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Staphylococcus colonization of the skin and antimicrobial peptides

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Abstract

Staphylococci are the most abundant skin-colonizing bacteria and the most important causes of nosocomial infections and community-associated skin infections. Molecular determinants of staphylococcal skin colonization include surface polymers and proteins that promote adhesion and aggregation, and a wide variety of mechanisms to evade acquired and innate host defenses. Antimicrobial peptides (AMPs) likely play a central role in providing immunity to bacterial colonization on human epithelia. Recent research has shown that staphylococci have a broad arsenal to combat AMP activity, and can regulate expression of AMP-resistance mechanisms depending on the presence of AMPs. While direct *in vivo* evidence is still lacking, this suggests that the interplay between AMPs and AMP resistance mechanisms during evolution had a crucial role in rendering staphylococci efficient colonizers of human skin.

Keywords

antimicrobial peptides; colonization; innate host defense; *Staphylococcus aureus*; *Staphylococcus epidermidis*

Distribution & frequency of staphylococcal colonization on the human skin

Members of the genus *Staphylococcus* are common colonizers of the skin in mammals and birds [1]. Two main groups are distinguished by their ability to coagulate blood: coagulase-positive staphylococci, with the most important species being *Staphylococcus aureus*, and coagulase-negative staphylococci, which comprise most species including *Staphylococcus epidermidis*. Humans are colonized by many different staphylococcal species. Some, such as *S. epidermidis* or *Staphylococcus hominis*, are found on virtually all body parts. Some others have more distinct preferences for certain parts of the human body, such as *Staphylococcus auricularis*, which is found mostly in the ear canal [2]. In general, the largest densities of staphylococci are found in sweat glands and on mucous membranes surrounding body openings.

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Most reports on staphylococcal colonization have *S. aureus* as a subject, owing to its eminent role in human infection. The nose is the most important site of *S. aureus* colonization [3], but *S. aureus* is also found in the pharynx, perineum, axillae and on the skin (predominantly on the hands, chest and abdomen) [4–6]. Persistent colonization with *S. aureus* is observed in approximately 20% of the population, while 30% carry *S. aureus* transiently, and approximately 50% are noncarriers [7,8]. In persistent *S. aureus* carriers, who all have *S. aureus* in their noses, the frequency of colonization of other body sites is increased compared with the general population [9]. Persistent carriage rates are higher in children than adults [4]. Interestingly, there has been a drop in persistent carriage rates over time, which is likely due to improved personal hygiene [9].

S. epidermidis is the staphylococcal species that is most frequently isolated from the human skin [10]. It predominantly colonizes the nose, axillae and the head [10]. Other frequent human skin colonizers include *S. hominis* and *Staphylococcus capitis*, the latter found mostly on the head and more frequently during puberty [11]. Less frequent colonizers of the human skin include *Staphylococcus haemolyticus* and *Staphylococcus warneri*. In addition, humans may be transiently colonized by species that normally live on pet or farm animals, such as *Staphylococcus sciuri* or Staphylococcus intermedius [12–14].

Colonization & disease

Whereas almost all staphylococcal species have been reported as causes of opportunistic infections [15], some species stand out as more frequent and serious pathogens. Most notably, *S. aureus* is a dangerous human pathogen that can cause severe and life-threatening diseases, such as severe sepsis, pneumonia, toxic shock syndrome and endocarditis [16]. Other staphylococcal species tend to cause subacute and chronic rather than fulminant infections [15], with *Staphylococcus lugdunensis* being somewhat more aggressive than other coagulase-negative staphylococci [17,18]. In addition, *S. aureus* and *S. epidermidis* are the most frequent causes of nosocomial infections on indwelling devices [18–20]. Several other coagulase-negative staphylococci, such as *S. haemolyticus*, *Staphylococcus simulans* and *S. warneri*, may also cause device-related and other, usually subacute, infections, but are often not further distinguished in the clinical microbiology laboratory [15]. Finally, *Staphylococcus saprophyticus* is the second most important cause of urinary tract infections [21].

Antibiotic resistance is frequent in many staphylococci and significantly complicates and increases the cost of treatment [22,23]. *S. aureus* strains resistant to the antibiotic methicillin (methicil-lin-resistant *S. aureus* [MRSA]) are now common in hospitals [24], and more recently are also spreading in a pandemic fashion in the community (community-associated MRSA [CA-MRSA]) [25]. Remarkably, MRSA has been estimated to cause more deaths annually in the USA than HIV/AIDS [26]. Methicillin resistance is frequent also in *S. epidermidis* [27], and may originally have been transferred to *S. aureus* from this species [28]. This indicates that coagulase-negative staphylococci have an indirect importance for the pathogenesis of *S. aureus* as a reservoir of resistance genes that adds to their own pathogenic potential.

