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Staphylococcus epidermidis – the "accidental" pathogen

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While nosocomial infections by *Staphylococcus epidermidis* have gained much attention, this skin colonizer has apparently not evolved to cause disease, but maintain the commonly benign relationship with its host. Accordingly, *S. epidermidis* does not produce aggressive virulence determinants. Rather, factors that normally sustain the commensal lifestyle of *S. epidermidis* seem to rise to additional benefit during infection. Furthermore, we are beginning to comprehend the roles of *S. epidermidis* in balancing the epithelial microflora and serving as a reservoir of resistance genes. In this review, the molecular basis of the commensal and infectious lifestyles of *S. epidermidis* will be discussed.

Whereas previously only regarded as an innocuous commensal microorganism on the human skin, *Staphylococcus epidermidis* is nowadays seen as an important opportunistic pathogen. It is now the most frequent cause of nosocomial infections, at a rate about as high as that due to its more virulent cousin *Staphylococcus aureus*[1]. In particular, *S. epidermidis* represents the most common source of infections on indwelling medical devices. This likely stems from the fact that *S. epidermidis* is a permanent and ubiquitous colonizer of human skin, and the resulting high probability of device contamination during insertion[2]. While *S. epidermidis* infections only rarely develop into life-threatening diseases, their frequency and the fact that they are extremely difficult to treat represent a serious burden for the public health system. The costs related to vascular catheter-related bloodstream infections caused by *S. epidermidis* amount to an estimated $ 2 billion annually in the United States alone[3–5]. Treatment is complicated by specific antibiotic resistance genes and the formation of biofilms, multicellular agglomerations that have intrinsic resistance to antibiotics and mechanisms of host defense[3]. Furthermore, recent investigation has identified specific molecular determinants facilitating *S. epidermidis* immune evasion and ability to cause chronic disease. Interestingly, many of these determinants are believed to have original functions in the non-infectious lifestyle of this microorganism, emphasizing the accidental nature of *S. epidermidis* infections. A better understanding of *S. epidermidis* physiology not only during infection, but also in its commensal status is urgently needed to evaluate therapeutic strategies against *S. epidermidis*.

S. epidermidis – the species

Staphylococci are common bacterial colonizers of the skin and mucous membranes of humans and other mammals[4]. *S. epidermidis* in particular is the most frequently isolated species from human epithelia. It colonizes predominantly the axillae, head, and nares[5]. Analysis of the *S. epidermidis* genome indicated that the species is well equipped with genes assumed to provide protection from the harsh conditions encountered in its natural habitat[9, 10]. For example, to cope with extremes of salt concentration and osmotic pressure, *S. epidermidis* has eight sodium ion/proton exchangers and six transport systems for osmoprotectants[9].

S. epidermidis belongs to the group of coagulase-negative staphylococci (CoNS), which is distinguished from coagulase-positive staphylococci such as *S. aureus* by lacking the enzyme coagulase. The species shows a high degree of diversity with 74 identified sequence types

(STs)[6]. Most isolates belong to clonal complex (CC) 2, which comprises the most frequently isolated ST2. Possibly, the successful spread of ST2 may be due to the fact that all ST2 isolates contain IS256 insertion sequences and *ica* genes[7], two factors found correlated with *S. epidermidis* invasiveness[13–16]. In addition, most ST2 isolates show *in vitro* capacity to form biofilms[7]. Genome information is available for two strains of *S. epidermidis*: the biofilm-negative ATCC12228[8] and the biofilm-positive clinical isolate RP62A[9]. Of note, no genome sequence is available yet for an isolate of the most frequently found and potentially most invasive ST2.

An opportunistic pathogen

As part of the human epithelial microflora, *S. epidermidis* usually has a benign relationship with its host. Furthermore, it has been proposed that *S. epidermidis* may have a probiotic function by preventing colonization of more pathogenic bacteria such as *S. aureus*[17]. However, there is no clear evidence indicating that *S. epidermidis* secretes factors that impact colonization of other microorganisms *in vivo*.

In contrast to the relatively scarce information on the non-infectious lifestyle of *S. epidermidis*, *S. epidermidis* infections and mechanisms by which *S. epidermidis* promotes disease have gained much interest. Among CoNS, *S. epidermidis* clearly causes the greatest number of infections[2, 9]. In clinical microbiology, CoNS are often not further specified, as the major interest is in making a distinction between *S. aureus* and other staphylococci. However, based on reports that have performed species identification[1, 5], one can assume that the vast majority of non-specified CoNS infections are due to *S. epidermidis*. Particularly, *S. epidermidis* represents the most frequent causative agent involved with infections of any type of indwelling medical devices, such as peripheral or central intravenous catheters (CVCs)[9]. These infections usually commence with the introduction of bacteria from the skin of the patient or that of health care personnel during device insertion and have increased in number most likely owing to the increased use of such devices1, 18. *S. epidermidis* now accounts for at least 22% of bloodstream infections in intensive care unit patients in the USA, which occur in at least 4–5/1000 CVC insertions[1, 18]. In addition to the abundance of *S. epidermidis* on the skin, this high frequency is likely due to elaborate mechanisms to colonize catheter surfaces, which will be discussed later in this article. Furthermore, *S. epidermidis* may be involved in prosthetic joint, vascular graft, surgical site, central nervous system shunt, and cardiac device infections[9]. Last but not least, second only to *S. aureus*, *S. epidermidis* causes ~ 13% of prosthetic valve endocarditis (PVE) infections, with a high rate of intracardiac abscesses (38%) and 24% mortality[10]. However, PVE and other serious complications are rare among *S. epidermidis* infections, which altogether may be characterized as predominantly subacute and chronic.

The fact that *S. epidermidis* usually does not cause severe infections raises the interesting question why it is advantageous for this species – as opposed to its virulent cousin *S. aureus* – to maintain a low level of virulence. Massey *et al.* have developed a mathematical model outlining that for a strain with a high level of asymptomatic transmission such as *S. epidermidis*, avirulent strains out-compete virulent strains – in contrast to *S. aureus*, for which asymptomatic transmission is low and virulent strains out-compete avirulent strains[11]. This model is based on the assumption that *S. epidermidis* is more readily transmissible than *S. aureus*. The authors explain this by (i) the widespread colonization of *S. epidermidis* on human epithelia, while *S. aureus* almost exclusively colonizes the nares, (ii) colonization of all humans with *S. epidermidis* in contrast to *S. aureus*, which is only found in some individuals, and (iii) specific genetic factors involved in colonization and bacterial interference, such as cross-inhibiting quorum-sensing signals (Box 1). However, while quorum-sensing interference

favors at least one subtype of *S. epidermidis* over *S. aureus in vitro*[21, 22], there is no evidence that it plays a role *in vivo*[17].

In accordance with the low virulence potential of *S. epidermidis* and the Massey *et al.* model, the following paragraphs will show that *S. epidermidis* is well equipped with determinants promoting persistence, such as immune evasion molecules, rather than those which aggressively attack the host, such as toxins.

Evasion of host defenses

Pathogen survival in the human body requires evasion of host defenses. While a limited subset of host defense mechanisms such as antimicrobial peptides (AMPs) are present on the human skin[12], *S. epidermidis* has to cope with multiple additional mechanisms of host defense after penetration through the epithelial barrier. The innate immune system reacts first and in a rather non-specific way to any invading microorganism, including *S. epidermidis*. For example, as a key part of innate host defense, neutrophils ingest bacteria and kill them using reactive oxygen species and AMPs[13]. *S. epidermidis* has several mechanisms to evade being ingested and killed by neutrophils, as outlined below.

