Pathogenesis of infections due to coagulase-negative staphylococci

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As a group, the coagulase-negative staphylococci (CoNS) are among the most frequently isolated bacteria in the clinical microbiology laboratory and are becoming increasingly important, especially as causes of hospital-acquired infections. These bacteria are normal inhabitants of human skin and mucous membranes and, therefore, one of the major challenges of daily diagnostic work is to distinguish clinically significant CoNS from contaminant strains. This overview addresses current knowledge of the pathogenesis of infections due to CoNS and particularly focuses on virulence factors of the species *Staphylococcus epidermidis. S epidermidis* has been identified as a major cause of nosocomial infections, especially in patients with predisposing factors such as indwelling or implanted foreign polymer bodies. Most important in the pathogenesis of foreign-body-associated infections is the ability of these bacteria to colonise the polymer surface by the formation of a thick, multilayered biofilm. Biofilm formation takes place in two phases. The first phase involves the attachment of the bacteria to polymer surfaces that may be either unmodified or coated with host extracellular matrix proteins. In the second phase, the bacteria proliferate and accumulate into multilayered cell clusters that are embedded in an extracellular material. The bacterial factors involved in both phases of biofilm formation are discussed in this review. In addition, the most important aspects of the pathogenic potential of *S saprophyticus, S lugdunensis*, and *S schleiferi* are decribed, although, compared with *S epidermidis*, much less is known in these species concerning their virulence factors.

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Coagulase-negative staphylococci as nosocomial pathogens

Data taken from the National Nosocomial Infections Surveillance System from January 1990 until May 1999 showed that coagulase-negative staphylococci (CoNS) are the most commonly reported pathogens (37·3%, compared with 12·6% for *Staphylococcus aureus)* isolated from bloodstream infections in intensive care unit patients.[1] However, recognition of infection is hampered by the difficulty in distinguishing the infecting strain from the normal flora. CoNS have long been dismissed as culture contaminants since they are commonly seen among the normal flora of human skin and mucous membranes. They are ubiquitous commensals of the body including the mouth and throat, while certain species often inhabit characteristic ecological niches. For example, *S capitis* is seen mostly on the head, while *S auricularis* is isolated almost exclusively from the external auditory meatus. Treatment is also difficult because many CoNS carry multiple antibiotic resistances and even glycopeptide resistance is emerging. Nowadays CoNS are assuming greater importance as true pathogens and their increasing incidence have been noted.[2,3]

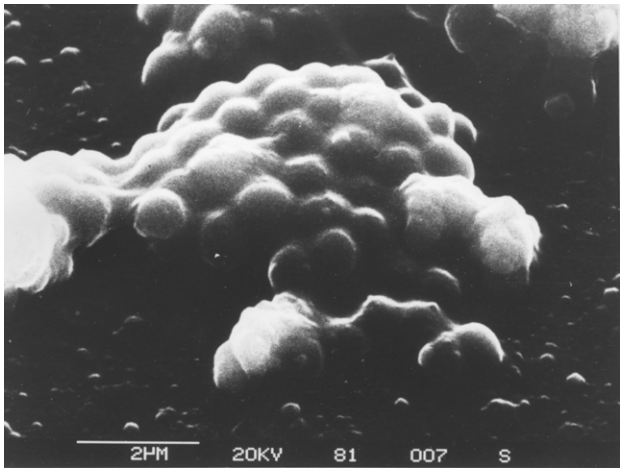

Figure 1. Most important in the pathogenesis of foreign-body-associated infections due to S epidermidis *is the colonisation of the polymer surface by formation of a biofilm composed of multilayered cell clusters that are embedded in an extracellular slime substance. The presence of such large biofilms has been shown by scanning electron microscopy on explanted intravascular catheters.*[16]

Of 32 CoNS species validly published, only half are seen in specimens of human origin. Most recently, CoNS were isolated from clinical specimens that significantly differed from all other *Staphylococcus* species based on phenotypic characteristics and 16 rRNA gene sequencing. Therefore, a novel staphylococcal species designated *S pettenkoferi* was proposed.[4]

CoNS can be divided into two groups depending on whether they are resistant to or susceptible to novobiocin. Those that are indigenous to human beings include the novobiocin-susceptible species *S epidermidis, S haemolyticus, S hominis, S lugdunensis, S schleiferi*, as well as the novobiocin-resistant species *S saprophyticus* and *S xylosus.*[3,5]