Molecular factors that determine staphylococcal pathogenesis have been extensively investigated. Aggressive virulence determinants such as toxins are mostly found in *S. aureus* [29], while other species mostly lack the production of toxins, in accordance with their much more limited aggressiveness. The reader is referred to other reviews that focus on the molecular basis of virulence in staphylococci [15,30,31]. Interestingly, in *S. epidermidis* as the most intensively studied species other than *S. aureus*, for the most part, factors that have been implicated in pathogenesis appear to have original functions in the noninfectious lifestyle of this bacterium. This indicates that these less aggressive species have not evolved to become pathogenic, but infection has to be regarded as an ‘accident’ rather than a program [31].

The accidental nature of infection with species such as *S. epidermidis* suggests that the frequency of infections is to a large part determined by the abundance of these species on the human body, from where infection is believed to originate. The most important sources of infection with *S. epidermidis* and many other staphylococci are likely the skin and mucous membranes of patients or healthcare personnel [31]. In the case of *S. aureus*, infection from the nose likely plays the most important role [32]. While in the case of community-associated skin infections a direct infection from an existing abscess on another person's skin is possible, colonization is commonly seen as a prerequisite for most staphylococcal infections. Accordingly, in *S. aureus* carriers, infection rates are higher than in noncarriers [33,34], and patients are usually infected by the same strains with which they are colonized [32]. This underlines the immense importance of studying colonization to understand the sources of staphylococcal disease.

Molecular factors involved in colonization

Both bacterial and host factors are believed to play a role in colonization. Host factors, including host defense systems, will be discussed in detail later. Among the bacterial determinants, one can distinguish between those facilitating adhesion to host surfaces and those involved in physiological and metabolic adaptations to the host environment, which also includes evasion of the host immune defense.

Adhesion to host tissue is achieved by a large family of staphylococcal surface proteins that bind with varying degrees of specificity to host matrix proteins, such as fibronectin, fibrinogen, vitronectin, laminin and von Willebrand factor. Members of this family are called microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) [35,36]. They are typically composed of an extended part that spans the staphylococcal cell wall, and an exposed part that interacts with the host protein. MSCRAMMs may be covalently or noncovalently bound to the staphylococcal cell wall. Covalent linkage is catalyzed by the sortase enzyme family, which recognize a conserved motif near the C-terminal end of the surface protein and link it to the peptide bridge of peptidoglycan [37,38]. There is significant functional redundancy among staphylococcal MSCRAMMs, most likely to ensure that this critical step in tissue colonization is accomplished reliably and successfully.

Larger bacterial agglomerations may develop using extracellular matrix components that link staphylococcal cells together in a biofilm-like structure. These include sugar-based polymers such as teichoic acids and the exopolysaccharide poly-*N*-acetylglucosamine (PNAG) (or polysaccharide intercellular adhesin), as well as secreted proteins [39]. For the sugar-based polymers, electrostatic interaction is believed to play a major role for cell–cell aggregation. Teichoic acids and other surface polymers have a negative charge [40], while PNAG is a cationic polymer [41] in which positive charges are introduced by a dedicated surface-located *N*-acetylglucosamine deacetylase [42]. This reaction and the resulting positive net charge of the PNAG molecule are crucial for aggregation, which likely occurs by interaction with the anionic polymers. Proteins involved in aggregation include the accumulation-associated protein Aap of *S. epidermidis* [43], which needs zinc ions [44] and proteolytic processing [45] for aggregation activity. Aap has been reported to form large fibrils on the bacterial surface [46]. In addition, recent research has indicated additional functions in cell–cell adhesion for several MSCRAMMs of *S. aureus*, such as protein A [47,48] or fibrinogen-binding proteins [49]. While one can assume that aggregation factors in general would be advantageous for colonization, there is also evidence to the contrary. Namely, it has been shown that absence of the *ica* genes coding for PNAG biosynthesis favors persistence of *S. epidermidis* on the arms of human subjects when applied together with the corresponding isogenic wild-type strains harboring these genes [50]. This indicates that results from bio-film research and *in vitro* aggregation assays may not be generally predictive for skin colonization.

Colonization of the skin requires resistance to environmental influences that change much more dramatically than in more protected parts of the human body. Sweating and drying of the skin mean considerable changes in osmolarity, salt concentration and pH value, in addition to mechanical stress. Among the nonhalophilic bacteria, the staphylococci are distinguished by an exceptionally high capacity to withstand these influences, particularly high concentrations of salt. *S. aureus*, for example, can survive up to 3.5 M NaCl [7]. Resistance to changing osmolarity is mediated by the accumulation of osmoprotectants, such as choline or glycine betaine, in the bacterial cells, for which staphylococci have a series of osmoprotectant transport systems and enzymatic conversion systems [51,52]. The increased frequency of *S. epidermidis* and some other coagulase-negative staphylococci as colonizers on undamaged human skin, compared with *S. aureus*, may be due to the increased presence or expression of such protective systems. Alternatively, direct bacterial interference between those species may play a role, as discussed below.