The role of the specific, acquired immune response to *S. epidermidis* infection is less well understood. The fact that our immune system has difficulties clearing long-lasting *S. epidermidis* infections despite production of antibodies against *S. epidermidis* proteins[25] indicates that acquired host defense may not be very efficient against *S. epidermidis*. This may be due in part to *S. epidermidis* exopolymers that protect from antibody recognition. Furthermore, our immune system may have evolved not to react in a strong manner to prevalent colonizing bacteria.

Biofilm formation

Biofilms are multicellular, surface-attached agglomerations of microorganisms. They have a characteristic physiology and architecture that form the basis of biofilm resistance to many antibiotics and mechanisms of host defense[3]. In accordance with this general notion, *S. epidermidis* shows significant, genome-wide adaptation to the biofilm mode of growth including down-regulation of basic cell processes such as nucleic acid, protein and cell wall biosyntheses[14]. These gene regulatory changes may explain limited activity of many antibiotics that target actively growing cells, such as penicillins[15], aminoglycosides[16], and quinolones[17], against *S. epidermidis* biofilms.

Biofilm formation proceeds via initial adhesion and subsequent aggregation into multicellular structures (Fig. 1). Thus, the development of a biofilm requires adhesive forces for the colonization of surfaces and the interaction of cells among each other. Disruptive forces are needed for the formation of fluid-filled channels that are important for nutrient delivery to all biofilm cells and give the mature biofilm its typical three-dimensional structure. Disruptive forces are also involved in the detachment of cell clusters from the biofilm, which limits biofilm expansion and may lead to the dissemination of infection[18].

Adhesion to abiotic surfaces such as catheters is mainly governed by bacterial cell surface hydrophobicity[19]. Specific proteins that impact surface adhesion in *S. epidermidis*, such as the abundant surface protein AtlE[20], a bifunctional adhesin/autolysin, and the Bap/Bhp protein[21] likely contribute to the hydrophobic character of the cell surface.

In vivo, matrix proteins fast cover abiotic surfaces such as those of indwelling medical devices. *S. epidermidis* has a vast array of surface proteins called MSCRAMMs (Microbial surface components recognizing adhesive matrix molecules) (Tab. 1) with the potential to interact with

matrix proteins. MSCRAMMs may be covalently bound to the bacterial surface by sortase A[22], or via yet incompletely understood non-covalent interaction with, probably, surface polymers such as teichoic acids[23] (Fig. 2). Binding activity to fibrinogen and collagen has been demonstrated for the covalently anchored proteins SdrG and SdrF[36, 37], respectively, and for the non-covalently bound autolysins AtlE and Aae, which show a less specific interaction and may bind to fibrinogen, fibronectin, and vitronectin[32, 38].

The most intensively studied MSCRAMM of *S. epidermidis* is SdrG (Fbe), a fibrinogen-binding protein that belongs to the serine/aspartate (SD) repeat family. Three members of this family, SdrF, SdrG, and SdrH, are present in most strains of *S. epidermidis*[39]. SdrG has been described as necessary and sufficient to promote *S. epidermidis* adhesion to fibrinogen *in vitro*[37, 40] and promotes CVC-associated infection *in vivo*[24]. SdrG binds to the thrombin cleavage site in the Bbeta chain of fibrinogen using a “dock, lock, and latch” mechanism[42]. This mechanism is believed to lead to a greatly stabilized MSCRAMM-ligand interaction. Emphasizing the importance of SdrG for *S. epidermidis* infection, expression of SdrG increases in an *in vivo* environment[43] and antibodies to SdrG are present in human blood[39]. Recently, an important role during ventricular assist device driveline-related infection has also been demonstrated for SdrF[25]. In addition, several further *S. epidermidis* MSCRAMMs have been predicted and undergone preliminary characterization[26], although their role in matrix protein binding and virulence is not yet understood.

After initial adhesion, biofilms develop via intercellular aggregation that is mediated by many different surface macromolecules. Among those, exopolysaccharide and some proteins appear be dedicated predominantly to the formation of the extracellular biofilm matrix. In addition, teichoic acids[46, 47] and extracellular DNA originating from lysed cells[27], may have accessory functions in aggregation, which are likely dependent on their polyanionic character (Fig. 1).

Many *S. epidermidis* strains produce a poly-N-acetylglucosamine (PNAG) homopolymer also named PIA (polysaccharide intercellular adhesin) that surrounds and connects *S. epidermidis* cells in a biofilm (Fig. 3)[28]. This polymer, which differs from other poly-N-acetylglucosamine polymers found in nature (such as chitin) by its β 1–6 linkage[28], has recently also been detected in many other microorganisms including *Yersinia pestis* and *Escherichia coli*[50, 51]. Production of PNAG/PIA is crucial for biofilm formation *in vitro*[52, 53] and has a significant impact on *S. epidermidis* biofilm-associated infection in most animal models [54–58]. The biosynthesis of PNAG/PIA is accomplished by the gene products of the *ica* (intercellular adhesion) locus[29]. IcaA and IcaD produce a chain from activated N-acetylglucosamine monomers, whose elongation is dependent on the IcaC protein, likely due to the assumed exporter function of the latter30. Partial de-acetylation of the N-acetylglucosamine residues is accomplished by the cell surface-located enzyme IcaB after export31. De-acetylation introduces positive charges in the otherwise neutral polymer that are important for surface binding of PNAG/PIA and its multiple biological functions in biofilm formation and immune evasion discussed below31. Production of PNAG/PIA is subject to a variety of regulatory influences[32], including by many global virulence regulators[61–67], but excluding the quorum-sensing regulator *agr*[33]. While it is less well understood which environmental signals control PNAG/PIA expression, particularly *in vivo*, the complexity of regulation underpins the importance of PNAG/PIA for *S. epidermidis* pathophysiology.

More recently, it was recognized that PNAG/PIA is not absolutely essential for biofilm formation in all *S. epidermidis* strains, as biofilm formation has been demonstrated in strains lacking the *ica* genes[34] and *ica*-negative *S. epidermidis* strains were isolated from biofilm-associated infection[70]. In some strains, biofilm formation may thus be mediated additionally or exclusively by specific surface proteins, namely Bap/Bhp[21] and Aap[35]. The Aap protein requires proteolytic activation[36] and zinc ions[37] for its biofilm-promoting effect. Zn^{2+} is crucial

for the modular association of so-called G5 tandem repeats[37], which may underlie the formation of Aap-made fibril-like structures on the bacterial surface[38] (Fig. 2). The same domains are known to interact with N-acetylglucosamine and thus potentially bind PIA/PNAG, forming a protein/polysaccharide biofilm network[39]. Based on the prevention of *in vitro* biofilm formation by a chelating agent, it has been suggested that biofilm formation in the strong biofilm forming strain *S. epidermidis* RP62A is solely dependent on Aap[37]. In support of this observation, monoclonal antibodies against Aap prevent biofilm formation in this strain[40]. However, this hypothesis is at variance with another report that did not find an impact of protein-mediated biofilm formation in the same strain[41]. Thus, the contribution of proteins to *S. epidermidis* biofilm formation and the involved mechanisms will certainly require intensive further investigation. In addition, the finding that biofilms solely made by proteins are not as robust as those with PNAG/PIA[70] indicates that both proteins and exopolysaccharide participate in efficient *S. epidermidis* biofilm formation.

