# *Staphylococcus epidermidis* — the 'accidental' pathogen

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Abstract | Although nosocomial infections by *Staphylococcus epidermidis* have gained much attention, this skin-colonizing bacterium has apparently evolved not to cause disease, but to 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 give rise to additional benefits 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, I discuss the molecular basis of the commensal and infectious lifestyles of *S. epidermidis*.

Biofilm
A multicellular agglomeration of microorganisms that forms on a surface. Biofilms have a characteristic three-dimensional structure and physiology.

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Previously regarded as an innocuous commensal microorganism on the human skin, *Staphylococcus epidermidis* is now seen as an important opportunistic pathogen. Together with its more virulent cousin *Staphylococcus aureus*, *S. epidermidis* ranks first among the causative agents of nosocomial infections[1]. In particular, *S. epidermidis* represents the most common source of infections on indwelling medical devices. This probably 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]. Although *S. epidermidis* infections only rarely develop into life-threatening diseases, their frequency, and the fact that they are extremely difficult to treat, represents 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 US$2 billion annually in the United States alone[3–5]. Treatment is complicated by the presence of specific antibiotic resistance genes and the formation of biofilms, which are multicellular agglomerations that have intrinsic resistance to antibiotics and mechanisms of host defence[6]. Furthermore, recent investigation has identified specific molecular determinants that facilitate immune evasion by *S. epidermidis* and its ability to cause chronic disease. Interestingly, many of these determinants are thought 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 during its commensal phase is urgently needed to evaluate therapeutic strategies against this pathogen.

## *S. epidermidis*: the species

Staphylococci are common bacterial colonizers of the skin and mucous membranes of humans and other mammals[7]. *S. epidermidis* is the most frequently isolated species from human epithelia, and predominantly colonizes the axillae (armpits), head and nares (nostrils)[8]. Analysis of the *S. epidermidis* genome indicated that the species is well equipped with genes that are predicted 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 coagulase-negative staphylococci (CoNS), which are distinguished from coagulase-positive staphylococci, such as *S. aureus*, by their lack of the enzyme coagulase. Although not as discriminatory as for *S. aureus*, recent endeavours using multilocus sequence typing have given insight into the composition of *S. epidermidis* as a species[11,12]. Using a recently improved scheme[13], it was found that *S. epidermidis* strains show a high level of diversity, with 74 identified sequence types (STs)[14]. Most isolates belong to clonal complex 2 (CC2), which includes the most frequently isolated ST2 (REF. 14). The successful spread of ST2 may be due to the fact that all ST2 isolates contain IS*256* insertion sequences and *ica* genes[15], two factors that have been found to correlate with *S. epidermidis* invasiveness[16–19]. In addition, most ST2 isolates show an *in vitro* capacity to form biofilms[15]. Genome information is available for two strains of *S. epidermidis*: the biofilm-negative *S. epidermidis* ATCC 12228

Box 1 | **Cross-inhibition of the *agr* quorum sensing system**

Quorum sensing in staphylococci is accomplished by the *agr* system, which consists of an auto-inducing peptide (AIP) precursor peptide maturation and export enzyme (AgrB) and a two-component signal transduction system (AgrC and AgrA)[146]. Quorum sensing-controlled target genes of *agr* are regulated directly by the DNA-binding protein AgrA or through the regulatory RNAIII[147,148]. AIPs (or pheromones) are 7 to 9 amino acids in length and have a conserved cysteine residue, the sulphhydryl group of which reacts with the carboxy-terminal carboxy group to form a thiolactone that is essential for activity[149,150]. Binding of the AIP to AgrC stimulates AgrC to autophosphorylate, which in turn leads to phosphorylation and activation of AgrA. AgrA activates the P2 promoter, which controls expression of *agrB*, *agrD*, *agrC* and *agrA*, thereby closing the quorum sensing circuit. It also activates the P3 promoter, which drives expression of RNAIII and the embedded phenol-soluble modulin δ-toxin (encoded by *hld*).

In general, AIPs of self activate the *agr* response, whereas AIPs of non-self (different species or subgroups) inhibit the *agr* response, unless the groups are closely related (for example, *Staphylococcus aureus agr* types I and IV)[146,151]. *Staphylococcus epidermidis agr* type I is the type that is by far most frequently isolated from infections. The AIP of *S. epidermidis agr* type I inhibits all *S. aureus agr* types except for the rare type IV, whereas only *S. aureus* type IV inhibits *S. epidermidis* type I[152]. Interference by quorum sensing cross-inhibition between *S. aureus* and *S. epidermidis* therefore seems to be in favour of *S. epidermidis*, but it is not known whether this has a role during colonization *in vivo*.

(REF. 10) and the biofilm-positive clinical isolate *S. epidermidis* RP62A[9]. Notably, no genome sequence is yet available for any isolate of ST2, the most frequently found and potentially most invasive ST.

## 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 the host by more severe pathogens, such as *S. aureus*[20]. However, there is no clear evidence indicating that *S. epidermidis* secretes factors that have an impact on the colonization of other microorganisms *in vivo*.

There is little information on the non-infectious, colonizing lifestyle of *S. epidermidis*. However, *S. epidermidis* infections and the mechanisms by which *S. epidermidis* promotes disease have become increasingly studied. Among the CoNS, *S. epidermidis* causes the greatest number of infections[2,5]. In clinical microbiology, the CoNS are often left uncharacterized, as the main goal is to distinguish between *S. aureus* and other staphylococci. However, from the studies in which species identification has been performed[1,5], it can be assumed that most non-specified CoNS infections are due to *S. epidermidis*. In particular, *S. epidermidis* represents the most frequent causative agent of infections of indwelling medical devices, such as peripheral or central intravenous catheters (CVCs)[5]. 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, probably owing to the increased use of such devices[1,21]. Bloodstream infections occur in at least 4–5 out of every 1,000 CVC insertions performed on patients in intensive care in the United States; at least 22% of these infections are caused by *S. epidermidis*[1,21]. In addition to the abundance of *S. epidermidis* on the skin, this high frequency of infection is probably due to the elaborate mechanisms used to colonize catheter surfaces (discussed below). Furthermore, *S. epidermidis* may be involved in prosthetic joint, vascular graft, surgical site, central nervous system shunt and cardiac device infections[5]. Notably, and 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[22]. However, PVE and other serious complications are rare among *S. epidermidis* infections, which can be characterized as predominantly subacute and chronic.