Bacterial interference

Before turning to the interaction of staphylococci with the human host, it should be discussed whether bacterial interference – either between staphylococcal strains or between staphylococci and other bacteria – determines colonization independently of host contribution.

Bacteriocins, secreted bacterial products that kill other microorganisms, have frequently been proposed to enhance survival of the producer strains in a competitive fashion [53,54]. Many staphylococci produce peptide bacteriocins [54]. Often, these belong to the lantibiotic class that is characterized by lanthionine bridges, which render the peptide extremely resistant to proteolytic degradation [55]. Some strains of *S. epidermidis* produce lantibiotics, such as epidermin, epilancins K7 and 15X, and Pep5, which have high bactericidal potency against many Gram-positive bacteria [56–60]. Other lantibiotics have been described in *S. warneri* [61,62], *Staphylococcus gallinarum* [63] and *S. aureus* [64]. Commonly, genes encoding bacteriocins are coupled to genes that provide producer protection, thus giving an at least hypothetical advantage over other bacteria that are susceptible to that substance [65]. Producer immunity to lantibiotics may be accomplished, for example, by highly specific export systems [66–68].

However, there is no direct evidence to suggest that bacteriocins in staphylococci contribute significantly to competitive fitness. Additionally, the fact that bacteriocin production is limited to a small subset of staphylococcal strains strongly argues against a general role in bacterial interference, as these strains do not seem to have spread more widely. Interestingly, we know from the numerous available *S. aureus* genome sequences that many *S. aureus* strains have genes that encode the complete bio-synthetic gene cluster needed to produce an epidermin-like lantibiotic [51,69,70]. Notably, this includes the clonal complex 8 with the pandemic CA-MRSA strain USA300. However, lantibiotic production has never been described in any of these strains. Thus, the biological role of these genes is unknown.

Phenol-soluble modulins (PSMs) and similar peptides are short amphipathic and α-helical peptides that are genome-encoded and produced by a majority of *S. aureus*, *S. epidermidis* and likely many other staphylococcal strains [71–73]. Some *S. aureus* PSMs have strong cytolytic capacity toward human neutrophils and other cell types [73]. Some PSM and PSM-like peptides, such as the well-characterized δ-toxin of *S. aureus* or a PSM-like gonococcal growth inhibitor from *S. haemolyticus*, have been reported to exhibit bactericidal activity [74–77]. However, activity was mostly limited to very specific target bacteria or occurred only in synthetic derivatives. In general, bactericidal activity is rare among PSMs. Furthermore, PSMs lack the cationic character that is typical of antimicrobial peptides (AMPs). This generally

prohibits activity against bacteria, suggesting that PSMs have evolved to harm eukaryotic rather than prokaryotic cells [78].

Quorum-sensing is a gene regulatory process that leads to changes in gene expression in response to bacterial cell density [79]. It requires the secretion and sensing of a signal [80], which in staphylococci is a post-translationally modified peptide [81–83]. Quorum-sensing occurs in almost all bacteria, but the staphylococcal accessory gene regulator (*agr*) quorum-sensing system is quite unique inasmuch as different subgroups exist whose signals can be cross-inhibitory [84,85]. While frequently proposed to play a role in bacterial interference, there is no experimental evidence to suggest that cross-inhibition by *agr* affects competitive colonization *in vivo* [86].

Interaction with host defenses during colonization

The human immune system comprises two major parts. The acquired or adaptive immune system is antigen- and thus pathogen-specific, and requires antigen presentation. It allows for immunological memory and a strong immune response, but only reacts slowly after pathogen invasion. A main part of the adaptive immune response are antibodies (or immunoglobulins) that are produced by B cells and mark the pathogen for destruction. The major immunoglobulin subtype secreted on the mucosae is IgA.

Owing to the fact that the human body is in constant contact with staphylococci, the role of adaptive immunity in controlling staphylococcal colonization and infection is complex and poorly understood. We know that humans have circulating antibodies against many staphylococcal proteins [87], but there is no general protective immunity from staphylococcal infection. However, there is evidence to suggest that the adaptive immune system impacts staphylococcal infection and colonization. First, the antibody repertoire [88,89] and the outcome of bacterial infection in *S. aureus* carriers are different from those in noncarriers [33,34]. While the risk for infection in *S. aureus* noncarriers is lower [34], carriers have a lower risk for serious complications, such as death from bacteremia [90]. This indicates an involvement of acquired immunity. Second, *S. aureus* has multiple mechanisms to evade human acquired immune defenses. For example, SSL7, which is a member of the staphylococcal superantigen-like proteins and interacts with IgA molecules [91], may play a key role in *S. aureus* evasion of antibody-based defenses on the skin and mucosal surfaces. Protein A, which is well known from laboratory research due to its capacity to nonspecifically bind IgG, produces a camou-flage coat composed of nonspecific antibodies on the *S. aureus* surface, thus preventing binding of specific antibodies [92].