Clinical manifestations of infections due to most CoNS markedly differ from those of *S aureus* infections. Normally, the clinical picture is subtle and non-specific, and the clinical course more subacute or even chronic without fulminant signs of infection. Coagulase-negative staphylococcal

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bacteraemia is rarely life-threatening, especially if treated promptly and adequately. However, frank sepsis syndrome and fatal outcome may occur, especially in immunocompromised patients and/or if one of the more virulent species, such as *S lugdunensis*, is involved.[6–8]

The importance of CoNS as nosocomial pathogens has prompted more interest in their detailed characterisation. Research on CoNS has proceeded on several fronts, including development of more accurate methods for identifying species, for distinguishing infecting from contaminating isolates, and for epidemiological typing of strains. Furthermore, various virulence factors involved in the pathogenesis of infections due to CoNS, especially of polymer-associated staphylococcal infection, have been isolated and characterised during the past decade.

Spectrum of disease due to novobiocin-susceptible CoNS

Novobiocin-susceptible CoNS, particularly *S epidermidis*, have emerged as a major cause of nosocomial infections, and of nosocomial bacteraemia in particular. These microorganisms usually infect immunocompromised patients, such as premature babies and patients hospitalised for chemotherapy, other malignant diseases, or organ transplantation.[7–9]

In intravenous heroin users who develop right-sided endocarditis *S epidermidis* is the most frequently isolated causative organism. The drug injected intravenously may cause microlesions of the tricuspid valvular endothelium. Repeated episodes of *S epidermidis* bacteraemia as a result of non-sterile injections might then lead to tricuspid valve endocarditis.

The most important group of particularly susceptible patients for infection due to novobiocin-susceptible CoNS comprises those with indwelling or implanted foreign polymer bodies, with *S epidermidis* responsible for 50–70% of catheter-related infections. About 50% of all patients receive an intravascular device during their hospital stay, and polymer-associated infection rates for central-line-associated bloodstream infections range between 4·5/1000 central-line days in medical/surgical intensive care units and 14·6/1000 central-line days in burn units.[2] CoNS, especially *S epidermidis*, are also the predominant species in other polymer-associated infections. Depending on the kind of device and its insertion site, different infection syndromes generate several clinical presentations.[9,10]

Furthermore, there is growing evidence that other, more chronic, polymer-associated clinical syndromes may also be at least partly associated with CoNS, particularly with *S epidermidis*. These syndromes include the aseptic loosening of hip or other joint prostheses,[11] fibrous capsular contracture syndrome after mammary augmentation with silicone prostheses,[12] and late-onset endophthalmitis after implantation of artificial intraocular lenses after cataract surgery.[13] In these studies, identical clones were isolated at different times and/or at various multiple sites, indicating the significance of the isolated bacteria.[11–13]

The only infection in the immunocompetent adult host that is generally accepted to be caused by *S epidermidis* without the involvement of a foreign body is native valve endocarditis. There are reports of osteomyelitis, wound infection, otitis media, endophthalmitis, urinary tract infection, meningitis, and even pneumonia caused by *S epidermidis* but there is no definitive evidence, particularly for meningitis and pneumonia, that *S epidermidis* can cause these diseases in the absence of a foreign body.[2,6,10]

Pathogenesis of polymer-associated infection due to *S epidermidis*

The pathogenesis of foreign-body-associated infections with *S epidermidis* is characterised by the ability of this species to colonise the polymer surface by the formation of a thick, multilayered biofilm. Small numbers of bacteria from the patient's skin or mucous membranes, where these bacteria normally occur, probably contaminate the polymer during the surgical implantation of the device. Sometimes the bacteria are acquired from the hands of the surgical or clinical staff. Recently published guidelines for the prevention of intravascular catheter-related infections may help to reduce the incidence of infections related to the insertion of foreign bodies.[14] Biofilm formation is a two-step process: first, the bacteria rapidly adhere to the polymer material. During the following accumulation phase, the bacteria proliferate to form multilayered cell clusters on the polymer surface, which are embedded in extracellular material.[15] The presence of such large adherent biofilms on the surfaces of foreign bodies has been shown by scanning electron microscopy (figure 1).[16] In the past 5 years, significant progress has been made in the definition of molecular mechanisms involved in *S epidermidis* biofilm formation.

Attachment to unmodified polymer surfaces.