The fact that *S. epidermidis* does not usually cause severe infections raises the interesting question of why it is advantageous for this species to maintain a low level of virulence. Massey *et al.* have developed a mathematical model outlining how for a species with a high level of asymptomatic transmission, such as *S. epidermidis*, avirulent strains out-compete virulent strains, whereas for species in which asymptomatic transmission is low, such as *S. aureus*, virulent strains out-compete avirulent strains[23]. This model is based on the assumption that *S. epidermidis* is more readily transmissible than *S. aureus*. The authors explain that this assumption is valid because of the widespread colonization of *S. epidermidis* on human epithelia (*S. aureus* almost exclusively colonizes the nares), the colonization of all humans with *S. epidermidis* (*S. aureus* is only found in some individuals) and the specific genetic factors involved in colonization and bacterial interference, such as cross-inhibiting quorum sensing signals (BOX 1). However, although quorum sensing interference favours at least one subtype of *S. epidermidis* over *S. aureus in vitro*[24,25], there is no evidence that it has a role *in vivo*[20].

In accordance with the low virulence potential of *S. epidermidis* and the Massey *et al.* model, *S. epidermidis* is well equipped with determinants that promote persistence, such as immune evasion molecules, rather than those that aggressively attack the host, such as toxins (discussed below).

## Evasion of host defences

Pathogens must evade host defences to survive in the human body. Although only a limited subset of host defence mechanisms, such as the production of antimicrobial peptides (AMPs), are present on human skin[26], *S. epidermidis* has to cope with various additional mechanisms of host defence after penetration of the epithelial barrier. The innate immune system is the

Quorum sensing
A method of 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* and controls a series of genes involved in metabolism and virulence.

Antimicrobial peptide
A peptide such as a defensin or cathelicidin, which have antimicrobial activity. Antimicrobial peptides are secreted by the host, for example, by epithelial cells or into neutrophil phagosomes.

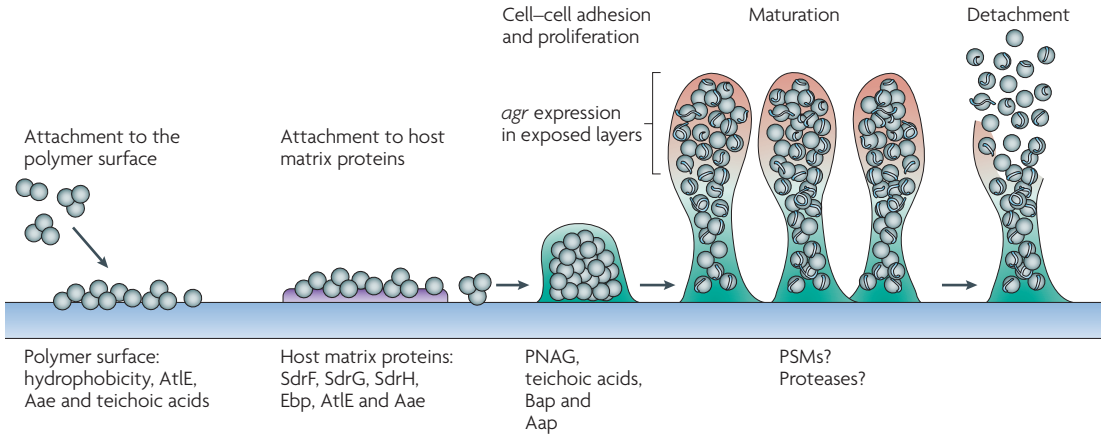


Figure 1 **Biofilm development in *Staphylococcus epidermidis*.** Attachment to uncoated material is mainly dependent on cell surface hydrophobicity, whereas dedicated surface proteins mediate adhesion to host matrix-covered devices. After adhesion to the surface, exopolysaccharide (for example, poly-*N*-acetylglucosamine (PNAG), also known as PIA), specific proteins (Bap (also known as Bhp) and Aap) and accessory macromolecules (such as teichoic acids) aid 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. The gene expression profile is markedly different in the biofilm compared with in the planktonic mode of growth and includes downregulation of basic cell processes. PSM, phenol-soluble modulin.

Innate host defence
A part of the immune system that provides the first line of defence, a fast response to invading microorganisms, based on recognition of pathogen-associated molecular patterns. The innate immune system consists mainly of phagocytes, platelets and secreted antimicrobial peptides.

Neutrophil
The most abundant leukocyte in human blood. Neutrophils are the main cells that eliminate invading microorganisms by uptake and subsequent killing through reactive oxygen species and antimicrobial proteins and peptides.

Acquired host defence
A 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. The acquired response provides long-term humoral (antibody-based) and cell-mediated immunity, but is delayed.

Sortase
An 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 the carboxyl terminus.

Teichoic acid
An anionic cell envelope glycopolymer produced by Gram-positive bacteria, composed of many identical sugar–phosphate-repeating units. Teichoic acids can be linked to peptidoglycan (wall teichoic acids) or to the cytoplasmic membrane through a lipid anchor (lipoteichoic acids).

first line of defence against an invading microorganism such as *S. epidermidis* and acts in a non-specific way. For example, as a key part of innate host defence, neutrophils ingest bacteria and kill them using reactive oxygen species and AMPs[27]. *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 the production of antibodies against *S. epidermidis* proteins[28], indicates that the acquired host defence system might not be efficient against *S. epidermidis*. This may be due, in part, to *S. epidermidis* exopolymers that protect the cells from antibody recognition. Furthermore, our immune system may have evolved to react less strongly 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 defence[6]. In accordance with this general notion, *S. epidermidis* shows substantial, genome-wide adaptation to the biofilm mode of growth, including downregulation of basic cell processes such as nucleic acid, protein and cell wall biosyntheses[29]. These gene-regulatory changes may explain the limited activity of many antibiotics that target actively growing cells (for example, penicillins[30], aminoglycosides[31] and quinolones[32]) against *S. epidermidis* biofilms.

Biofilm formation proceeds by the initial adhesion of cells to a surface and their subsequent aggregation into multicellular structures (FIG. 1). Therefore, the development of a biofilm requires adhesive forces for both the colonization of surfaces and the cell–cell interactions. 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[33].

Adhesion to abiotic surfaces such as catheters is mainly governed by bacterial cell surface hydrophobicity[34]. Specific proteins that affect surface adhesion in *S. epidermidis*, such as the abundant surface protein AtlE[35], a bifunctional adhesin and autolysin, and the Bap protein (also known as Bhp)[36], are likely to contribute to the hydrophobic character of the cell surface.

*In vivo*, matrix proteins quickly 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) (TABLE 1), which have the potential to interact with matrix proteins. MSCRAMMs can be covalently bound to the bacterial surface by sortase A[37] or through as yet incompletely understood, non-covalent interactions with surface polymers such as teichoic acids[38] (FIG. 2). Binding to fibrinogen and collagen has been demonstrated for the covalently anchored proteins SdrG (also known as Fbe) and SdrF[39,40], respectively, whereas the non-covalently bound autolysins AtlE and Aae show a less-specific interaction and can bind to fibrinogen, fibronectin and vitronectin[35,41].

