (MICs) and minimum biofilm eliminating concentrations (MBECs) of 10 anti-staphylococcal antimicrobial agents were measured for 15 *S. lugdunensis* isolates collected from patients with endocarditis, medical device infections, or skin and soft tissue infections. Planktonic isolates were susceptible to all agents studied, but biofilms were resistant to high concentrations of most of the drugs. MBEC testing showed that vancomycin was not bactericidal against 93% of *S. lugdunensis* isolates, suggesting widespread vancomycin tolerance in this species. These data provide insight into the response of *S. lugdunensis* isolates when challenged with various levels of antimicrobial agents in clinical use (198).

Other skin microbes, such as *P. acnes* are avid biofilm formers and play a significant role in the pathogenesis of acne vulgaris when present in hair follicles (172,199). *Propionibacterium acnes* has been shown to readily form biofilms and therefore is a concern when associated with wound infections (200,201). Research from these studies indicates that biofilm formation should be considered in the diagnosis and treatment of invasive *P. acnes* infections (172,201). *Propionibacterium acnes* also forms biofilms on medical devices and this has major implications for delayed joint prosthesis infection (201). In addition, *in vitro* and *in vivo* biofilm formation has been demonstrated for other micro-organisms involved in skin diseases. For example, *S. aureus*, *S. pyogenes* and *C. jeikeium* isolated from human skin have all been reported to form biofilms (172,202,203).

Biofilms and wound healing

Over the years there have been many studies describing the microbiology of wounds and the prevalence of selected phenotypes. These historical studies have given credence to the microbial etiology and the potential consequences of microbial presence in a chronic wound. Interpretation of the data has, however, been problematic, particularly because of the problems previously highlighted with culture techniques, and more recently through the recognition of biofilms and their potential impact.

Biofilms are important in a number of mucosal chronic infections including those of the urinary tract, periodontium, respiratory tract and chronic wound (198,204). Studies have shown that in *P. aeruginosa* chronic wound biofilms, bacteria are aggregated in an extracellular matrix in the form of microcolonies with few planktonic micro-organisms evident (205). Using scanning electron microscopy, James *et al*. (206) showed that the majority of chronic wounds (60%) had a biofilm presence, compared with only 6% of acute wounds.

A study by Scheirle *et al*. (197) showed that *S. aureus* and *S. epidermidis* biofilms formed in a murine wound model caused disruption of normal re-epithelialisation, whilst Wolcott *et al*. (207) proposed that bacterial biofilms in chronic wounds 'hijack' the host response to enable the production of nutrients for the bacteria to survive, and at the same time secreted factors which dampened the host response to evade destruction.

The role of molecular analysis in analysing microbial composition

Approximately 1–2% of all known bacteria can be cultured in the laboratory (208). Given the growing recognition of VBNC bacteria and the emergence of molecular methods available to our diagnostic laboratories, non-culture techniques are increasingly being used in studies of chronic wounds (209). Molecular methods have incorporated 16S ribosomal DNA-PCR together with denaturing gradient gel electrophoresis (DGGE). DGGE provides detail on the diversity of a microbial community without the limitations associated with bacterial culture. DGGE limits are that it assumes nucleic extraction efficiency and subsequent PCR is equivalent for all members of the population and that each band resolved represents a different species. Furthermore, the procedure does not distinguish between viable and non-viable micro-organisms and is not quantitative. Nevertheless, DGGE does provide a valuable alternative to establish the diversity and complexity of a microbial including that associated with biofilms (210). Many studies have highlighted the value of 16S RNA analysis in elucidating community composition (211). Davies *et al*. (212) used 16S ribosomal DNA-PCR and DGGE to analyse the microflora of healing and non-healing chronic venous leg ulcers. The work highlighted the complexity of the microbial community as well as the limitations of culture methodology (211,212). Similarly, Dowd *et al*. (213) also described the complexity of the wound bed microflora and the lack of correlation between culture and non-culture techniques. A number of disparities between non-culture and traditional methods in analysing microbial populations in wounds exist (214,215).

Relationship between microbial colonisation and delayed wound healing

Chronic wounds, by definition are wounds that have a biological or physiological reason for not healing and have been reported to comprise 60–80% of all human infectious diseases (216). The relationship between the wound microflora and delayed healing remains an ongoing debate and is still poorly understood.

Key Points

- given the growing recognition of VBNC bacteria and the emergence of molecular methods available to our diagnostic laboratories, non-culture techniques are increasingly being used in studies of chronic wounds
- molecular methods have incorporated 16S ribosomal DNA-PCR together with denaturing gradient gel electrophoresis (DGGE)

The extracellular adherence protein of *S. aureus* delays wound closure by its potent anti-angiogenic and anti-inflammatory properties mediated by the inhibition of leucocytes (217).