Microbial adherence to biomaterials largely depends on bacterial cell surface characteristics and on the nature of the polymer material. The initial interactions involve non-specific physicochemical forces such as van der Waal's forces, hydrophobic interactions, and polarity (figure 2).

Initial adherence and cell surface hydrophobicity have been associated with bacterial surface-associated proteins. The two antigenically related staphylococcal surface proteins—SSP-1 (280 kDa) and SSP-2 (250 kDa)—are fimbria-like polymers and contribute to the adherence of *S epidermidis* strain 354 to polystyrene.[17] The genes encoding these proteins have not been identified.

Other proteins also seem to be involved in the initial attachment phase of biofilm formation. After Tn917 mutagenesis, we isolated biofilm-negative mutants from the clinical isolate *S epidermidis* O-47.[18] *S epidermidis* mut1 had a reduced ability to attach to polystyrene and lacked surface-associated proteins. Complementation of mut1 with the DNA fragment that was inactivated in mut1 fully restored biofilm formation. Sequencing of the DNA fragment showed that initial attachment of the cells to a polystyrene surface is mediated, at least in part, by the surface-associated autolysin AtlE (see below).[19]

Recently, the biofilm-associated protein Bap, was reported to contribute to *S aureus* biofilm formation.[20]

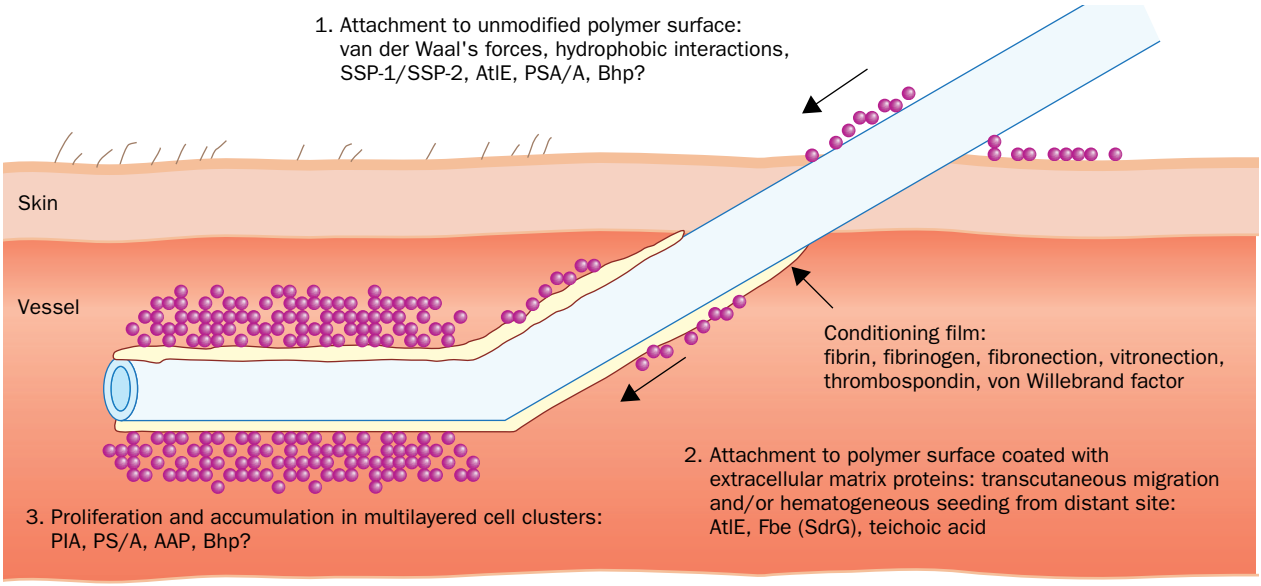

Figure 2. Schematic model of the phases involved in S epidermidis *biofilm formation and bacterial factors involved. Bhp whose homologous counterpart in* S aureus *has been shown to be involved in* S aureus *biofilm formation is labelled with a question mark. SSP-1/SSP-2=staphylococcal surface proteins, AtlE=autolysin, PS/A=polysaccharide/adhesin, Bhp=biofilm-associated (Bap)-homologous protein, Fbe=fibrinogen-binding protein, SdrG=serine-aspartate-repeat-containing protein G, PIA=polysaccharide intercellular adhesin, AAP=accumulation-associated protein.*