Biofilm detachment

In contrast to intercellular aggregation, *S. epidermidis* biofilm structuring and detachment are poorly understood. We know that biofilm detachment in *S. epidermidis* is controlled by the quorum-sensing system *agr*, as biofilms that are *agr*-dysfunctional produce thicker biofilms and have an obvious defect in detachment[68, 78]. In *S. aureus* a model has been proposed that involves *agr* expression at the exposed layers of a biofilm, promoting detachment of cell clusters from the biofilm surface, thereby controlling biofilm expansion[42]. Likewise, *S. epidermidis agr* activity is limited to the biofilm surface[43], indicating a common staphylococcal mechanism of quorum-sensing-controlled biofilm detachment. Two detachment mechanisms have been proposed: enzymatic degradation of biofilm exopolymers and disruption of non-covalent interaction by detergent-like molecules (Fig. 1). With regard to enzymatic degradation of proteinaceous biofilm factors as suggested in *S. aureus*[44], evidence for such a function of proteases in biofilm detachment in *S. epidermidis* has not been obtained. However, *S. epidermidis* produces a series of exoproteases with relatively low substrate specificity that may serve to degrade surface proteins[81–83]. As for degradation of biofilm exopolysaccharide, staphylococci do not appear to have a dedicated enzyme for PNAG/PIA hydrolysis in contrast to several other bacteria with PNAG/PIA production[84, 85]. Alternatively, detergent-like molecules may disrupt non-covalent such as electrostatic and hydrophobic interactions, as for example between the cationic PNAG/PIA and anionic surface polymers, or between hydrophobic parts of the bacterial surface. The short amphipathic phenol-soluble modulins (PSMs) that include the *S. epidermidis* δ-toxin have been proposed to have such a function[45] (Fig. 4). Both *S. epidermidis* PSMs and exoproteases are strictly *agr*-regulated[87, 88], lending support to the idea that they may be candidates for biofilm structuring activity.

In general, knowledge about the molecular mechanisms of biofilm formation and its regulation in *S. epidermidis* is almost exclusively based on *in vitro* research. The contribution to pathogenesis of some determinants such as PNAG/PIA[54–58], AtlE[46], Fbe (SdrG)[24], SdrF25, and the regulators *agr*[43], *luxS*[47], and *sigB*[90] has been demonstrated using animal models. Furthermore, there is evidence indicating that important biofilm factors are expressed *in vivo*[58, 91]. Nevertheless, there is an urgent need for more detailed *in vivo* research providing mechanistic insight into *S. epidermidis* biofilm-associated infection. A recently constructed bioluminescent strain of a biofilm-forming clinical isolate of *S. epidermidis* may be helpful in these endeavors[48].

Protective exopolymers

S. epidermidis produces exopolymers, namely poly-γ-glutamic acid (PGA) and PNAG/PIA, that protect from important mechanisms of innate host defense. The pseudopeptide polymer PGA, which is synthesized by the gene products of the *cap* locus, is crucial for *S.*

epidermidis resistance to neutrophil phagocytosis and AMPs, despite comparatively low production[49]. Except for *Bacillus anthracis*[50], *S. epidermidis* is the only organism known so far in which PGA has a function in pathogenesis. Furthermore, PGA promotes growth of *S. epidermidis* at high salt concentrations and is induced under these conditions[49]. This is reminiscent of PGA production in many halophilic bacteria, where PGA is believed to contribute to osmotolerance[51], and indicates a role of PGA during *S. epidermidis* colonization. Finally, expression of the *cap* genes appears to be increased during the biofilm mode of growth[14]. Interestingly, PGA is present in many CoNS, but absent from *S. aureus*[9].

In addition to its role as part of the extracellular biofilm matrix, the already described exopolysaccharide PNAG/PIA has been found to protect *S. epidermidis* from neutrophil killing, complement deposition, immunoglobulins, and AMPs[96, 97], and from *Caenorhabditis elegans* immune defenses in a nematode infection model[98]. The cationic PNAG/PIA protects from AMPs of cationic and anionic charge, indicating that its mechanism of action may not be limited to electrostatic repulsion of AMPs of the same charge[52]. It may thus also work by sequestering oppositely charged AMPs in a way similar to the proposed mechanism of protection from tobramycin by *Pseudomonas aeruginosa* alginate[53].

Pathogen-associated molecular patterns

Pathogen-associated molecular patterns (PAMPs) are structures on the bacterial surface that the innate immune system recognizes as non-self via dedicated pathogen recognition receptors (PRRs), such as the Toll-like receptors (TLRs)[52]. Recognition of PAMPs acivates host defense mechanisms that include phagocytosis and cytokine release54. PAMPs such as lipoproteins and lipoteichoic acids are common in Gram-positive bacteria. Furthermore, there are reports suggesting that several additional molecules that are specific to *S. epidermidis* may stimulate innate host defense. For example, PNAG/PIA was reported to stimulate the Toll-like receptor 2 (TLR2)[55]. Recognition of PIA/PNAG by the human immune system would constitute an interesting example of the hide-and-seek interplay between pathogen and host, as a substance that *S. epidermidis* uses for immune evasion would trigger innate host defense mechanisms. However, this has not been confirmed using genetic deletion mutants, which is important to rule out that contaminating strongly pro-inflammatory substances such as lipoproteins were the basis of the observed effect, a situation that has led to frequent misidentification of alleged TLR2 stimulators[102–104]. Similarly, pro-inflammatory capacities of *S. epidermidis* PSMs[56] have not yet been confirmed using synthetic peptides or gene deletion mutants. However, their similarity to *S. aureus* PSMs, for which activity has been confirmed[57], indicates that the described pro-inflammatory effect of *S. epidermidis* PSMs is genuine; although activation of TLR2 by PSMs[107] needs verification. Finally, an unusual short-chain pro-inflammatory lipoteichoic acid has been described in *S. epidermidis*[58]. However, the chemical characterization of the purified molecule does not indicate a teichoic acid-related polymer, and thus the identity of this molecule and the reported pro-inflammatory activity certainly require confirmation. Thus, there is a clear need for further characterization of *S. epidermidis* molecules that activate host defenses.

Sensing of antimicrobial peptides

In a way similar to the recognition of *S. epidermidis* PAMPs by the human immune system, *S. epidermidis* has mechanisms to sense the presence of harmful molecules produced by the host. Specifically, an AMP-sensing system has been identified, termed *aps*, that is activated by a variety of AMPs and triggers up-regulation of staphylococcal AMP-defensive systems[59], including D-alanylation of teichoic acids[60], lysylation of phospholipids by the MprF enzyme[111], and the VraFG proteins[61] (Fig. 5) The former two mechanisms decrease the anionic charge of the bacterial surface, thus preventing efficient attraction of cationic AMPs, while the latter likely function as an AMP exporter, removing AMPs from the cytoplasmic membrane.

Thus, the Aps system – the first example of an AMP sensor in Gram-positive bacteria - has a function similar to the Gram-negative PhoP/PhoQ AMP sensor[113], but is not evolutionarily related. Importantly, activation and protective response of the *aps* system is limited to cationic AMPs. Furthermore, the *aps* system represents an exceptional example of a 3-component sensor/regulator that contains an essential component of unknown function, ApsX, in addition to the classical components of a two-component system, the histidine kinase ApsS (GraS) and the response regulator protein, ApsR (GraR).

Toxins

In *S. aureus* and many other bacteria, toxins are the most important contributors to aggressive virulence. In contrast to the vast toxin repertoire of *S. aureus*, *S. epidermidis* toxin production is mostly limited to PSMs. While strain-specific production of enterotoxins has been described[114, 115], *S. epidermidis* is not generally accepted as an enterotoxin producer. In contrast, all except naturally *agr* dysfunctional *S. epidermidis* strains produce PSMs[68, 87] (Fig. 4). These already mentioned peptides characteristically are short, amphipathic, and α-helical and have pro-inflammatory and sometimes cytolytic function. *S. epidermidis* δ-toxin (also called PSMγ), a 24-amino acid peptide that differs from its *S. aureus* homologue only in one amino acid position, has been suggested to be involved in necrotizing enterocolitis in neonates[62]. Some *S. epidermidis* PSMs are related to *S. aureus* PSMs that have pronounced capacity to lyse human neutrophils[57]. However, the PSM production pattern in *S. epidermidis* shows strong production solely of the moderately cytolytic δ-toxin and non-cytolytic β-type PSMs[14]. Thus, the PSM production pattern, in addition to the general absence of highly aggressive toxins in *S. epidermidis*, contrasts the high cytolytic potential of *S. aureus*. This underpins the Massey *et al.*[11] model proposing an evolutionary advantage for low aggressiveness of *S. epidermidis*.