The evolutionarily older innate immune system reacts fast by recognizing invariant parts on invading microorganisms, which triggers elimination by professional phagocytes such as neutrophils and macrophages. Among these, neutrophils are the first to arrive at the site of an infection, and play the most important role in eliminating invading bacteria. After ingestion (phagocytosis), bacteria are killed in the neutrophil phagosome by reactive oxygen species and AMPs and proteins [93]. However, there are no phagocytes on the skin, where the innate immune system is mainly comprised of secreted AMPs. Evasion of AMP activity will be described below in detail. For a more comprehensive portrayal of staphylococcal immune evasion mechanisms, the reader is referred to other review articles [94,95].

AMPs on the human skin

Antimicrobial peptides are part of the innate immune system in many organisms from almost all phyla, and developed early in evolution [96–98]. In lower organisms, they may constitute the only or major part of host defense. Many AMPs have activity against a wide range of

pathogens including bacteria, fungi and viruses. AMPs have often been suggested as potential novel antimicrobial compounds [99,100].

Antimicrobial peptides are synthesized as proforms that need to be processed to active, mature peptides. Most AMPs share cationic character and pronounced amphipathy as common structural motifs. Although for many AMPs the mode of action is incompletely understood, these motifs are believed to contribute to binding to the commonly anionic bacterial surface and integration into the cytoplasmic membrane, where many AMPs are thought to form channels or pores.

In humans, the role of AMPs as a key part of innate host defense has only recently been recognized. AMPs are produced by many cell types of the human immune system, including neutrophils, where they are secreted into phagosomes to kill ingested microorganisms [93], mast cells [101] and T cells [102]. On the skin, keratinocytes are the major source of AMPs, but AMPs are also produced by other cell types, such as hair bulb cells and sebocytes [103, 104]. In addition to their microbicidal activity, more recent research has shown that AMPs may also have a signaling function, inasmuch as they activate components of the human acquired immune system, such as T and dendritic cells [105–107]. Table 1 shows the most important human AMPs. It also includes antimicrobial proteins and enzymes, which may be grouped with AMPs in a wider sense, although they are not peptides *sensu strictu* (i.e., smaller than approximately 50 amino acids). Almost all of these AMPs are produced by keratinocytes, and many by sebocytes, except dermcidin (DCD), which is mainly secreted by eccrine sweat glands [108].

Most AMPs *sensu strictu* that are expressed in humans belong to the β-defensin family. Defensins are amphipathic peptides found in many vertebrates [109]. They have a β-sheet structure and are subcategorized according to the number and location of disulfide bridges. Human β defensins 1–4 are produced by human keratinocytes and are well-characterized [109,110]. Computational genomic research predicts many other potential defensin genes on the basis of conserved cysteine residues. The expression and roles of those peptides are unknown. Human β-defensin 1 (*hBD1*) is constitutively expressed, whereas *hBD2* and *hBD3* are inducible by bacterial infection or cytokines [111]. hBD2–4 may also be suppressed by retinoic acid [111]. The activity of human β-defensins strongly depends on salt concentration. Under physiological conditions, only hBD3 has activity against staphylococci [112], and has thus been used in many studies investigating AMP resistance mechanisms in these bacteria (see below). In addition to their antimicrobial activity, defensins elicit the production of cytokines, such as IL-8, and have chemotactic activity [113].

Cathelicidins are a family of AMPs named after resemblance to the precursor forms of the protein cathelin [114]. The N-terminal cathelin domain keeps the AMP precursor inactive until proteolytic cleavage releases the active C-terminal peptide, which may vary in structure. However, most mature cathelicidins are α-helical, amphipathic and cationic. This is also true for the only cathelicidin found in humans, the peptide LL-37 [115]. LL-37 is proteolytically cleaved from its precursor, which is called hCAP18 (human antimicrobial protein 18 kDa) [116,117]. LL-37 is but one processed form of hCAP18. Other forms, such as RK-31 and KS-30 (termed after the first two amino acids and total length), may be produced by alternative cleavage, especially on the skin [118]. KS-30 and RK-31 have increased antimicrobial activity, and also differ from LL-37 regarding the potency to elicit cytokine release. Similar to the defensins, LL-37 may induce chemotaxis and cytokine release [113]. Interestingly, 1,25-dehydroxyvitamin D3 is a powerful inducer of cathelicidin gene transcription, thus giving vitamin D an important role in skin infection [119].