The most intensively studied MSCRAMM of *S. epidermidis* is SdrG, a fibrinogen-binding protein that belongs to the serine/aspartate repeat family. Three members of this family, SdrF, SdrG and SdrH, are present in most strains of *S. epidermidis*[42]. SdrG has been described as necessary and sufficient to promote *S. epidermidis* adhesion to fibrinogen *in vitro*[40,43] and promotes CVC-associated infection *in vivo*[44]. SdrG binds to the thrombin cleavage

Table 1 | **Virulence factors of *Staphylococcus epidermidis***

| Virulence factor | Gene | Function | Refs |
|---|---|---|---|
| ***Biofilm formation through primary attachment to abiotic surfaces*** | | | |
| AtlE | *atlE* | An abundant bifunctional autolysin and adhesin that affects surface hydrophobicity | 35 |
| Aae | *aae* | A bifunctional autolysin and adhesin | 41 |
| Teichoic acids | Multiple biosynthetic genes | In *Staphylococcus aureus*, teichoic acids affect attachment (through the binding of autolysins?) | 49 |
| ***Biofilm formation through primary attachment to matrix proteins*** | | | |
| SdrF | *sdrF* | Binds to collagen | 39,47 |
| SdrG (also known as Fbe) | *sdrG* (also known as *fbe*) | Binds to fibrinogen | 43 |
| SdrH | *sdrH* | Putative binding function only | 42 |
| Ebp | *ebp* | Binds to elastin (in *S. aureus*) | 161 |
| AtlE and Aae | *atlE* and *aae* | Bind to various matrix proteins | 35,41 |
| ***Intercellular aggregation*** | | | |
| PNAG (also known as PIA) | *icaA*, *icaD*, *icaB* and *icaC* | An intercellular polysaccharide adhesin | 52,56 |
| Biofilm-associated protein Bap (also known as Bhp) | *bap* (also known as *bhp*) | An intercellular protein adhesin | 36 |
| Accumulation-associated protein Aap | *aap* | An intercellular protein adhesin precursor that requires proteolytic processing for its activation | 75,76 |
| Teichoic acids | Multiple biosynthetic genes | Components of the biofilm matrix | 50 |
| ***Protective exopolymers*** | | | |
| PNAG | *icaA*, *icaD*, *icaB* and *icaC* | Protects from IgG, AMPs, phagocytosis and complement | 97,98 |
| PGA | *capA*, *capB*, *capC* and *capD* | Protects from AMPs and phagocytosis | 94 |
| ***Resistance to AMPs*** | | | |
| SepA protease | *sepA* | Involved in AMP degradation | 122 |
| Dlt, MprF, VraF and VraG | *dltA*, *dltB*, *dltC*, *dltD*, *mprF*, *vraF* and *vraG* | Analogous to *S. aureus*, these proteins function in the D-alanylation of teichoic acids (Dlt), lysylation of phospholipids (MprF) and putative AMP export (VraF and VraG) | 111–113 |
| Aps system | *apsR* (also known as *graR*), *apsS* (also known as *graS*) and *apsX* | This system senses AMPs and regulates AMP resistance mechanisms | 110 |
| ***Toxins*** | | | |
| PSMs | *psmα*, *psmδ*, *psmε*, *hld*, *psmβ1* and *psmβ2* | Pro-inflammatory cytolysins | 29,92,106 |
| ***Exoenzymes*** | | | |
| Cysteine protease (SspB and Ecp); *S. aureus* staphopain homologue | *sspB* | Unknown: tissue damage? | 87 |
| Metalloprotease or elastase (SepA); *S. aureus* aureolysin homologue | *sepA* | Involved in lipase maturation, AMP resistance and, potentially, tissue damage | 86,122,153 |
| Glutamylendopeptidase and serine protease (GluSE, SspA and Esp); *S. aureus* V8 protease homologue | *sspA* | Degradation of fibrinogen and complement factor C5 | 87,88 |
| Lipases GehC and GehD | *gehC* and *gehD* | Persistence in fatty acid secretions? | 154–156 |
| ***Other factors*** | | | |
| Staphyloferrins | *sfna* locus (*S. aureus* staphyloferrin A) | Siderophores (iron acquisition) | 157,158 |
| SitA, SitB and SitC | *sitA*, *sitB* and *sitC* | An iron importer | 159 |
| FAME | Unidentified | Detoxification of bactericidal fatty acids | 160 |

AMP, antimicrobial protein; FAME, fatty acid modifying enzyme; IgG, immunoglobulin G; PGA, poly-γ-glutamic acid; PNAG, poly-*N*-acetylglucosamine; PSM, phenol-soluble modulin.