Colonization and pathogenesis

Several studies have attempted to identify determinants that distinguish *S. epidermidis* strains that may cause infection from those that live on the skin. These studies focused on putative virulence determinants or used genome-wide approaches such as comparative genomic hybridization[14–16, 117]. Two main putative determinants of *S. epidermidis* invasiveness were identified in these studies: the *ica* genes encoding production of PNAG/PIA and the insertion element IS256. IS256 is believed to contribute to genetic adaptation that may play a role during infection[63]. For example, it may serve to abolish production of PNAG/PIA or function of the *agr* global virulence regulator by inserting into the *ica* or *agr* loci, respectively[78, 118]. As for PNAG/PIA, correlation with invasiveness may be due to the roles of this exopolymer in biofilm formation and immune evasion. In addition, results from a human colonization model indicate that *ica*-negative strains may even have a selective advantage over *ica*-positive strains on the skin[64]. However, there is also evidence suggesting that when corrected for clonal relatedness, there may not be any differences between commensal and infectious strains [117].

Several lines of evidence indicate that most “virulence factors” of *S. epidermidis* have original roles in the commensal lifestyle of *S. epidermidis* (Fig. 6). The roles of PNAG/PIA, PGA, and the SepA protease in protecting from AMPs indicate a key role of these polymers also during life on the skin[60, 93, 120], where AMPs are a major determinant of innate host defense. Furthermore, intercellular adhesion by PIA and biofilm-related proteins can be assumed to be vital in an environment such as the skin, where there is considerable mechanical stress for the bacteria. Finally, the role of PGA in osmotolerance[49] suggests an original function of this polymer in the non-infectious lifestyle of *S. epidermidis*. Moreover, there is no clear evidence indicating differences between infectious and commensal strains for the multitude of *S. epidermidis* MSCRAMMs, indicating that these proteins are valuable during both infection

and colonization. This makes sense, as adhesion to host tissue is considered imperative during both these lifestyles. Together, this suggests that *S. epidermidis* should be regarded as an "accidental" pathogen, whose clinical importance stems less from a dedicated infectious lifestyle, but rather from (i) the frequency of contamination events and (ii) mechanisms such as adhesion and immune evasion that are beneficial for the bacteria during both colonization and chronic infection.

Antibiotic resistance and prophylaxis

Specific antibiotic resistance genes are widespread in *S. epidermidis.* Most notably, resistance to methicillin as an antibiotic of first choice against staphylococcal infections is at 75–90% among hospital isolates of *S. epidermidis*, which is even higher than the corresponding rate for *S. aureus* (40–60%)[65]. High-level resistance to methicillin is encoded on mobile genetic elements (MGEs), namely the staphylococcal cassette chromosome *mec* (SCC*mec*), which contains the *mecA* gene encoding a penicillin-binding protein, PBP2a, with decreased affinity for methicillin compared to other PBPs[66]. In *S. epidermidis*, 10 different SCC*mec* structures were identified, with the short SCC*mec* type IV element[67] being the most abundant (36%)[68]. SCC*mec* type IV poses a particular problem, as it does not impose a fitness cost to its host and may thus spread without selective antibiotic pressure[125]. Interestingly, closely related strains may carry different SCC*mec* types, indicating frequent loss and acquisition of SCC*mec* elements by *S. epidermidis*[68].

In addition to methicillin resistance, *S. epidermidis* strains have acquired resistance to several other antibiotics, including rifamycin, flouroquinolones, gentamicin, tetracycline, chloramphenicol, erythromycin, clindamycin, and sulfonamides[9]. Very rarely, there is resistance to streptogramins, linezolid, and tigecycline. Most antibiotic resistance genes are plasmid-encoded and more often found in methicillin-resistant than methicillin-susceptible strains[69]. This likely has no molecular reasons, but is due to the fact that both resistance to methicillin and other antibiotics is frequent among endemic nosocomial strains. Despite widespread resistance to methicillin and other antibiotics, 80% of *S. epidermidis*-infected catheters may still be treated with antibiotics such as vancomycin without catheter removal[70]. However, intermediate resistance to vancomycin has been described[71]. Additionally, staphylococcal biofilm formation significantly decreases the activity of vancomycin and other antibiotics[129– 131].

The frequency of antibiotic resistance in *S. epidermidis* reflects antibiotic overuse. Furthermore, the ubiquity of *S. epidermidis* as a human commensal microorganism renders this bacterium an optimal carrier and reservoir for antibiotic resistance genes, particularly those that do not inflict a major fitness cost to the bacteria, such as SCC*mec* elements. Accordingly, there is evidence suggesting that methicillin resistance cassettes were transferred from *S. epidermidis* to *S. aureus* [124], [132]. Especially the acquisition of SCC*mec* type IV by community-associated methicillin-resistant *S. aureus* (CA-MRSA)[67] may have had an enormous impact on public health. It enabled the combination of methicillin resistance at no cost for fitness paired with exceptional virulence, which is the main molecular basis of the epidemic caused by CA-MRSA72. In addition, there is recent evidence indicating that CA-MRSA acquired other MGEs from *S. epidermidis* by horizontal gene transfer that may be important for efficient colonization [73]. These findings emphasize an important role of *S. epidermidis* in human disease by providing a "reservoir" function for the transfer of genetic elements to enhance pathogenic success of *S. aureus*.

Together, these considerations highlight the need for prophylactic measures against *S. epidermidis* infections. Vaccination and decolonization, often discussed for other pathogens including *S. aureus*, appear not appropriate for *S. epidermidis*. First, there is no anti-

staphylococcal vaccine and several lines of evidence indicate that it may be very difficult to use traditional active immunization for staphylococci[135, 136]. Second, eradication of *S. epidermidis* as a common part of the human microflora may not only be difficult to achieve owing to the fact that re-colonization from other individuals will be fast; it may also turn out to be counterproductive as it may allow potentially more harmful microorganisms to take the place of *S. epidermidis*. Thus, it is commonly agreed upon that the best way to deal with *S. epidermidis* infections is by prevention, which includes sterilization of medical equipment and body parts of patients and health care personnel in possible contact with indwelling medical devices during surgery[9].

Unidirectional horizontal gene transfer?

Interestingly, while *S. epidermidis* appears to frequently transfer MGEs to *S. aureus*[132, 134], it does not contain toxin genes, although acquisition of toxin genes from *S. aureus* using a similar mechanism would seem easy. The recent investigation of CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) sequences, short repeats that are involved in preventing uptake of conjugative elements such as phages and conjugative plasmids, may provide an explanation of why the transfer of MGEs between *S. epidermidis* and *S. aureus* is unidirectional[74]. These sequences have only been found in *S. epidermidis*, in one of the two genome-sequenced strains[9], but in none of the many *S. aureus* genomes that are known. While CRISPR-mediated prevention of MGE uptake in *S. epidermidis* clearly needs to be further evaluated, this mechanism may represent a molecular cause for the absence of a highly diverse toxin repertoire and the resulting lack of aggressive virulence in *S. epidermidis*.