Dermcidin is constitutively expressed by eccrine sweat glands and its processed forms have activity against many bacteria, including staphylococci [108,120]. While the DCD-1L and DCD-1 processed forms of DCD are negatively charged [108], a further processed form that is cationic (SSl-25) also has antimicrobial activity, suggesting that charge is of no importance to the mode of action of DCD-derived peptides [121].

Adrenomedullin is a 52 amino acid peptide with a multitude of functions that include hormone regulation, neurotransmission and vasodilatation [122]. It has high antimicrobial activity against many bacteria, particularly *Propionibacterium acnes* [123]. However, its potency against *S. aureus* and *S. epidermidis* is only moderate [124].

Lysozyme is an enzyme that cleaves the β-1,4 glycosidic bond in bacterial peptidoglycan between the *N*-acetylglucosamine and *N*-acetylmuramic acid residues [125]. It is found in many body fluids, such as mucosal secretions and tears. Lysozyme seems to be produced in skin cells, but only in the cytoplasm, and thus its contribution to cutaneous defense is unclear. Lysozyme is active against Gram-positive and Gram-negative organisms [126]. However, several staphylococcal species, including *S. aureus* and *S. epidermidis*, have an enzyme that *O*-acetylates peptidoglycan, which confers resistance to lysozyme [127,128].

Skin-derived antileukoproteinase is expressed by keratinocytes and inhibits neutrophil elastase, thereby controlling inflammation. It also inhibits bacterial growth, including that of *S. aureus*, but is not bactericidal [129,130].

Psoriasin (S100A7) is an 11-kDa AMP whose activity is dependent on zinc ions [131]. It is active against and inducible by contact with *E. coli*, but does not have activity toward staphylococci. In contrast, calprotectin, another member of the S100 AMP family, while not expressed in skin cells, is produced by neutrophils and controls proliferation of *S. aureus* in pus-filled abscesses [132].

Finally, the cationic RNase7 is produced by many tissue types including keratinocytes, and has strong activity against a broad range of bacteria including *S. aureus* [133].

The role of AMPs in infectious diseases & evidence for a role of AMPs in controlling bacterial colonization

Direct evidence for a contribution of human AMPs to controlling bacterial colonization or infection of the skin is scarce. Concentrations of AMPs needed to obtain *in vitro* activity are often much higher than what is estimated to be actually present on the skin. Possibly, local concentrations in epithelial microenvironments may be higher than what expression levels would suggest, and sufficient to kill microorganisms *in vivo*. Furthermore, the conditions used in minimal inhibitory concentration or killing assays *in vitro* are not standardized, may vary significantly, and very likely do not adequately reflect the physiological conditions on the skin. In many cases – such as for the human β-defensins – mimicking physiological conditions with regard to salt concentration seems to impair rather than increase AMP activity [110,112]. On the other hand, growth in low-nutrient media with serum components and carbonate, which probably better resembles skin conditions, appears to increase bacterial susceptibility to AMPs [134]. This indicates that physiological components that may increase the activity of AMPs *in vivo* might be lacking from most *in vitro* assays being used. Thus, while the physiological conditions present in the microenvironments on the human skin can only be guessed and hardly reproduced *in vitro*, *in vitro* assays used so far may have led to an underestimation of AMP potency *in vivo*.

Some, although circumstantial, evidence for a key role of AMPs in controlling bacterial colonization and infection of the skin is derived from differential AMP expression in certain diseases, such as atopic dermatitis, psoriasis and acne vulgaris [135]. Atopic dermatitis is a chronic inflammatory skin disease that is associated with recurrent infections. Lesions and unaffected skin in atopic dermatitis patients are colonized by *S. aureus* to a dramatically higher degree compared with healthy individuals [136,137], while the expression of inducible AMPs (LL-37, hBD-2 and hBD-3) and DCD-derived peptides is much lower in atopic dermatitis patients [138–140]. Furthermore, in the inflammatory skin disease psoriasis, many AMPs are overexpressed, which has not only facilitated purification of many AMPs [141], but may also explain the lower risk for bacterial infection observed in psoriatic skin [135]. Finally, the most important bacterial causative agent of acne vulgaris, *Propionibacterium acnes*, triggers overexpression of some AMPs such as hBD-1 and hBD-2 [142,143]. Together, these observations suggest that differential expression of certain AMPs may be triggered by bacterial pathogens and affect bacterial colonization and infection.

Furthermore, evidence for AMP importance *in vivo* has been achieved using knockout mice. This approach is difficult and only works for some selected AMPs, as AMP production and genes are very different between mice and men. However, the *CAMP* and *Cnlp* genes, encoding the human and mouse versions of the cathelicidins LL-37 and CRAMP, respectively, are very similar [144,145]. In an important study, it has been shown that mice deleted in the *Cnlp* genes have increased susceptibility for infection by Group A streptococci, representing the first evidence from knockout mice indicating a key role of AMPs in bacterial infection [146].