S aureus mutants with a Tn917 insertion in *bap* had reduced adherence to a polystyrene surface, and their ability for intercellular adhesion and biofilm formation was markedly reduced. Thus, Bap seems to participate in both phases of biofilm formation. The *bap* mutants were less persistent in a mouse foreign body infection model by comparison with the wild type delineating the in-vivo importance of Bap. The *bap* gene codes for a novel cell-surface-associated protein with a predicted molecular mass of 238·7 kDa. Bap has been found to be present in only 5% of 350 bovine mastitis *S aureus* isolates tested and was absent in all 75 clinical human *S aureus* isolates tested. However, a gene encoding the Bap-homologous protein Bhp (Tormo et al, unpublished; EMBL database accession number AAK29746) is encoded by the genome of the clinical isolate *S epidermidis* RP62A. Bhp has a predicted molecular mass of 258 kDa and may contribute to *S epidermidis* biofilm formation.

Aside from proteins, a polysaccharide, capsular polysaccharide/adhesin (PS/A), has been associated with initial adherence and slime production. PS/A-deficient Tn917 mutants are less virulent in a rabbit model of endocarditis.[21] Moreover, immunisation with PS/A results in protection against infection.

Attachment via interaction with host extracellular matrix proteins.

The direct interaction between bacteria and the unmodified polymer surface plays a critical part in the early stages of the adherence process in vitro and probably also in vivo. After implantation of the medical device, the polymer material rapidly becomes coated with plasma and extracellular matrix proteins such as fibronectin, fibrinogen, vitronectin, thrombospondin, and von Willebrand factor.[22–24] Thus, in later stages of infection, plasma and extracellular matrix proteins deposited on the polymer surface facilitate colonisation by pathogens—eg, *S aureus*, which produces specific cell-surface receptors for those host factors.[25,26] *S epidermidis* also seems to produce surface proteins that may be involved in the interaction with host factors and therefore in biofilm formation on protein-coated implants. Adherence of clinical isolates of coagulase-positive and coagulase-negative staphylococci to biomaterials is enhanced by surface-bound fibronectin.[27] Adherence of all *S aureus* strains tested is significantly promoted by immobilised fibrinogen, while adherence of *S epidermidis* strains to fibrinogen varies significantly among strains.

Several genes for host-factor-binding proteins from *S aureus* have been cloned and sequenced, such as fibronectin-binding proteins and fibrinogen-binding proteins. Much less is known about host-factor-binding proteins from *S epidermidis*. We noted that the surface-associated autolysin AtlE from *S epidermidis* not only is involved in initial attachment of the cells to an unmodified polystyrene surface (see above), but also binds to the extracellular matrix protein vitronectin.[19] This finding suggests that AtlE may also have a role in colonisation of vitronectin-coated foreign material. The importance of AtlE in *S epidermidis* pathogenicity has been shown: the *atlE* mutant was significantly less virulent than the wild type O-47 in an intravascular catheter-associated infection model in rats.[28] Recently, the gene encoding an autolysin (Aas) from *S saprophyticus* has been cloned and sequenced (see below).[29] Aas exhibits significant homology to AtlE, binds to fibronectin, and agglutinates sheep erythrocytes (figure 3). An isogenic *aas* mutant showed decreased binding to fibronectin. The discovery of multifunctional roles of AtlE

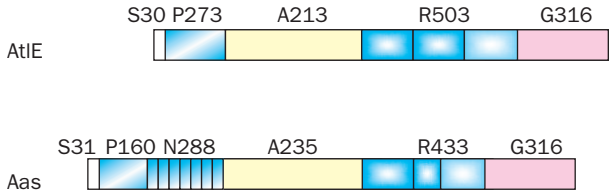

Figure 3. Model of the structural organisation organisation of the autolysins AtlE (1335 aminoacids) and AaS (1463 aminoacids) from S epidermidis *and* S saprophyticus*, respectively (modified from references 19 and 29). The relative positions and sizes (number of amino acids) of their signal sequences (S), propeptides (P), N-terminal repeats (N, Aas only), amidase domains (A), repeats (R) R1, R2, R3, and glucosaminodase domains (G) are indicated.*

and Aas has led to the proposal of a new class of staphylococcal adhesins—the autolysin/adhesins. Bacterial autolysins are peptidoglycan hydrolases that are involved in cell division, cell separation, cell-wall turnover, and antibiotic-induced lysis of bacterial cells. Autolysins are also considered virulence factors. Some autolysins seem to be involved in adherence—eg the autolysin Ami of *Listeria monocytogenes* is involved in adherence to eucaryotic cells. Recently, the eukaryotic cell-binding activity of Ami has been localised to the cell-wall-anchoring domain, which contains multiple repeats with glycine-tryptophane (GW)-motifs.[30] Such GW-containing modules are also seen in the three repetitive domains R1, R2, and R3 of AtlE and Aas and could be involved in the ligand-binding activity of these staphylococcal autolysins.[30]