Outlook

To evaluate potential novel strategies to combat *S. epidermidis* infections, we need to better understand the relationship between the commensal and infectious lifestyles of this bacterium. To that end, we should more thoroughly investigate determinants that ensure survival of *S. epidermidis* in its natural habitat, which most notably will include the development and use of skin colonization models. Furthermore, the interaction of *S. epidermidis* with other bacteria and the reservoir function of *S. epidermidis* for genes that may be transferred to *S. aureus* will need to be elucidated in more detail. For several of these tasks, it would be helpful to determine the genome sequence of additional *S. epidermidis* strains, particularly those of ST2 that appear to be most widely distributed among infectious isolates. Finally, the molecular mechanisms influencing biofilm-associated infection of *S. epidermidis* will need to be explored using *in vivo* approaches.

The staphylococcal accessory gene regulator (*agr*) quorum-sensing system and cross-inhibition by *agr* autoinducing peptides (AIPs)

Quorum-sensing in staphylococci is accomplished by the *agr* system, which consists of an AIP precursor peptide maturation and export enzyme (AgrB) and a two-component signal transduction system (AgrC, AgrA)[75]. Quorum-sensing-controlled target genes of *agr* are regulated directly by the DNA-binding protein AgrA or via the regulatory RNAIII[139, 140]. AIPs (or pheromones) are 7 to 9 amino acids in length and have a conserved cysteine residue, whose sulfhydryl group reacts with the C-terminal carboxy group to form a thiolactone that is essential for activity[141, 142]. Binding of the AIP to AgrC stimulates AgrC to auto-phosphorylate, which in turn leads to phosphorylation and activation of AgrA. AgrA activates the P2 promoter controlling expression of *agrBDCA*, thereby closing the quorum-sensing circuit. It also activates the P3 promoter that drives expression of RNAIII and the embedded PSM, δ-toxin (*hld*).

In general, AIPs of self activate, whereas AIPs of non-self (different species or subgroups) inhibit the *agr* response, unless the groups are closely related (e.g. *S. aureus agr* types I and IV)[138, 143]. The *S. epidermidis agr* type I is by far the most frequently isolated type from infections. The AIP of *S. epidermidis agr* type I inhibits all *S. aureus agr* types except for the rare type IV, while only *S. aureus* type IV inhibits *S. epidermidis* type I[76]. Interference by quorum-sensing cross-inhibition between *S. aureus* and *S. epidermidis* seems therefore to be in favor of *S. epidermidis*, but it is not known whether this plays a role during colonization *in vivo*.

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Glossary

Biofilms	Multicellular agglomerations of microorganisms on a surface with a characteristic three-dimensional structure and physiology.
Quorum-sensing	Cell density-dependent gene regulation in bacteria. Quorum-sensing systems in Gram-positive bacteria commonly contain peptide-based secreted signals and a membrane-located sensor. The staphylococcal quorum-sensing system is termed *agr* (accessory gene regulator) and controls a series of genes involved in metabolism and virulence.
Pseudopeptide	A peptide formed by peptide bonds via carboxyl groups other than the α-carboxyl group.
Innate host defense	Part of the immune system that provides first line of defense, fast response to invading microorganisms, based on recognition of pathogen-associated molecular patterns (PAMPs). Consists mainly of phagocytes, platelets, and secreted antimicrobial peptides (AMPs).
PAMPs	or pathogen-associated molecular patterns. Surface structures on pathogens that the innate immune system recognizes as non-self and which trigger activation of innate host defense commonly by binding to toll-like receptors (TLRs).
Acquired host defense	Part of the immune system that depends on antigen-dependent clonal expansion of T and B cells after antigen presentation by professional antigen-presenting cells. Provides long-term humoral (e.g., antibody-based) and cell-mediated immunity, but is delayed in response.
Neutrophils	short for neutrophil granulocytes (or polymorphonuclear leukocytes - PMNs), the most abundant leukocytes in human blood. Neutrophils are the primary cells in charge of eliminating invading microorganisms by uptake and subsequent killing via reactive oxygen species and antimicrobial proteins and peptides.
Antimicrobial peptides	or AMPs, peptides with antimicrobial activity, such as defensins, cathelicidins, etc., secreted for example by epithelial cells or into neutrophil phagosomes.
Sortase	Enzyme that covalently links secreted bacterial surface proteins to peptidoglycan. Most of these proteins are substrates of sortase A and are characterized by an LPXTG amino acid motif at their C-terminus.
Teichoic acids	Anionic cell envelope glycopolymer in Gram-positive bacteria composed of many identical sugar-phosphate repeating units. May be

	linked to peptidoglycan (wall teichoic acids) or the cytoplasmic membrane via a lipid anchor (lipoteichoic acids).
Two-component system	or TCS, bacterial sensory system composed of a membrane-located sensor (histidine kinase) and a cytoplasmic DNA-binding regulatory protein (response regulator), whose autophosphorylation-dependent activation is triggered by an extracellular signal.
Enterotoxin	a protein toxin released by a microorganism into the intestine.
Methicillin	Penicillin derivative that is resistant to penicillinase (an enzyme widespread in staphylococci providing resistance to penicillin).
Mobile genetic elements	or MGEs, DNA such as plasmids or transposons that may be exchanged between bacteria by horizontal gene transfer, and which often carry virulence or antibiotic resistance genes.

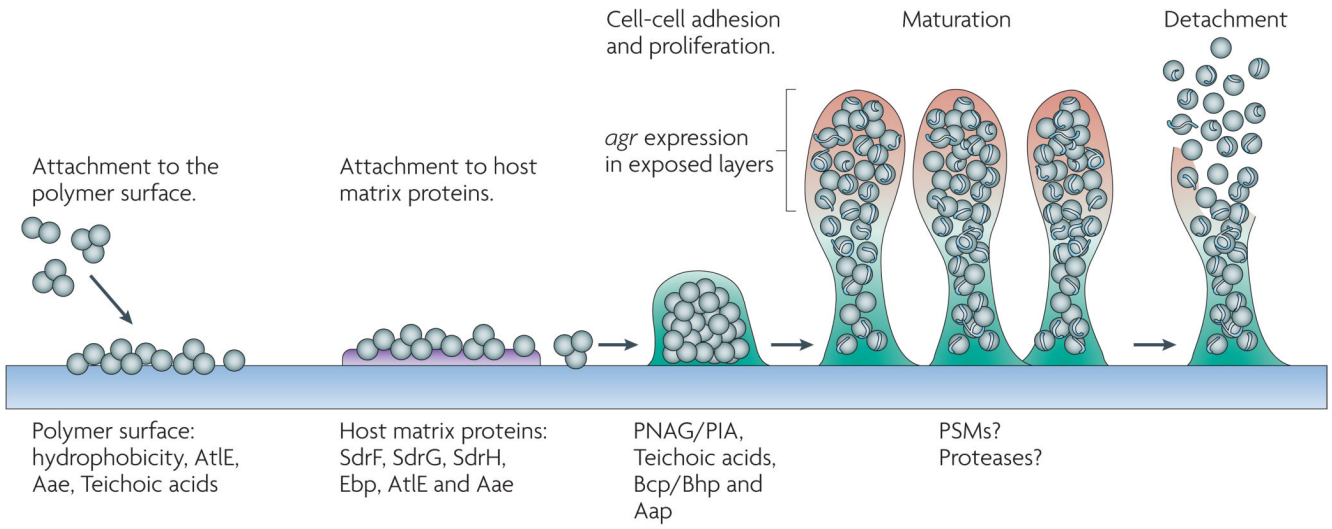

Figure 1. Biofilm development in *S. epidermidis*

Attachment to uncoated material is mainly dependent on cell surface hydrophobicity, while dedicated surface proteins mediate adhesion to host matrix-covered devices. Afterwards, exopolysaccharide, specific proteins, and accessory macromolecules provide intercellular aggregation. Mechanisms of biofilm maturation, structuring, and detachment are poorly understood, but possibly involve quorum-sensing controlled expression of detergent-like peptides and proteolytic activity in exposed layers of the biofilm. Genome-wide gene expression is significantly different in the biofilm compared to the planktonic mode of growth and includes down-regulation of basic cell processes.