Moreover, the AMPs hBD-2 and hBD-3 likely play a key role during selection of carrier versus noncarrier strains during nasal colonization. It has been suggested that *S. aureus* carrier strains achieve a competitive advantage over noncarrier stains by delaying the innate host response and downregulating expression of these defensins in nasal epithelial cells [147].

Finally, the fact that bacteria have developed specific resistance mechanisms to AMPs [148], which will be presented in the next paragraph, clearly underlines the importance of AMPs in battling bacterial colonizers. Thus, although direct evidence has been hard to achieve, AMPs are now commonly agreed to form a key part of innate host defense on the human skin.

Antimicrobial peptide resistance mechanisms in staphylococci

Bacteria have developed several efficient mechanisms to combat the activity of AMPs [149]. Secreted bacterial proteases may degrade AMPs. Specific secreted bacterial proteins can sequester AMPs, and thus prevent them from reaching their cellular target. In addition, there are many membrane-located transporters that export AMPs in a drug exporter-like fashion. Moreover, many mechanisms alter the bacterial cell surface net charge to minimize attraction of the commonly cationic AMPs.

Staphylococci, as the most important bacterial colonizers of the human skin, have developed mechanisms belonging to all of these four categories (Table 2). *S. aureus* and *S. epidermidis* produce several secreted proteases with broad substrate specificities that are able to degrade AMPs. For example, the *S. epidermidis* protease SepA has been shown to degrade hBD-3 and DCD [150]. The homologous *S. aureus* aureolysin degrades and inactivates LL-37 [151]. The 136 amino acid protein staphylokinase, which is bacteriophage-encoded and secreted by lysogenic staphylococcal strains, binds to α-defensins, thereby abolishing antimicrobial activity [152]. The *vraF* and *vraG* genes, which encode an ABC transporter and are found in both *S. aureus* and *S. epidermidis*, have been demonstrated to confer resistance to AMPs [153]. Likely, VraFG functions by expelling AMPs from the cytoplasmic membrane.

Finally, there are many AMP resistance mechanisms in staphylococci that involve alteration of surface charge. Usually, these lead to a decrease of the anionic character of surface or cytoplasmic membrane structures, thus preventing attraction of cationic AMPs. Several of these mechanisms have first been described in staphylococci. The enzyme MprF produces lysinylated phospholipids, whose integration into the cytoplasmic membrane decreases the overall negative charge of this direct target structure for many AMPs [154]. The proteins encoded in the *dlt* locus are responsible for introducing alanyl residues in teichoic acids [155]. The free amino groups of the carboxy-esterified alanyl residues act to partially counterbalance the strongly anionic character of teichoic acids, thus minimizing AMP attraction [156]. However, surface polymers may also act to eliminate AMPs using different mechanisms. The cationic exopolysaccharide PNAG provides protection from AMPs of both positive and negative charge [157]. Thus, it may function either by repulsion or sequestration, or possibly by providing a structural barrier. Finally, some AMP resistance mechanisms may also provide protection from specific antibiotics, which is likely due to structural and physico–chemical similarities with AMPs, for example in the case of the cationic cyclic lipopeptide daptomycin [158].

Sensing antimicrobial peptides

Host adaptations to bacterial AMP resistance mechanisms exemplify the interplay between innate host defense and bacteria during evolution [148]. These include, for example, the production of anionic AMPs, such as DCD, to subvert bacterial resistance mechanisms that exclusively target cationic AMPs, or the development of protease-resistant AMPs such as the heavily bridged defensins. Bacteria, in addition to having developed AMP resistance mechanisms, have learned to sense the presence of sub-lethal concentrations of AMPs [159]. This means that expression of resistance genes can be limited to times when they are needed, which is beneficial for the bacteria, because expression of AMP resistance genes often involves significant physiological changes that likely represent a considerable energetic burden.

In Gram-negative bacteria, the PhoP/PhoQ sensor/regulator first described in *Salmonella* senses the presence of cationic AMPs and mainly triggers alteration of lipopolysaccharide [159,160]. The first AMP sensor in Gram-positive bacteria has only recently been described in *S. epidermidis* [161]. Interestingly, it is composed of three essential parts, ApsS (or GraS, the sensor part), ApsR (or GraR, the DNA-binding regulator) and ApsX, an unusual third component of unknown function. The Aps system, which has also been investigated in *S. aureus* [153], upregulates three loci coding for key AMP resistance mechanisms in staphylococci: the *vraF*/*vraG* transporter genes, the *dlt* operon and the *mprF* gene. While genes similar to *apsS* and *apsR* exist in other bacteria, *apsX* homologs are found only in staphylococci, indicating that the *aps*-based sensing of AMPs may be a unique staphylococcal property [161]. Furthermore, AMPs trigger in an apparently less specific way the altered expression of global regulatory systems including *agr*, *sarA* and *saeRS*, leading to increased secretion of proteases, such as of *S. epidermidis* aureolysin, that degrade AMPs [150]. Thus, the capacities to express a broad series of AMP resistance mechanisms and respond to the presence of AMPs may contribute to the exceptional ability of staphylococci to colonize human epithelia.