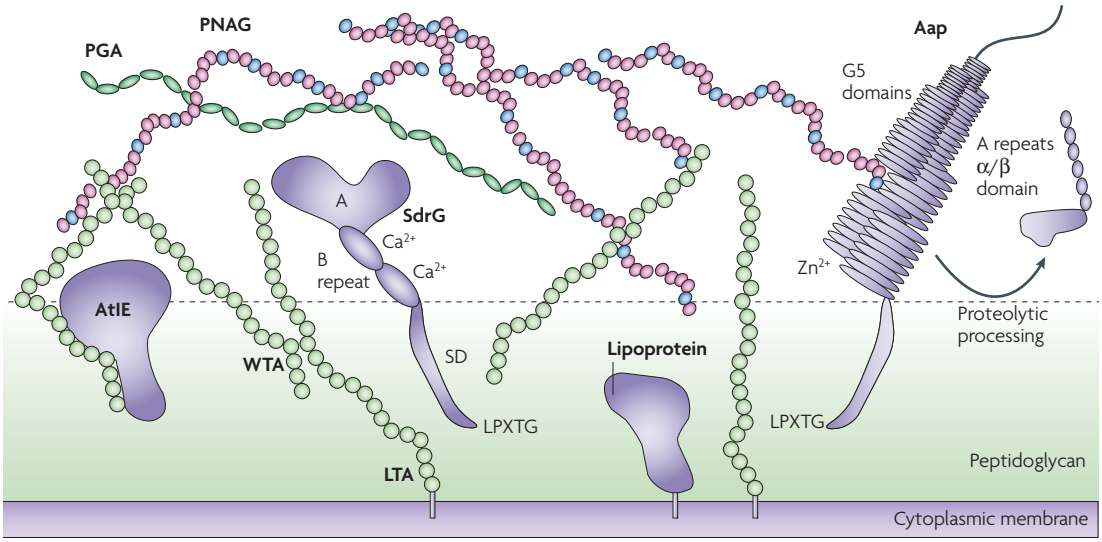


Figure 2 | **The *Staphylococcus epidermidis* cell surface.** Proteins such as SdrG and Aap can be attached to the cell surface through sortase-catalysed covalent anchoring. These proteins harbour a characteristic LPXTG motif at the carboxyl terminus, the threonine residue of which is linked to peptidoglycan. Many autolysins, such as AtlE, are anchored non-covalently, probably through interactions with teichoic acids. Furthermore, lipoproteins are attached to the surface through a fatty acid anchor that penetrates the cytoplasmic membrane. AtlE is a bifunctional adhesin and autolysin that contributes to biofilm formation through its surface hydrophobicity and by binding to host matrix proteins. SdrG is an example of the Sdr protein family of MSCRAMMs (microbial surface components recognizing adhesive matrix molecules). Its serine/aspartate (SD) repeat region spans the peptidoglycan layer and its A region binds fibrinogen. The B repeats harbour a $Ca^{2+}$ binding EF-hand domain. Aap proteins aggregate through $Zn^{2+}$-dependent G5 domains and form fibrils that are likely to connect cells in the biofilm matrix. G5 domains also bind *N*-acetylglucosamine and can therefore interact with poly-*N*-acetylglucosamine (PNAG, also known as PIA). In a step that is crucial for the function of Aap in intercellular aggregation, the amino terminal region of the protein, comprising A repeats and the globular α/β domain, is proteolytically removed. PNAG is cationic and probably interacts with negatively charged surface polymers such as lipoteichoic acids (LTAs), wall teichoic acids (WTAs) and poly-γ-glutamic acid (PGA). Green shading represents negative charge and blue shading represents positive charge.

site in the Bβ-chain of fibrinogen using a 'dock, lock and latch' mechanism[45]. This mechanism is thought to lead to a stabilized MSCRAMM–ligand interaction. Expression of SdrG increases in an *in vivo* environment[46] and antibodies to SdrG are present in human blood[42], emphasizing the importance of SdrG for *S. epidermidis* infection. Recently, an important role has also been demonstrated for SdrF during ventricular assist device driveline-related infection[47]. Several additional *S. epidermidis* MSCRAMMs have been predicted and have undergone preliminary characterization[48], although their role in matrix protein binding and virulence is not yet understood.

After initial adhesion, biofilms develop through intercellular aggregation that is mediated by many different surface macromolecules. These include exopolysaccharide and certain proteins, which seem to be predominantly dedicated to the formation of the extracellular biofilm matrix. In addition, teichoic acids[49,50] and extracellular DNA originating from lysed cells[51] can have accessory functions in aggregation, which are likely to be dependent on their polyanionic character (FIG. 1).

Many *S. epidermidis* strains produce a poly-*N*-acetylglucosamine (PNAG) homopolymer, also named PIA, that surrounds and connects *S. epidermidis* cells in a biofilm[52] (FIG. 3). This polymer, which differs from other PNAG polymers found in nature (such as chitin) by its β1–6 linkage[52], has recently also been detected in many other microorganisms, including *Yersinia pestis* and *Escherichia coli*[53,54]. Production of PNAG is crucial for biofilm formation *in vitro*[55,56] and has a substantial impact on *S. epidermidis* biofilm-associated infection in most animal models[57–61]. The biosynthesis of PNAG is accomplished by the gene products of the *ica* (intercellular adhesion) locus[56]. IcaA and IcaD produce a chain from activated *N*-acetylglucosamine (GlcNAc) monomers, the elongation of which is dependent on the IcaC protein, probably owing to the predicted exporter function of IcaC[62]. Partial de-acetylation of the GlcNAc residues is accomplished by the cell surface-located enzyme IcaB after export[63]. De-acetylation introduces positive charges into the otherwise-neutral polymer that are important for surface binding of PNAG and its various biological functions in biofilm formation and immune evasion, which are discussed below[63]. Production of PNAG is subject to a range of regulatory influences[64], including many global virulence regulators[65–71] but excluding the quorum sensing regulator *agr*[72]. It is less well understood which environmental signals control PNAG expression, particularly *in vivo*, but the complexity of this regulation highlights the importance of PNAG for *S. epidermidis* pathophysiology.

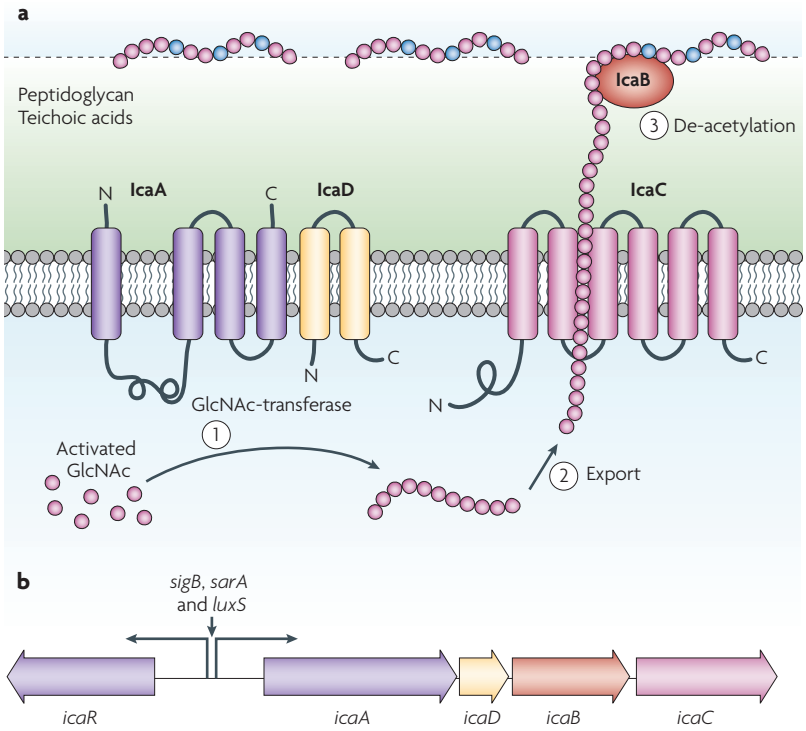


Figure 3 | **The exopolysaccharide poly-*N*-acetylglucosamine. a** | The exopolysaccharide poly-*N*-acetylglucosamine (PNAG; also known as PIA), a partially de-acetylated β1-6-linked *N*-acetylglucosamine (GlcNAc) homopolymer involved in immune evasion and biofilm aggregation, is synthesized by the membrane-located GlcNAc transferase IcaA, which needs the accessory IcaD membrane protein for activity (step 1). The growing PNAG chain is probably exported by the IcaC membrane protein (step 2). After export, IcaB de-acetylase, located on the cell surface, removes some of the *N*-acetyl groups, giving the polymer a cationic character that is essential for surface attachment (step 3). **b** | The Ica proteins are encoded by the *ica* gene locus containing the *icaADBC* operon and the *icaR* gene, which encodes a regulatory protein. Expression of the *icaADBC* operon is regulated either directly at the *icaA* promoter or through expression of IcaR, both of which are controlled by a series of global regulatory proteins (SigB, SarA and LuxS). Furthermore, insertion and excision of the IS*256* element can turn PNAG expression off and on. Green shading represents negative charge and blue shading represents positive charge. C, carboxyl; N, amino.