Besides the autolysin/adhesins, other *S epidermidis* proteins also seem to bind to host proteins. The 119 kDa fibrinogen-binding protein Fbe was identified in the *S epidermidis* strain HB by the phage display technique.[31] Fbe is a member of another recently identified protein family, the serine-aspartate (SD)-repeat-containing (Sdr) family of cell wall-anchored surface proteins. The fibrinogen-binding clumping factor (ClfA) from *S aureus* was the first member of the Sdr protein family that was described. Common features of this protein family are a signal peptide at the N-terminus, a known or putative ligand-binding region A, the SD-repeat region that is predicted to span the cell wall, and a C-terminal cell-wall anchor domain.

The gene encoding Fbe has been cloned and sequenced.[31] The general organisation of Fbe corresponds to that of the other Sdr proteins. Sequence comparison showed that Fbe is similar to the clumping factors ClfA and ClfB. This similarity is not limited to the SD-repeats, but is also seen within the fibrinogen-binding region A. By contrast to ClfA, which binds to the γ chain of fibrinogen, and like ClfB, Fbe binds to the β chain of fibrinogen. A role of Fbe in adhesion has been shown: an *fbe*-deficient mutant showed reduced adherence to fibrinogen immobilised on a polyethylene surface and to peripheral venous catheters that were removed from patients.[32] Addition of purified recombinant Fbe completely inhibited the adherence of *S epidermidis* to immobilised fibrinogen. Fbe antibodies also efficiently blocked that adherence reaction, suggesting the possibility of immunoprophylactic therapy against device-associated infections.[33,34] The potential of immunoprotection seems even more attractive since PCR analysis showed that the *fbe* gene is present in 40 of 43 clinical isolates of *S epidermidis*.[31]

Other cell-surface-associated proteins containing SD repeats—namely SdrG (approximately 95% identical to Fbe), SdrF, and SdrH—were identified in *S epidermidis*.[35] The *sdrG* and *sdrH* genes are present in all 16 strains tested, whereas *sdrF* is present in only 12 strains. SdrG and SdrH protein antibodies were seen in 16 convalescent patient serum samples, suggesting that their genes are expressed during infection.[35] Like Fbe, SdrG binds to the β chain in fibrinogen. More specifically, SdrG was seen to bind to the N-terminal segment (peptide β6-20) of this polypeptide, which is located near to the thrombin cleavage site. It was also shown that SdrG inhibits thrombin-induced fibrinogen clotting by interfering with the release of fibrinopeptide B.[36] It was speculated that binding of SdrG might prevent the release of chemotactic elements, such as fibrinopeptide B. This may reduce the influx of phagocytic neutrophils, thereby aiding the survival of the bacteria in the host.[36] The function of the other members of this new protein family in *S epidermidis* remains unknown.

Most recently, it has been shown that besides proteins, cell-wall teichoic acid is involved in the adherence of *S epidermidis* to fibronectin.[37] Adherence of *S epidermidis* to immobilised fibronectin is significantly promoted by teichoic acid in a dose-dependent way; preincubation of either the bacteria or fibronectin coated surfaces with teichoic acid promoted *S epidermidis* adherence. This finding suggests that teichoic acid can function as a bridging molecule between the bacteria and fibronectin-coated polymer.

Accumulation phase

After adherence to the foreign body surface, the bacteria multiply and accumulate as multilayered cell clusters. This biofilm accumulation involves intercellular adhesion. Tn917 mutants that were accumulation-negative on a polymer surface were seen to lack the *S epidermidis* polysaccharide intercellular adhesin (PIA).[18,38] Purification and structural analysis showed that PIA consists of two forms of the polysaccharide, a major polysaccharide I (>80%) and a minor polysaccharide II (<20%).[39] Polysaccharide I is a linear β-1,6-linked glucosaminoglycan mainly composed of at least 130 2-deoxy-2-amino-D-glucopyranosyl residues of which 80–85% are N-acetylated. Polysaccharide II is structurally related to polysaccharide I, but with a lower content of non-N-acetylated glucosaminyl residues and small amounts of phosphate and ester-linked succinyl residues.