A recent study by Gontcharova *et al*. (218) compared the bacteriology of wounds and associated intact skin using Tag-encoded FLX amplicon pyrosequencing (bTEFAP) to identify bacterial species and showed a significantly more diverse bacteriology of intact skin compared with wounds. Higher levels of anaerobic bacteria, including *Peptoniphilus*, *Finegoldia* and *Anaerococcus* species were evident in wounds, whilst opportunistic wound pathogens were in lower levels in intact skin. In a later study using only traditional culturable techniques, Westgate *et al*. (219) investigated the presence of bacterial biofilms within equine wounds. Fifty-one wounds and control skin sites were sampled and the biofilm forming potential of all isolated bacteria determined. Stained tissue samples provided evidence of biofilms in 61.5% (8 out of 13) of equine wounds. In total, 340 bacterial isolates were identified from all the equine wounds and skin samples. *Pseudomonas aeruginosa* and *Enterococcus faecium* were the most frequently isolated bacterial species from equine wound and skin samples respectively. *Staphylococcus* was the most commonly isolated genus in both environments.

It is becoming increasingly accepted that one of the major barriers to wound healing is the presence of a polymicrobial biofilm (220,221). Bacteria found within biofilms exist within a complex community of both readily culturable and viable but not culturable states. Within the biofilm the bacterial communities are highly tolerant to many antimicrobial interventions necessitating the need for anti-biofilm strategies for the management of the wound (221–225).

The diversity of healthy skin is considerable. The theory is that the more abundant the microflora of intact skin is, then the greater the protection from the spread of infection or accumulation of both opportunistic and strict pathogenic populations. A number of studies have highlighted that the properties of the skins microflora of intact skin provides significant advantages (226,227).

In the study by Gontcharova *et al*. (218) it was found that the genera *Corynebacterium*, *Streptococcus* and *Anaerococcus* were found in abundance in chronic wounds and intact skin. However, the study found that in wounded skin, levels were higher and the results of the study indicated that *Corynebacterium* was a significant opportunistic contributor to chronic wounds. Also within this study, *Streptococcus* spp was only found in 17 of 29 intact skin samples, at an average of only 1.54% compared to ~20% in wounds. The authors proposed that this is evidence for elevated levels of certain bacteria potentially contributing to a wound biofilm, bioburden and infection.

Corynebacterium, are important opportunistic human pathogens (228). The genus *Corynebacterium* is known to harbour an array of different species including *C. jeikeium*, which is a lipophilic and multidrug resistant bacterium of the human skin flora (229). Anaerobes are now recognised as a major population in chronic wound biofilms (230–232). The most commonly encountered genera have included *Finegoldia* and *Peptoniphilus* together with other anaerobes (231–234). This group of bacteria is known to be able to survive the detrimental effects of oxygen by co-existing or co-aggregating with aerobic bacteria (235,236).

It has been reported that deep within a biofilm, oxygen diffusion is limited so that these areas allow for proliferation and protection of anaerobes (237).

Intact skin is known to act as a barrier to *E. coli* with this species reported to be unable to survive and colonise on skin (238). However, within wounds that do not possess a functional skin barrier, many bacteria, including *E. coli* are able to colonise and grow in and around the wound.

From the studies of Gontcharova *et al*. (218) and Westgate *et al*. (219) it was found that the microbiology of intact skin and wound samples was very similar. Thus it is probable that the chronic wound environment promotes propagation and accumulation of key opportunistic pathogenic populations which lead to a delay in wound healing and heightened risk of infection.

CONCLUSIONS

The complex ecosystem that comprises the skin microflora is multifaceted, but to date, the ecological studies that have been undertaken in different regions of skin have relied solely on culture techniques that are unable

to accurately indicate either the numbers or the diversity of aerobic or anaerobic bacteria. Clinical science has established that resident microbial communities on adult and infant skin have a major role to play in human health and the AMPs produced on the skin surface are important for maintaining the ecological stability of the skin's microbiota and therefore its defence (239). Despite a plethora of scientific and clinical dermatology research, a poor understanding of the biology of the cutaneous microflora remains (240). Hence, limited comprehension of skin microbiology and the related implications for health and wound infections continues. If patient care in respect of prevention and management of skin infections is to advance, a deeper understanding of skin microbiology and associated host factors is required as these impinge on bacterial community (biofilm) interactions and therefore will affect the 'microbiological-host balance'. This includes developing informed insight in respect of what precisely constitutes a skin infection, and when and how to treat.

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Key Points

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- clinical science has established that resident microbial communities on adult and infant skin have a major role to play in human health and the AMPs produced on the skin surface are important for maintaining the ecological stability of the skin's microbiota and therefore its defence
- if patient care in respect of prevention and management of skin infections is to advance, a deeper understanding of skin microbiology and associated host factors is required as these impinge on bacterial community (biofilm) interactions and therefore will affect the 'microbiological-host balance'
- this includes developing informed insight in respect of what precisely constitutes a skin infection, and when and how to treat

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