More recently, it was recognized that PNAG is not essential for biofilm formation in all *S. epidermidis* strains: strains that lack the *ica* genes can form biofilms[73] and *ica*-negative *S. epidermidis* strains have been isolated from biofilm-associated infections[74]. In some strains, biofilm formation may be additionally or exclusively mediated by specific surface proteins, namely Bap[36] and Aap[75]. The Aap protein requires proteolytic activation[76] and zinc ions[77] for its biofilm-promoting effect. $Zn^{2+}$ is crucial for the modular association of so-called G5 tandem repeats[77], which may underlie the formation of Aap-based fibril-like structures on the bacterial surface[78] (FIG. 2). The same domains are known to interact with GlcNAc and can therefore potentially bind to PNAG, forming a protein–polysaccharide biofilm network[79]. Because *in vitro* biofilm formation can be prevented by a chelating agent in the strong biofilm-forming strain *S. epidermidis* RP62A, it has been suggested that biofilm formation in this strain is solely dependent on Aap[77]. In support of this observation, monoclonal antibodies against Aap prevent biofilm formation in this strain[80]. However, this hypothesis is inconsistent with other reports that supported dependence on PNAG[81] and did not find protein-mediated biofilm formation to be important in the same strain[82]. Therefore, the contribution of proteins to *S. epidermidis* biofilm formation and to the mechanisms involved will require intensive further investigation. In addition, the finding that biofilms created solely with proteins are not as robust as those created with PNAG[74] indicates that both proteins and exopolysaccharide participate in efficient *S. epidermidis* biofilm formation.

***Biofilm detachment.*** In contrast to intercellular aggregation, biofilm structuring and detachment are poorly understood in *S. epidermidis*. We know that biofilm detachment in *S. epidermidis* is controlled by the quorum sensing system *agr*, because biofilms that are dysfunctional in the *agr* system are thicker and have an obvious defect in detachment[72,83]. In *S. aureus,* a model has been proposed that involves *agr* expression in the exposed layers of a biofilm and promotes the detachment of cell clusters from the biofilm surface, thereby controlling biofilm expansion[84]. Likewise, *S. epidermidis agr* activity is limited to the biofilm surface[83], indicating that there is 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 interactions by detergent-like molecules (FIG. 1). Enzymatic degradation of proteinaceous biofilm factors has been suggested as a mechanism of biofilm detachment in *S. aureus*[85], but evidence for such a function of proteases in *S. epidermidis* has not been obtained. However, *S. epidermidis* does produce a series of exoproteases with low substrate specificity that may serve to degrade surface proteins[86–88]. As for degradation of biofilm exopolysaccharide, staphylococci do not seem to have a dedicated enzyme for PNAG hydrolysis, in contrast to several other bacteria that produce PNAG[89,90]. Alternatively, detergent-like molecules can disrupt non-covalent (such as electrostatic and hydrophobic) interactions that occur, for example, between the cationic PNAG and anionic surface polymers or between hydrophobic regions of the bacterial surface. The short amphipathic phenol-soluble modulins (PSMs) (for example, the *S. epidermidis* δ-toxin; FIG. 4) have been proposed to have such a function[91]. *S. epidermidis* PSMs and exoproteases are strictly *agr*-regulated[92,93], lending support to the idea that they may be involved in biofilm structuring.

***Protective exopolymers.*** *S. epidermidis* produces exopolymers, namely poly-γ-glutamic acid (PGA) and PNAG, that protect the bacterium from important mechanisms of innate host defence. 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 its low levels of production[94]. Except for *Bacillus anthracis*[95], *S. epidermidis* is the

Pseudopeptide
A peptide that is formed by peptide bonds through carboxyl groups other than the α-carboxyl group.

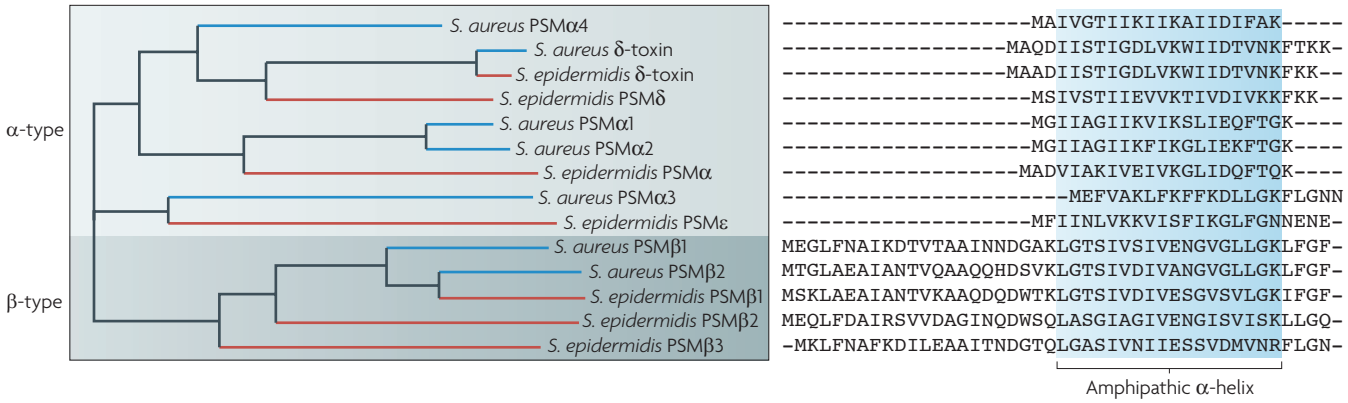


Figure 4 | **Phenol-soluble modulins.** A sequence alignment of *Staphylococcus epidermidis* and *Staphylococcus aureus* phenol-soluble modulins (PSMs) is shown. PSMs serve as immune evasion molecules to their bacterial producer and as pathogen-associated molecular patterns (PAMPs) for pathogen recognition to the host. All PSMs contain an amphipathic α-helix and amino-terminal *N*-formyl methionine, as they are secreted without post-translational processing, in an unknown manner. PSMs of the α-type are short, containing approximately 20–25 amino acids. The *S. aureus* PSMα peptides 1–4 are strongly cytolytic. PSMs of the β-type are longer (~45 amino acids) and do not have any substantial 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. Despite being part of the *psmβ* 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*.

only known organism in which PGA has a function in pathogenesis. Furthermore, PGA promotes the growth of *S. epidermidis* at high salt concentrations and is induced under these conditions[94]. This is reminiscent of PGA production in many halophilic bacteria, in which PGA is thought to contribute to osmotolerance[96], and indicates a role for PGA during *S. epidermidis* colonization. Finally, expression of the *cap* genes seems to be increased during the biofilm mode of growth[29]. Interestingly, PGA is present in many CoNS but is absent from *S. aureus*[9].