The *icaABC* genes that mediate cell clustering and PIA synthesis in *S epidermidis* were cloned and sequenced.[40] Later, an additional open reading frame (*icaD*) was identified; it is located between *icaA* and *icaB* and overlaps with both genes.[41] The functions of the respective proteins in PIA synthesis were analysed. It was shown that IcaA carries out the proposed N-acetylglucosaminyltransferase activity. However, IcaA shows only low transferase activity; co-expression of *icaA* with *icaD* leads to a significant increase in

Staphylococcal surface structures and their putative functions

Surface structure	Putative function
Staphylococcal surface proteins: SSP-1, SSP-2	Adherence of *S epidermidis* to unmodified polystyrene
Autolysin: AtlE	Adherence of *S epidermidis* to unmodified and/or Vn-coated polymer surface
Biofilm-associated protein: Bap	Adherence of *S aureus* to a polymer surface and biofilm accumulation
Bap-homologous protein: Bhp	*S epidermidis* biofilm formation
Capsular polysaccharide/adhesin: PS/A	Adherence of *S epidermidis* to a polymer surface and biofilm accumulation
Fibrinogen-binding protein: Fbe	Binding of *S epidermidis* to the β chain of fibrinogen
SD-repeat containing proteins: SdrG (homologous to Fbe), SdrF, SdrH	SdrG: binding of *S epidermidis* to the β chain of fibrinogen and inhibition of thrombin-induced fibrinogen-clotting. SdrF, SdrH: unknown
Intercellular adhesion gene cluster: *icaADBC*	PIA production
Polysaccharide intercellular adhesin: PIA (N-acetyl-glucosaminoglycan)	Intercellular adhesion and biofilm accumulation
Slime-associated antigen: SAA	Same composition and function as PIA
Accumulation-associated protein: AAP	Biofilm accumulation of *S epidermidis*
Autolysin: aas	Binding *S saprophyticus* to fibronectin and uroepithelial cells; haemagglutination
S saprophyticus surface-associated protein: Ssp	Adherence of *S saprophyticus* to uroepithelial cells

activity and to synthesis of N-acetylglucosamine oligomers with a maximum length of 20 residues. Only in the presence of *icaC* does IcaAD catalyse the synthesis of long-chain oligomers that react with PIA-specific antisera. The importance of PIA as a virulence factor has been shown: a PIA-negative mutant was significantly less virulent than the isogenic wild-type strain in a mouse model of subcutaneous foreign body infection and in a rat model of central-venous-catheter-associated infection.[42,43] Furthermore, heterologous expression of the *icaADCB* operon in *S carnosus* conferred haemagglutination on the haemagglutination-negative *S carnosus* wild type. Thus, the *icaADCB* operon mediated biofilm accumulation, PIA production, and haemagglutination.[44] In a study designed to investigate the pathogenic properties of strains of *S epidermidis* obtained from patients with prosthetic device-associated septicaemia, there was a strong association between pathogenesis and both biofilm formation and presence of the *ica* gene cluster. Meanwhile, saprophytic skin and mucosal isolates were usually biofilm negative and lacked the *ica* genes.[45]

It has been reported that PS/A production is also mediated by the *ica* gene cluster and that PS/A is chemically related to PIA.[46] Both antigens have a β-1,6-linked polyglucosamine backbone, but PS/A was distinguished from PIA by molecular size (>250 000 kDa vs ~28 kDa, respectively) and succinylation of most of the amino groups of the glucosamine residues. It has been shown that the synthesis of a similar if not identical polysaccharide from *S aureus* is mediated by an homologous *ica* gene cluster.[47] However, analysis of that polysaccharide suggests that most of the amino groups of the glucosamine residues are acetylated as described for PIA, rather than succinylated (G Pier, personal communication).