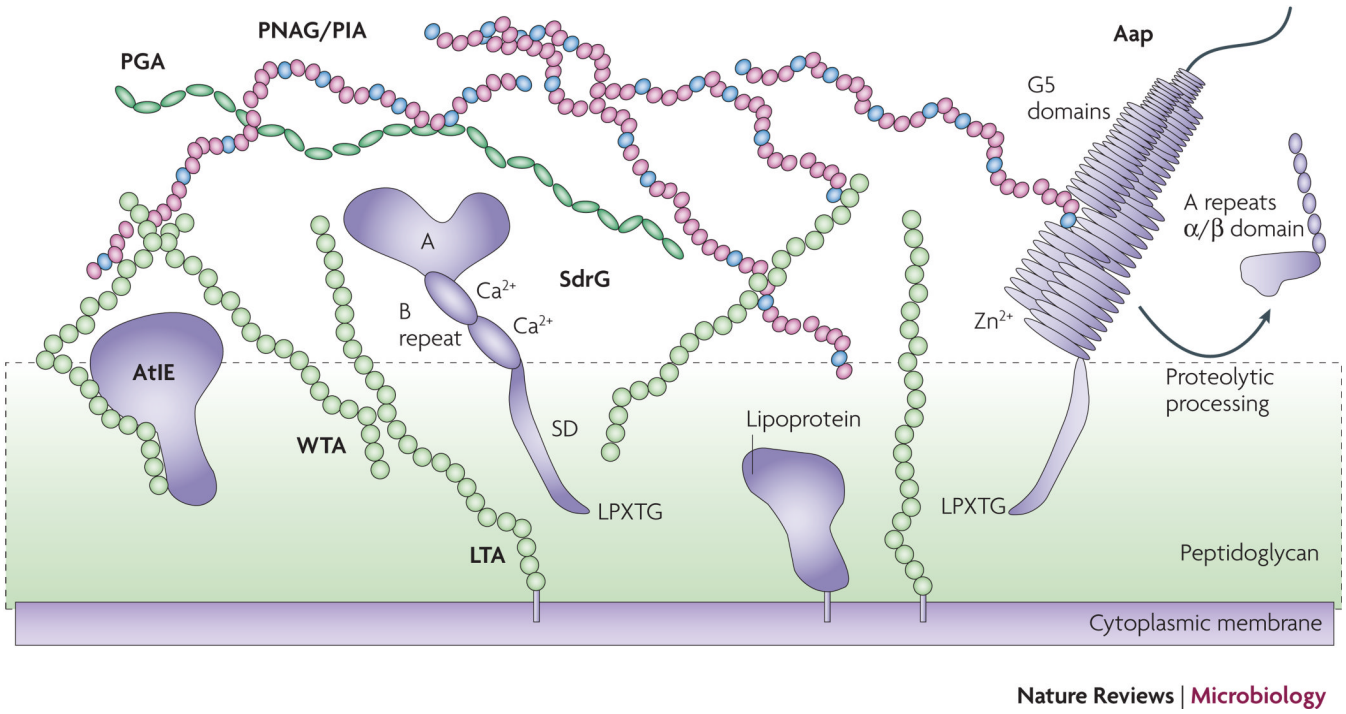

Figure 2. The *S. epidermidis* cell surface

Proteins such as SdrG and Aap may be attached to the cell surface via sortase-catalyzed covalent anchoring. These proteins harbor a characteristic LPXTG motif at the C-terminus, of which the threonine residue is linked to peptidoglycan. Many autolysins such as AtlE are anchored non-covalently, likely via interaction with teichoic acids. Furthermore, lipoproteins are surface-attached via their fatty acid anchor that penetrates the cytoplasmic membrane. AtlE is a bifunctional adhesin/autolysin that contributres to biofilm formation by its surface hydrophobicity and to host matrix protein binding. SdrG is an example of the Sdr protein family of MSCRAMMs. It stretches the peptidoglycan layer by its SD repeat region and binds fibrinogen via its A region. The B repeats harbor a Ca^{2+} binding EF-hand domain. Aap proteins aggregate via Zn^{2+}-dependent G5 domains and form fibrils that likely connect cells in the biofilm matrix. G5 domains also bind N-acetylglucosamine and may thus interact with the N-acetylglucosamine exopolysaccharide PNAG/PIA. PNAG/PIA is cationic and likely interacts with negatively charged surface polymers such as teichoic acids (lipoteichoic acids, LTA and wall teichoic acids, WTA) and poly-γ-glutamic acid (PGA).

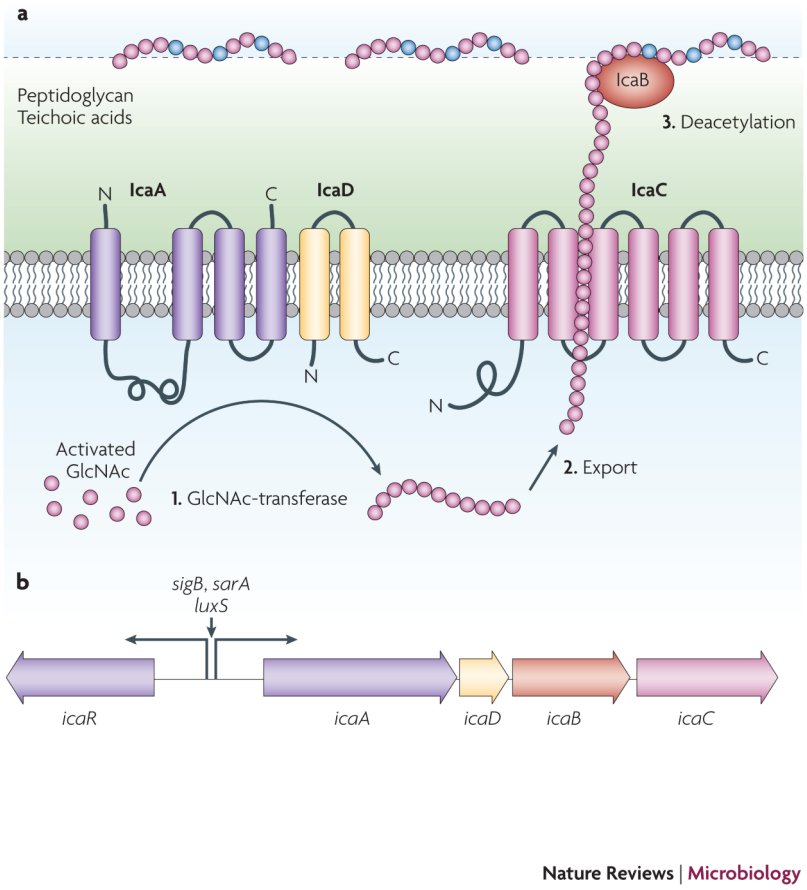

Figure 3. The exopolysaccharide PNAG/PIA

The immune evasion and biofilm aggregation exopolysaccharide PNAG/PIA, a partially de-acetylated β 1–6 linked N-acetylglucosamine homopolymer, is synthesized by the membrane-located N-acetylglucosamine transferase IcaA that needs the accessory IcaD membrane protein for activity. The growing PNAG/PIA chain is likely exported by the IcaC membrane protein. After export, the surface-located IcaB de-acetylase removes some of the N-acetyl groups, giving the polymer a cationic character that is essential for surface attachment. The Ica proteins are encoded in the *ica* gene locus, which contains the *icaADBC* operon and the *icaR* gene encoding a regulatory protein. Expression of the *icaADBC* operon is regulated either directly at the *icaA* promoter or via expression of IcaR by a series of global regulatory proteins. Furthermore, insertion and excision of the IS256 element may turn PNAG/PIA expression off and on.