In addition to its role as part of the extracellular biofilm matrix, PNAG has been found to protect *S. epidermidis* from neutrophil killing, complement deposition, immunoglobulins and AMPs[97,98], and also from *Caenorhabditis elegans* immune defences in a nematode infection model[99]. The cationic PNAG protects cells 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[98]. It may therefore also work by sequestering oppositely charged AMPs in a similar way to the proposed mechanism of protection from tobramycin by *Pseudomonas aeruginosa* alginate[100].

***Pathogen-associated molecular patterns.*** Pathogen-associated molecular patterns (PAMPs) are structures on the bacterial surface that the innate immune system recognizes as non-self through dedicated pathogen recognition receptors (PRRs), such as the Toll-like receptors (TLRs)[52]. PAMPs such as lipoproteins and lipoteichoic acids are common in Gram-positive bacteria. Recognition of PAMPs activates host defence mechanisms that include phagocytosis and cytokine release[101]. Furthermore, there are reports suggesting that several additional molecules that are specific to *S. epidermidis* may stimulate the innate host defence response. For example, PNAG was reported to stimulate TLR2 (REF. 102). Recognition of PNAG by the human immune system would be an interesting example of the hide-and-seek interplay between pathogen and host, as this would mean that a substance used by *S. epidermidis* for immune evasion can trigger innate host defence mechanisms. However, this has not been confirmed using genetic deletion mutants, which would be important to rule out the possibility that contaminating pro-inflammatory substances (for example, lipoproteins) were the basis of the observed effect; such contamination has led to frequent misidentification of alleged TLR2 stimulators[103–105]. Similarly, the pro-inflammatory capacities of *S. epidermidis* PSMs[106] have not yet been confirmed using synthetic peptides or gene deletion mutants. However, *S. epidermidis* PSMs are similar to *S. aureus* PSMs, for which host defence triggering activity has been confirmed[107], which indicates that the described pro-inflammatory effect of *S. epidermidis* PSMs is genuine, although the activation of TLR2 by PSMs[108] has not been verified. Finally, an unusual short-chain pro-inflammatory lipoteichoic acid has been described in *S. epidermidis*[109]. However, chemical characterization of the purified molecule did not indicate that this molecule is a teichoic acid-related polymer, and thus the identity of this molecule and its pro-inflammatory activity remains unknown. Therefore, there is a clear need for further characterization of *S. epidermidis* molecules that activate host defences.

***Sensing antimicrobial peptides.*** Just as the human immune system recognizes *S. epidermidis* PAMPs, *S. epidermidis* has mechanisms to sense the presence of harmful molecules produced by the host. An AMP-sensing system termed *aps* has been identified that is activated by a range of AMPs and triggers upregulation of staphylococcal AMP-defensive mechanisms[110] (FIG. 5). These mechanisms include the D-alanylation of teichoic acids[111] and

Pathogen-associated molecular pattern
A surface structure on pathogens that is recognized by the innate immune system as non-self and triggers activation of innate host defence, usually by binding to Toll-like receptors.

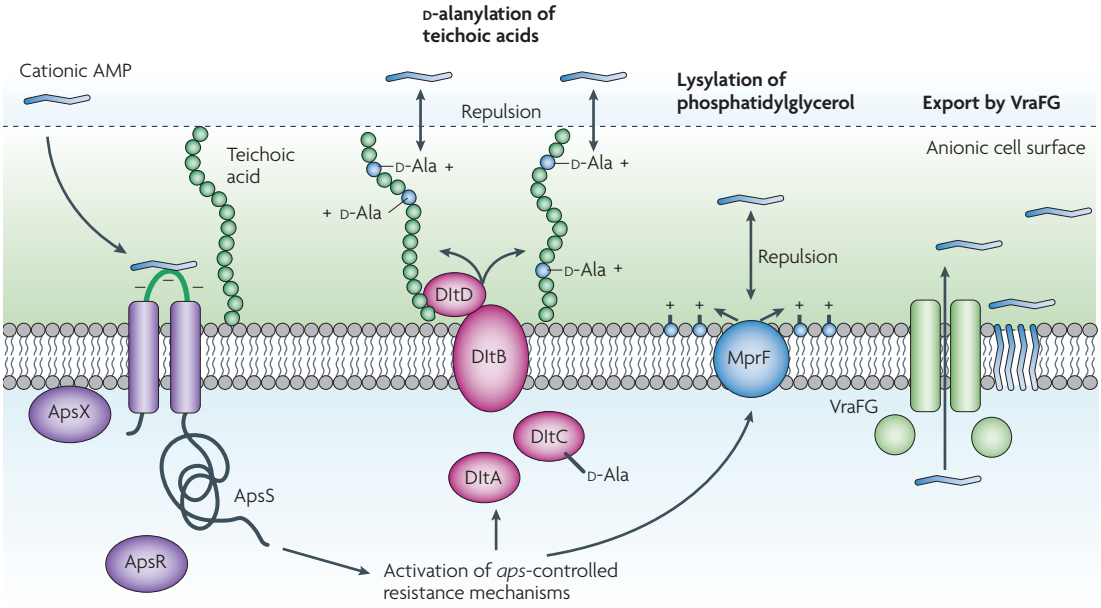


Figure 5 | **The antimicrobial peptide sensor and regulator Aps.** Cationic antimicrobial peptides (AMPs) attach to the negatively charged bacterial surface and membrane by electrostatic interactions, a prerequisite for AMP antimicrobial activity that is often based on pore formation in the bacterial cytoplasmic membrane. The *Staphylococcus epidermidis* ApsS AMP sensor has one short extracellular loop with a high density of negatively charged amino acid residues that interact with cationic AMPs. Transduction of this interaction signal through ApsS and the essential accessory ApsX, which has an unknown function, triggers the expression of key AMP resistance mechanisms. The D-alanylation of teichoic acids, which is carried out by the products of the *dlt* operon, and lysylation of phosphatidylglycerol, which is catalysed by the MprF enzyme, result in the decreased negative charge of the cell surface and membrane, respectively, leading to decreased attraction or repulsion of cationic AMPs. The VraF and VraG ABC transporter also promotes resistance to AMPs and probably functions as an AMP exporter. Green shading represents negative charge and blue shading represents positive charge.