Formerly, another antigenic marker of slime production and accumulation was identified and designated as slime-associated antigen (SAA). Changes in the purification procedure have shown that the composition of SAA differs from that originally described and that SAA mainly consists of N-acetylglucosamine. Hence, it has been concluded that SAA is PIA.[48]

Proteins also seem to be essential for accumulation and biofilm formation in *S epidermidis*. The 140 kDa extracellular protein AAP (accumulation-associated protein) that is lacking in the accumulation-negative mutant M7 was shown to be essential for accumulative growth in certain *S epidermidis* strains on polymer surfaces.[49,50] An antiserum specific for AAP inhibited accumulation by up to 98% of the wild type strain RP62A. The gene encoding AAP has been cloned and sequenced. AAP has features typical of Gram-positive surface proteins—eg, an N-terminal signal peptide, multiple repeat domains, and a C-terminal cell-wall anchor (M Hussain, personal communication). Biochemical and functional properties clearly differentiate AAP from other factors that have been implicated in biofilm formation. It is proposed that AAP has a role in the anchoring of PIA to the cell surface, since the mutant M7 produces PIA that is only loosely attached to the cell surface by contrast with the wild type (D Mack, personal communication).

Other potential virulence factors of *S epidermidis*

Extracellular enzymes and toxins

The establishment of an infection and the survival of the bacteria in the host depends on the ability to invade host tissues and to evade host defense systems, respectively. For this, staphylococci, in particular *S aureus*, have developed multiple mechanisms including production of several extracellular proteins and enzymes such as protein A, lipases, proteases, esterases, phospholipases, fatty-acid modifying enzymes (FAME), as well as production of haemolysins and toxins with superantigenic properties such as enterotoxins, exfoliative toxins, and toxic shock syndrome toxin-1 (TSST-1). Additionally, proteases may have a role in proteolytic inactivation of host defense mechanisms such as antibodies and platelet microbicidal proteins (PMPs) as well as in destruction of tissue proteins causing increased invasiveness.

In *S epidermidis*, an extracellular metalloprotease with elastase activity has been detected, and its gene has been cloned and sequenced.[51] Previously, an elastase from *S epidermidis* which degrades human sIgA, IgM, serum

albumin, fibrinogen, and fibronectin has been identified as a cysteine protease and thus assumed to be a virulence factor;[52] however, the corresponding gene has not yet been cloned. An extracellular serine protease is involved in epidermin processing (see below).[53] The genes of the lipases *gehC* and *gehSE1* from *S epidermidis* strains 9 and RP62A, respectively, showing a high degree of similarity (97·8% identical aminoacids) have been cloned and sequenced, and they have been proposed to be involved in skin colonisation.[54,55] Recently, a second lipase gene, *gehD,* from *S epidermidis* strain 9 has been characterised.[56] The characterisation and expression of FAME in *S epidermidis* has also been described.[57]

By contrast with *S aureus,* which produces all of the above mentioned toxins in a strain-dependent manner, *S epidermidis* is much less toxigenic. *S epidermidis* can produce δ-toxin, which differs from the *S aureus* δ-toxin in only three aminoacids.[58] The δ-toxin is encoded by *hld,* which is a component of the regulatory *agr* system and acts by formation of pores in the membrane leading to the lysis of erythrocytes and other mammalian cells. Reports ofunusual *S epidermidis* strains producing enterotoxin C or TSST-1 are controversial.[59]

Production of lantibiotics.

The production of lantibiotics is another feature of *S epidermidis* that may explain its effective colonisation of skin. Lantibiotics are bacteriocins, which are produced by *S epidermidis* and other Gram-positive bacteria—eg, *Bacillus subtilis* and lactobacilli—but not by *S aureus.* Lantibiotics, such as the well-characterised epidermin,[60] Pep5,[61] epilancin K7,[62] and epicidin 280,[63] are antibiotic peptides, which contain the rare thioether aminoacids lanthionine and/or methyllanthionine and are active against Gram-positive bacteria. Lantibiotic production may play a substantial part in bacterial interference on skin and mucous membranes by excluding competing organisms that are sensitive to their bactericidal activities. In general, these peptides are gene-encoded and post-translationally modified. The genes involved are organised in biosynthetic gene clusters located on plasmids.[60–63]

Iron acquisition inside the host

The common prerequisite for all bacterial pathogens to establish an infection is the ability to proliferate within the mammalian host. As do all bacteria, staphylococci need iron for their growth; however, the free iron concentration (10^{-18} mol/L) in the extracellular body fluids, owing to the presence of high-affinity iron-binding glycoproteins such as transferrin or lactotransferrin, is much too low to support staphylococcal growth.