Expert commentary

Progress in our understanding of staphylococcal colonization of the skin and the molecular factors involved therein is linked to the development and use of suitable animal colonization models. Quite understandably, the staphylococcal research community has mostly been focused on infection and infection models. However, colonization has recently been recognized as an important area of research, predominantly owing to the occurrence of CA-MRSA and the likely high importance of colonization as a prerequisite for infection with these strains.

Several researchers have proposed decolonization strategies to prevent staphylococcal infections [162,163]. To find targets for vaccine development aimed at preventing colonization, we need to better understand which molecular factors staphylococci rely on to colonize the human skin and mucous membranes. While decolonization of coagulase-negative staphylococci, such as *S. epidermidis*, may not represent a good idea owing to the possible interference with the natural microflora and its balance, a stronger focus on the molecular biology of staphylococcal colonization will also lead to better knowledge about colonization by those strains.

Animal models for staphylococcal colonization are in their infancy. Models for nasal colonization have been used in mice and cotton rats [164,165]. However, prolonged colonization is difficult to achieve, and the time the animals need to clear staphylococci from the nose in these models is relatively short, with cotton rats showing longer colonization than mice. Monitoring permanent colonization, such as is seen in humans, is therefore so far not possible. However, with more laboratories using these models, they may become optimized over time. Rarely, human subjects have been used to monitor colonization [131], but for obvious reasons, this approach is limited to less virulent species such as *S. epidermidis*. Tissue culture studies may provide some insight into the interaction of staphylococci with, for example, keratinocytes, but such *in vitro* systems lack the complicated build-up of real skin to adequately reflect the complexity of that interaction.

The relative importance of AMPs in determining staphylococcal colonization and for the interplay between the innate immune system and bacteria in general is still a mystery. As discussed above, evidence to support a key role of AMPs in the cutaneous defense against microorganisms is mainly circumstantial. To better judge the relative importance of AMPs, *in vitro* assay conditions should be standardized and adjusted to reflect physiological settings more closely. While the use of animals is difficult in this area owing to the differences with humans regarding AMP genes, the immense progress in human genetics may provide evidence in the future derived from the investigation of gene composition in individuals prone to develop skin diseases.

Whether direct bacterial interference, such as most notably between *S. aureus* and *S. epidermidis*, plays a role in determining microflora composition on different human epithelia is equally mysterious. Bacteriocins as the most obvious candidates for bacterial interference seem to be limited to specific strains, for which no clear colonization advantage could be established. While the molecular basis of competition is quite easy to investigate *in vitro*, similarly to AMPs, only *in vivo* research and epidemiology will provide clear answers in this field.

Altogether, it appears that while much *in vitro* research still needs to be performed, classic laboratory microbiology is at its limits to further our understanding of the interplay between staphylococci and their host. Integrative efforts comprised of molecular biology, animal colonization models, human genetics and epidemiology will be needed. Furthermore, the presence of AMP resistance mechanisms in many bacteria, including staphylococci, indicates that the frequently suggested development of AMPs into therapeutics [99,100] may be problematic. On the other hand, efficient AMPs have evolved despite those mechanisms [148], and may thus provide a basis for the development of valuable alternatives to antibiotics, for example in the topical treatment of staphylococcal skin infections.

Five-year view

The CA-MRSA epidemic will probably drive the investigation of molecular factors enabling these strains to better colonize, and possibly compete with other strains. It is to be expected that these endeavors will also lead to a better understanding of staphylococcal colonization in

general. Researchers will focus more on using animal models of colonization. In contrast, achieving more direct evidence for a role of AMPs in controlling staphylococcal skin colonization is expected to take longer. In the meantime, the field will likely provide more detailed insight into the mechanisms of AMP resistance in staphylococci and their regulation.