the lysylation of phospholipids by the MprF enzyme[112], both of which decrease the anionic charge of the bacterial surface, thereby preventing efficient attraction of cationic AMPs. Additionally, the VraF and VraG proteins[113] possibly function as an AMP exporter by removing AMPs from the cytoplasmic membrane. Therefore, the *aps* system — the first example of an AMP sensor in Gram-positive bacteria — has a similar function to the PhoP–PhoQ AMP sensor found in Gram-negative bacteria[114], but is not evolutionarily related. Importantly, the activation and protective response of the *aps* system is limited to cationic AMPs. Furthermore, the *aps* system represents a unique example of a three-component sensor or 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 (also known as GraS) and the response regulator protein, ApsR (also known as GraR).

Two-component system
A bacterial sensory system composed of a membrane-located sensor (histidine kinase) and a cytoplasmic DNA-binding regulatory protein (response regulator). The autophosphorylation-dependent activation of two-component systems is triggered by an extracellular signal.

Enterotoxin
A protein toxin released by a microorganism into the intestine of its host.

## 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. Although strain-specific production of enterotoxins has been described[115,116], *S. epidermidis* is not generally accepted as an enterotoxin producer. By contrast, in an evaluation of ~200 *S. epidermidis* strains, all were found to produce PSMs except those strains that were naturally *agr*-dysfunctional[72,92,117] (FIG. 4). PSMs are characteristically short, amphipathic, α-helical peptides and have pro-inflammatory and sometimes cytolytic functions. The *S. epidermidis* δ-toxin (also called PSMγ), a 24-amino acid peptide that differs from its *S. aureus* homologue in only one amino acid position, has been suggested to be involved in necrotizing enterocolitis in neonates[118]. Some *S. epidermidis* PSMs are related to *S. aureus* PSMs that have a pronounced capacity to lyse human neutrophils[107]. However, the PSM production pattern in *S. epidermidis* shows strong production of only the moderately cytolytic δ-toxin and non-cytolytic β-type PSMs[29]. Therefore, the PSM production pattern in *S. epidermidis*, in addition to the general absence of highly aggressive toxins in this species, is in contrast with the high cytolytic potential of *S. aureus*. This underpins the Massey *et al.*[23] model, which proposes an evolutionary advantage for the low aggressiveness of *S. epidermidis*.

## Colonization and pathogenesis

Several studies have attempted to identify the determinants that distinguish *S. epidermidis* strains which can cause infection from those that live on the skin. These studies either focused on putative virulence determinants or used genome-wide approaches such as comparative genomic hybridization[17–19,119]. Two main putative determinants of *S. epidermidis* invasiveness were identified in these studies: the *ica* genes, which regulate the production of PNAG, and the insertion element IS*256*. IS*256* is

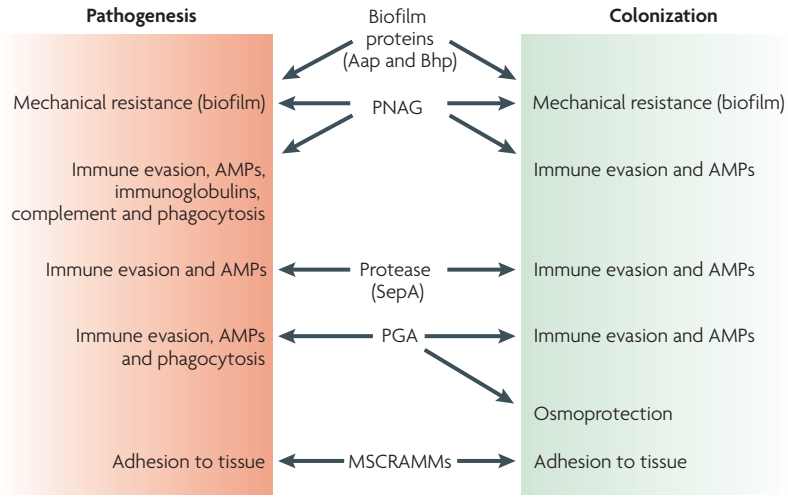


Figure 6 | ***Staphylococcus epidermidis* as a commensal and infectious microorganism.** Determinants that are thought to contribute to both the colonization and the pathogenesis of *S. epidermidis* are shown, along with their functions. In animal models, only the roles of poly-*N*-acetylglucosamine (PNAG; also known as PIA), poly-γ-glutamic acid (PGA) and the MSCRAMM (microbial surface component recognizing adhesive matrix molecule) SdrG in infection have been determined. Other roles are based on *in vitro* experiments and environmental challenges during colonization and infection. Regulators such as *agr* or *sigB* are not shown; these control many of the determinants shown and may therefore also have important functions during both *S. epidermidis* lifestyles. AMP, antimicrobial peptide.

thought to contribute to the genetic adaptation that may have a role during infection[65]. For example, it may serve to abolish the production of PNAG or the function of the *agr* global virulence regulator by inserting into the *ica* or *agr* loci, respectively[83,120]. The correlation of the presence of PNAG with the invasiveness of the bacteria 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 can even have a selective advantage over the *ica*-positive strains on the skin[121]. However, other evidence suggests that when corrected for clonal relatedness, there are no differences between commensal and infectious strains[119].

Several lines of evidence indicate that most 'virulence factors' of *S. epidermidis* originally had roles in the commensal lifestyle of this species (FIG. 6). The parts played by PNAG, PGA and the SepA protease in protecting the bacterium from AMPs indicate that these polymers also have a key role during life on the skin[63,94,122], where AMPs are a major determinant of innate host defence. Furthermore, intercellular adhesion by PNAG and biofilm-related proteins can be assumed to be vital in an environment such as the skin, where the bacterium experiences considerable mechanical stress. Additionally, the role of PGA in osmotolerance[94] suggests an original function for this polymer in the non-infectious lifestyle of *S. epidermidis*. Moreover, no clear differences have been observed in the number of MSCRAMMS from infectious and commensal strains of *S. epidermidis*, indicating that these proteins are valuable during both infection and colonization. This makes sense, as adhesion to host tissue is considered to be imperative during both lifestyles. *S. epidermidis* should therefore be regarded as an 'accidental' pathogen, the clinical importance of which stems less from a dedicated infectious lifestyle and more from the frequency of contamination events and the existence of 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*. In many countries, including the United States, 75–90% of all hospital isolates of *S. epidermidis* are resistant to methicillin, a first-choice antibiotic against staphylococcal infections; this is even higher than the corresponding rate for *S. aureus* (40–60%)[123]. In some countries, such as The Netherlands, efficient search-and-destroy programmes and strict hygiene measures have succeeded in keeping the prevalence of methicillin-resistant *S. aureus* in hospitals at a low level[124], whereas this has proven much less successful for methicillin-resistant *S. epidermidis*[125]. Resistance to methicillin is encoded on mobile genetic elements (MGEs), namely the staphylococcal cassette chromosome *mec* (SCC*mec*). This cassette chromosome contains the *mecA* gene, which encodes a penicillin-binding protein, PBP2a, with decreased affinity for methicillin compared with the affinities of other PBPs[126]. In *S. epidermidis*, 10 different SCC*mec* structures were identified; the short SCC*mec* type IV element[127] was the most abundant (36%)[128]. SCC*mec* type IV poses a particular problem, as it does not impose a fitness cost to its host and can therefore spread in the absence of selective antibiotic pressure[129]. Interestingly, closely related strains can carry different SCC*mec* types, indicating that *S. epidermidis* frequently loses and acquires SCC*mec* elements[128].