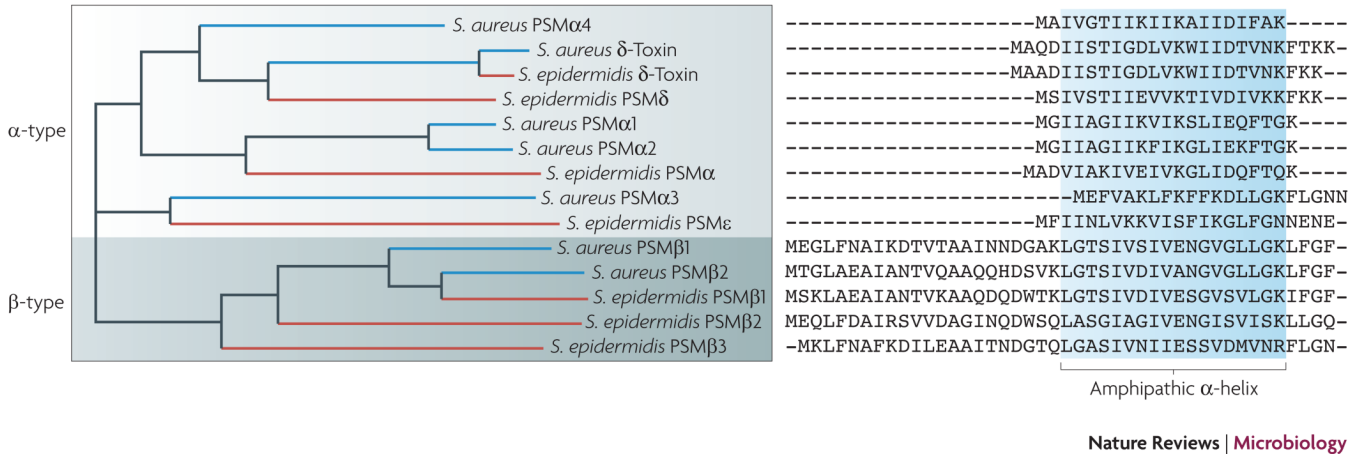

Figure 4. Phenol-soluble modulins

Sequence alignment of *S. epidermidis* and *S. aureus* PSMs. PSMs serve as immune evasion molecules to their bacterial producer and, on the other hand, as PAMPs for pathogen recognition to the host. All PSMs contain an amphipathic α-helix and N-terminal N-formyl methionine, as they are secreted as the direct translational product without processing in an unknown manner. PSMs of the α-type are relatively short, ~ 20–25 amino acids. Particularly the *S. aureus* PSMα peptides 1 through 4 are strongly cytolytic. PSMs of the β-type are longer, ~ 45 amino acids, and do not have considerable cytolytic activity. Only the δ-toxin, an α-type PSM with moderate cytolytic activity, and the β-type PSMs are secreted by *S. epidermidis* in large amounts. Although part of the *psm*β operon, the PSMβ3 peptide is not found in *S. epidermidis* culture filtrates for unknown reasons. The *psmβ1* gene is duplicated in some strains of *S. epidermidis*.

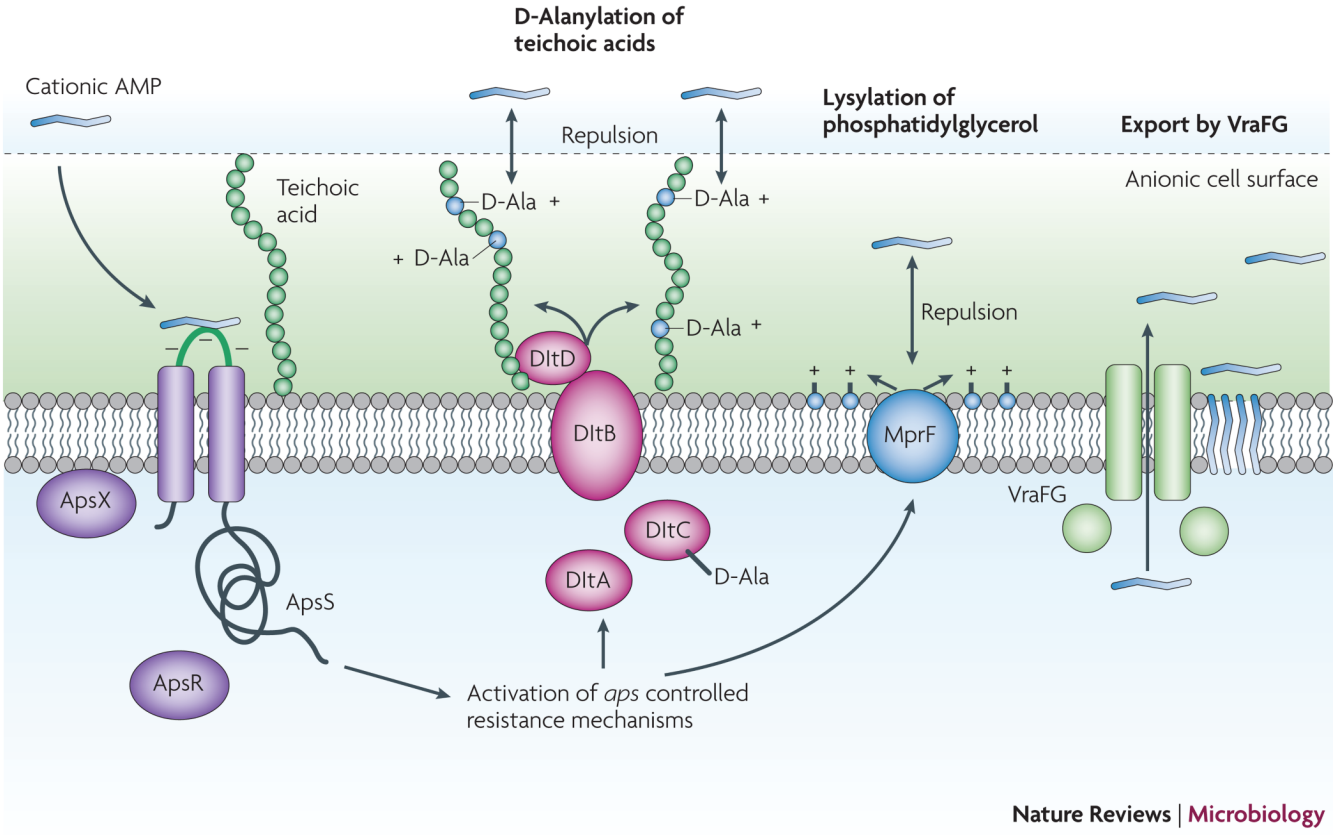

Figure 5. The Aps antimicrobial peptide sensor/regulator

Cationic AMPs attach to the negatively charged bacterial surface and membrane by electrostatic interaction, a prerequisite for AMP antimicrobial activity, which is often based on pore formation in the bacterial cytoplasmic membrane. The *S. epidermidis* ApsS AMP sensor has one short extracellular loop with a high density of negatively charged amino acid residues that interacts with cationic AMPs. Transduction of this signal via ApsS and the accessory, essential ApsX, which has a yet unknown function, triggers expression of key AMP resistance mechanisms. The D-alanylation of teichoic acids, encoded by the products of the *dlt* operon, and lysylation of phosphatidylglycerol, catalyzed by the MprF enzyme, result in a decreased negative charge of the cell surface and membrane, respectively, leading to decreased attraction, or repulsion, of cationic AMPs. The VraFG ABC transporter also promotes resistance to AMPs and likely functions as an AMP exporter.