Key issues

- Staphylococci are frequent colonizers of human epithelia. Many strains are permanent colonizers, while the human population is split into carriers and noncarriers with regard to *Staphylococcus aureus*. What distinguishes *S. aureus* carriers from noncarriers is not understood.
- The species *S. aureus* may cause fulminant infection, while infections by other staphylococcal species are mostly subacute. Colonization is usually a prerequisite for infection.
- Staphylococci produce many molecular factors that may play a role in colonization, such as surface-binding proteins and exopolymers. Furthermore, staphylococci show gene composition and expression aimed to withstand the harsh environmental conditions on human skin. However, the role of most of these components in colonization is hypothetical.
- There is no evidence so far that direct bacterial interference favors colonization by one staphylococcal strain over another, or other bacteria.
- Antimicrobial peptides are believed to play a key role in innate host defense on the human skin and in controlling bacterial colonization.
- Staphylococci have many mechanisms to subvert antimicrobial peptide activity, many of which are triggered by the presence of antimicrobial peptides.
- Colonization models will need to be used together with molecular biology approaches to provide direct evidence for a role of antimicrobial peptides (AMPs) in controlling staphylococcal colonization and a function of AMP resistance mechanisms in evading AMP activity *in vivo*, which is at present lacking.

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Table 1

Human antimicrobial peptides and proteins on the skin.

AMP	Size	Production in	General activity against bacteria	Staphylococci for which activity has been demonstrated
Defensins				
hBD-1	47 aa; 5.0 kDa	Keratinocytes, sebocytes	Gram-negative, (Gram-positive)	No activity at physiological salt concentration
hBD-2	41 aa; 4.3 kDa	Keratinocytes, sebocytes	Gram-negative, (Gram-positive)	No activity at physiological salt concentration
hBD-3	45 aa; 5.2 kDa	Keratinocytes	Gram-negative, Gram-positive	*S. aureus*, *S. epidermidis*
hBD-4	50 aa; 6.0 kDa	Keratinocytes	Gram-negative, Gram-positive	*S. aureus*
Cathelicidins				
LL-37	37 aa; 4.5 kDa	Keratinocytes, sebocytes	Gram-negative, Gram-positive	*S. aureus*, *S. epidermidis*
Others				
Dermcidin (DCD-1)	47 aa; 4.7 kDa	Eccrine sweat glands	Gram-negative, Gram-positive	*S. aureus*, *S. epidermidis*
Adrenomedullin	52 aa; 6 kDa	Keratinocytes, sebocytes, hair follicles, sweat glands	Gram-negative, Gram-positive	*S. aureus*, *S. epidermidis*
Elafin (SKALP)	57 aa; 6.0 kDa	Keratinocytes	Gram-negative, Gram-positive	*S. aureus*
Proteins & larger peptides				
Lysozyme	130 aa; 14.7 kDa	Keratinocytes, sebocytes, hair bulb	Gram-negative, Gram-positive	Some species (*S. aureus* and *S. epidermidis* are resistant)
Antileukoprotease (ALP, SLPI)	107 aa; 14 kDa	Keratinocytes	Gram-negative, Gram-positive	*S. aureus*, *S. epidermidis*
Psoriasin	101 aa; 11.5 kDa	Keratinocytes, sebocytes	Gram-negative, (Gram-positive)	*S. aureus*
RNase7	128 aa; 14.5 kDa	Keratinocytes	Gram-negative, Gram-positive	*S. aureus*

Parentheses indicate reduced activity.

aa: Amino acid; AMP: Antimicrobial peptide; DCD: Dermcidin; SKALP: Skin-derived antileukoproteinase.

Table 2

Prominent antimicrobial peptide resistance mechanisms in staphylococci.

Name	Gene(s)	Function	Present in *S. aureus*	Present in *S. epidermidis*
Secreted proteases				
Aureolysin	*sepA* (*S. epidermidis*) *aur* (*S. aureus*)	Degrades AMPs (LL-37, hBD-3, DCD-1)	+	+
Sequestration				
Staphylokinase	*sak* (bacteriophage-encoded)	Binds α-defensins	+ (some strains)	− (very rare)
Transporters				
VraF/VraG	*vraF*, *vraG*	Putative AMP exporter	+	+
Change of surface charge				
MprF	*mprF*	Lysylation of membrane phospholipids	+	+
Dlt locus	*dltA*, *dltB*, *dltC*, *dltD*	Alanylation of teichoic acids	+	+
IcaB (PNAG)	*icaA*, *icaB*, *icaC*, *icaD*	Production of PNAG exopolysaccharide; IcaB *N*-acetylglucosamine deacetylase introduces positive charge	+ (some strains)	+ (some strains)
AMP-triggered regulation				
Aps (Gra) RSX system	*apsS* (*graS*), *aps*R (*graR*), *apsX*	2-component sensor/ regulator with additional component (ApsX)	+	+
Global regulators: Agr, SarA, SaeRS	*agrB*, *agrD*, *agr C*, *agrA; sarA; saeR*, *saeS*	Agr is upregulated and Sar and Sae are downregulated by AMPs. Agr upregulates and SarA downregulates protease expression	+	+

DCD: Dermcidin; PNAG: Poly-*N*-acetylglucosamine.