In addition to methicillin resistance, *S. epidermidis* strains have acquired resistance to several other antibiotics, including rifamycin, fluoroquinolones, gentamycin, tetracycline, chloramphenicol, erythromycin, clindamycin and sulphonamides[5]. Resistance to streptogramins, linezolid and tigecycline also occurs, although rarely. Most antibiotic resistance genes are plasmid-encoded and are more often found in methicillin-resistant than methicillin-susceptible strains[130]. This is probably due to the fact that resistance to methicillin and other antibiotics is frequent among endemic nosocomial strains. Despite widespread resistance to methicillin and other antibiotics, 80% of catheters infected with *S. epidermidis* can still be treated with antibiotics such as vancomycin, without catheter removal[131]. However, intermediate resistance to vancomycin has also been described[132] and staphylococcal biofilm formation significantly decreases the activity of vancomycin and other antibiotics[133–135].

The frequency of antibiotic resistance in *S. epidermidis* reflects the overuse of antibiotics. 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 bacterium, such as SCC*mec* elements. Accordingly, there is evidence suggesting that methicillin resistance cassettes

Methicillin
A penicillin derivative that is resistant to penicillinase (an enzyme widespread in staphylococci that provides resistance to penicillin).

Mobile genetic element
DNA such as a plasmid or transposon that can be exchanged between bacteria by horizontal gene transfer. Mobile genetic elements often carry virulence or antibiotic resistance genes.

were transferred from *S. epidermidis* to *S. aureus*[128,136]. The acquisition of SCC*mec* type IV by community-associated methicillin-resistant *S. aureus* (CA-MRSA)[127] may have had an enormous impact on public health: it created a strain with both methicillin resistance, at no cost to fitness, and exceptional virulence, which was the molecular basis behind the epidemic caused by CA-MRSA[137]. CA-MRSA also acquired other MGEs that may be important for efficient colonization by horizontal gene transfer from *S. epidermidis*[138]. These findings show that *S. epidermidis* provides a 'reservoir' function for the transfer of genetic elements to enhance the pathogenic success of *S. aureus*, and therefore has an important role in human disease.

These considerations highlight the need for prophylactic measures against *S. epidermidis* infections. Vaccination and decolonization, measures that are often discussed for other pathogens including *S. aureus*, do not seem to be appropriate for *S. epidermidis*. First, there is no anti-staphylococcal vaccine and several lines of evidence indicate that it may be difficult to use traditional active immunization for staphylococci[139,140]. 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 is fast, but it may also turn out to be counterproductive, as it may allow potentially more harmful microorganisms to take the place of *S. epidermidis*. Therefore, it is commonly agreed that the best way to deal with *S. epidermidis* infections is by prevention, which includes sterilization of medical equipment and of body parts of patients and those health care personnel who are in contact with indwelling medical devices during surgery[5].

## Unidirectional horizontal gene transfer?

Interestingly, although there is evidence to suggest that *S. epidermidis* can frequently transfer MGEs to *S. aureus*[136,138], this transfer seems to be one way: *S. epidermidis* does not contain toxin genes, despite the fact that acquisition of such genes from *S. aureus* using a similar mechanism would seem easy. The recent investigation of clustered regularly interspaced short palindromic repeat (CRISPR) sequences, short repeats that are involved in preventing the uptake of conjugative elements such as phages and conjugative plasmids, may explain why the transfer of MGEs between *S. epidermidis* and *S. aureus* is unidirectional[141]. These sequences have been found in *S. epidermidis*, in one of the two genome-sequenced strains[9], but not in any of the many known *S. aureus* genomes. CRISPR-mediated prevention of MGE uptake in *S. epidermidis* clearly needs to be further evaluated, as this mechanism may represent a molecular basis for the absence of a highly diverse toxin repertoire and the resulting lack of aggressive virulence in *S. epidermidis*.

## Outlook

Knowledge about the molecular mechanisms of biofilm formation and their regulation in *S. epidermidis* is almost exclusively based on *in vitro* research. The contribution of some determinants such as PNAG[57–61], AtlE[142], SdrG[44], SdrF[47] and the regulators *agr*[83], *luxS*[71] and *sigB*[143] to pathogenesis has been demonstrated using animal models. Furthermore, there is evidence indicating that important biofilm factors are expressed *in vivo*[61,144]. Nevertheless, there is an urgent need for more detailed *in vivo* research that could provide 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 endeavours[145].

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 the determinants that ensure the survival of *S. epidermidis* in its natural habitat; the development of skin colonization models would be particularly valuable.

Finally, the interaction of *S. epidermidis* with other bacteria and its reservoir function for genes that can 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 sequences of additional *S. epidermidis* strains (particularly those of ST2) that seem to be most widely distributed among infectious isolates[15].


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## DATABASES
**Entrez Gene:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene
*hld* | *luxS* | *mecA*
**Entrez Genome Project:** http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=genomeprj
*Bacillus anthracis* | *Caenorhabditis elegans* | *Escherichia coli* | *Pseudomonas aeruginosa* | *Staphylococcus aureus* | *S. epidermidis* ATCC 12228 | *S. epidermidis* RP62A | *Yersinia pestis*
**UniProtKB:** http://www.uniprot.org
δ-toxin | Aap | AtlE | GraR | GraS | IcaA | IcaB | IcaC | IcaD | MprF | SdrF | SdrG | SdrH | TLR2

## FURTHER INFORMATION
**Michael Otto's homepage:** http://www3.niaid.nih.gov/labs/aboutlabs/lhbp/pathogenMolecularGeneticsSection/otto.htm

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