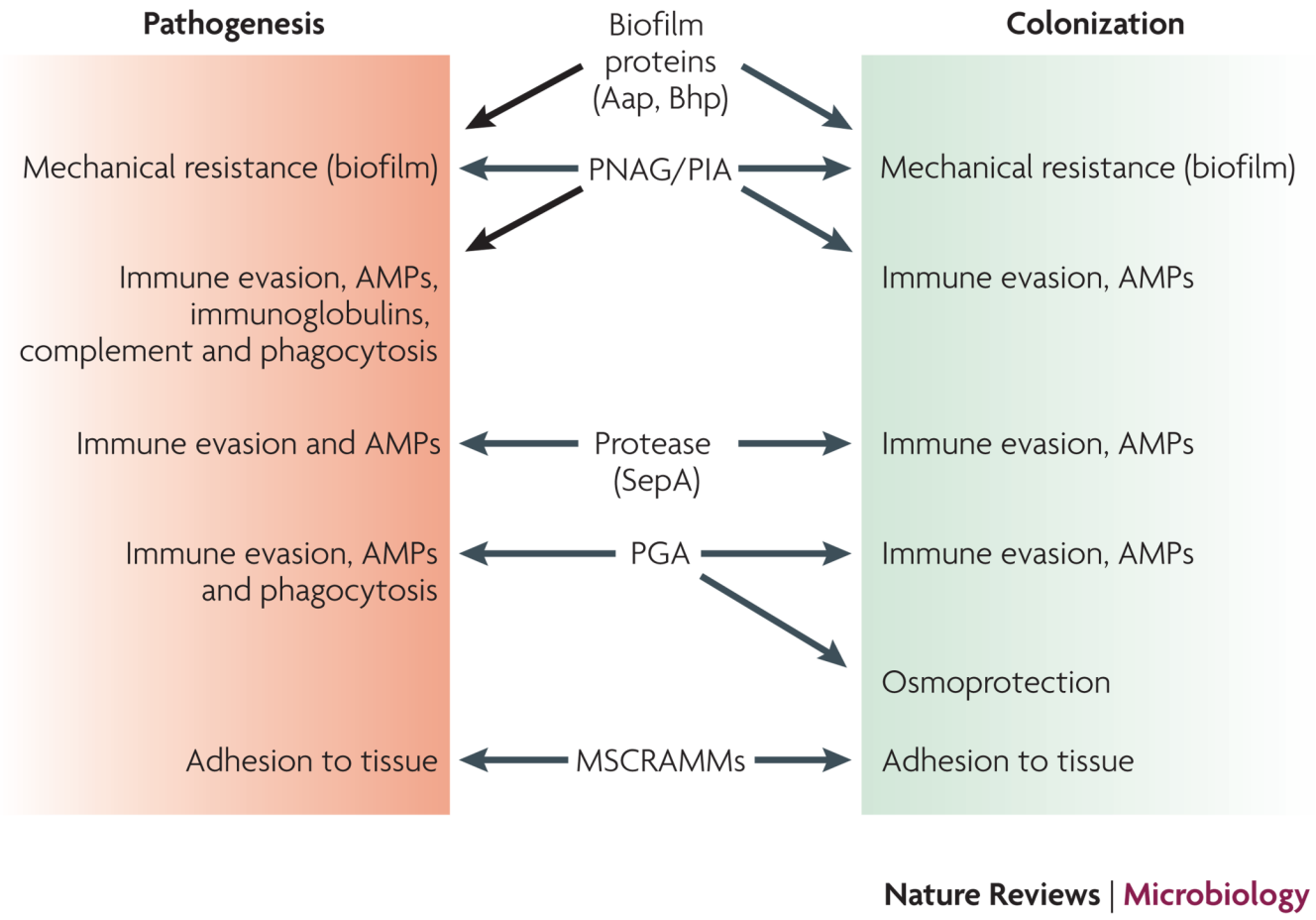

Figure 6. *S. epidermidis* as a commensal and infectious microorganism

Determinants that are believed to contribute to both *S. epidermidis* colonization and pathogenesis are shown. In animal models, only the roles of PNAG/PIA, PGA, and the MSCRAMM SdrG in infection have been demonstrated. Other roles are based on *in vitro* experiments and environmental challenges during colonization and infection. Not shown are regulators such as *agr* or *sigB* that control many of the depicted determinants and may thus also have important functions during both *S. epidermidis* lifestyles.

Tab. 1

Virulence factors of *S. epidermidis*

Virulence factor	Gene	Function	Reference
Immune evasion			
Biofilm formation			
Primary attachment			
(To abiotic surfaces)			
AtlE	*atlE*	Bifunctional autolysin/adhesin Abundant protein impacting surface hydrophobicity	20
Aae	*aae*	Bifunctional autolysin/adhesin	77
Teichoic acids	(multiple biosynthetic genes)	In *S. aureus* Impact attachment (via binding of autolysins?)	78
(To matrix proteins)			
SdrF	*sdrF*	Binds to collagen	36, 44
SdrG (Fbe)	*sdrG*	Binds to fibrinogen	79
SdrH	*sdrH*	Putative binding function only	39
Ebp	*ebp*	Binds to elastin	
AtlE/Aae	*atlE/aae*	Bind to multiple matrix proteins	32, 38
Intercellular aggregation			
PNAG/PIA	*icaADBC*	Intercellular polysaccharide adhesin	49, 53
Biofilm-associated protein Bap/Bhp	*bap/bhp*	Intercellular protein adhesin	21
Accumulation-associated protein Aap	*aap*	Intercellular protein adhesin precursor (requires proteolytic processing for activity)	71, 72
Teichoic acids		Component of biofilm matrix	80
Protective exopolymers			
PNAG/PIA	*icaADBC*	Protects from IgG, AMPs, phagocytosis, complement	96, 97
PGA	*capABCD*	Protects from AMPs, phagocytosis	49
Resistance to AMPs			
SepA protease	*sepA*	AMP degradation	81
Dlt, MprF, VraFG	*dltABCD mprF vraFG*	In analogy to *S. aureus* D-alanylation of teichoic acids (Dlt), lysylation of phospholipids (MprF), putative AMP export (VraFG)	110–112
Aps system	*apsR (graR), apsS (graS), apsX*	AMP sensor, regulator of AMP resistance mechanisms	59
Toxins			
Phenol-soluble modulins (PSMs)	*psmα,psmδ, psmε, hld, psmβ1, psmβ2*	Pro-inflammatory cytolysins	26, 87, 105
Exoenzymes			
Proteases			

Virulence factor	Gene	Function	Reference
Cysteine protease (SspB, Ecp)(*S.aureus* staphopain homologue)	*sspB*	Unknown, tissue damage?	82
Metalloprotease/elastase (SepA)	*sepA*	Tissue damage?, lipase maturation, AMP resistance	81, 120, 145
(*S.aureus* aureolysin homologue)			
Glutamylendopeptidase (GluSE, SspA, Esp), serine protease	*sspA*	Degradation of fibrinogen and complement factor C5	82, 83
(*S. aureus* V8 protease homologue)			
Lipases GehC, GehD	*gehC, gehD*	Persistence in fatty acid secretions?	146–148
Others			
Staphyloferrins	*sfna* locus (*S.aureus* staphyloferrin A)	Siderophores (iron acquisition)	149, 150
SitABC	*sitABC*	Iron importer	151
Fatty acid modifying enzyme (FAME)	unidentified	Detoxification of bactericidal fatty acids	83