The mechanisms by which staphylococci acquire iron from transferrin are not well understood. In general, there are two known mechanisms for iron acquisition. One mechanism involves the synthesis and secretion of low-molecular-mass iron chelators (siderophores), which remove iron from transferrin. The siderophore-iron complexes are then taken up by specific bacterial transport systems. The siderophores staphyloferrin A and B (481 and 448 Da, respectively), first isolated from *S hyicus,* were also seen to be produced by *S epidermidis* under iron-restricted conditions.[64] The second mechanism by which bacteria assimilate iron depends on direct contact between the host transferrin and a bacterial surface receptor.

Both *S epidermidis* and *S aureus* express several iron-repressible cell-wall-associated and cytoplasmic-membrane-associated proteins when isolated during infection in people, as well as when grown in vivo in experimental animal infections.[65] These proteins include a 42 kDa protein that is a cell wall glyceraldehyde-3-phosphate dehydrogenase and functions as a receptor for human transferrin,[66] as well as a 32 kDa cytoplasmic membrane-associated lipoprotein.[67] Cloning and sequencing of the DNA region encoding the 32 kDa cytoplasmic membrane-associated lipoprotein of *S epidermidis* showed that the corresponding gene (*sitC*) is part of a translationally coupled, iron-regulated operon (*sitABC*), which encodes an ABC-type transporter.[67] It is speculated that this novel ABC transporter is involved in either siderophore-mediated or transferrin-mediated iron uptake in *S epidermidis.*

S saprophyticus as cause of urinary tract infection

CoNS of the *S saprophyticus* group, mainly the species *S saprophyticus*, which is the most common bacterium in the novobiocin-resistant group, are true urinary pathogens causing both upper and lower urinary tract disease. Diseases of mild severity such as the dysuria syndrome in women or non-specific urethritis in men can be seen, but also cystitis, pyelonephritis, and even urosepsis may occur in the immuno-competent patient even in the absence of a foreign body.[68]

The most important group of particularly susceptible patients comprises young female outpatients. *S saprophyticus* accounts for up to 42% of all urinary tract infections (UTIs) in young women. Bacteriuria in hospitalised patients is only rarely caused by *S saprophyticus*, as is UTI in males. Symptoms of an UTI are present in more than 90% of women from whom *S saprophyticus* is cultured, and pyuria is present in between 70% and 85% of these women.[69-71] However, in a recent study, *S saprophyticus* was regarded as an organism of low virulence, because *S saprophyticus* was usually low in numbers, found with a low degree of pyuria, and the hosts were usually symptom-free.[72]

Unlike infections with enteric bacteria, there seems to be a seasonal predilection for *S saprophyticus* UTIs. Infections usually peak during late summer and fall, a pattern similar to that of sexually transmitted diseases. In addition, since young sexually active men and women are affected preferentially, it has been suggested that *S saprophyticus* infections may be sexually transmitted. However, a specific adherence—eg, to vaginal epithelium—has not been shown to date. Besides recent sexual intercourse, outdoor swimming and occupational meat processing have been identified as risk factors.[71]

Since urine colony counts for *S saprophyticus* are often lower than those for enteric bacteria ($<10^5$ cfu/mL), this staphylococcal species has been implicated as one cause of the dysuria-pyuria syndrome (acute urethral syndrome or abacteriuric pyuria).[73]

Pathogenicity of *S saprophyticus*

In past years several potential virulence factors that may explain the pathogenic potential of this *S saprophyticus* have been identified and characterised. This species seems to have a greater capacity to adhere specifically to uroepithelial cells than many other staphylococcal species. Thus, a surface-exposed 160 kDa protein with haemagglutinin/adhesin properties that mediates binding to uroepithelial cells might explain the tropism for kidney colonisation of *S saprophyticus*.[74–76] This protein is expressed preferably under anaerobic conditions and binds surface-exposed fibronectin on uroepithelial cells. Recently, the gene encoding this novel protein of *S saprophyticus* has been cloned and sequenced.[29] It encodes a protein with 1463 aminoacids and its reduced aminoacid sequence is homologous to the autolysins Atl and AtlE from *S aureus* and *S epidermidis*, respectively. Because the protein, designated Aas, lacks the motifs typical of Gram-positive surface proteins, shows a different overall organisation, and is multifunctional, this autolysin/adhesin seems to represent a new class of staphylococcal adhesins.[29]

Another surface-associated fibrillar protein, designated Ssp (for *S saprophyticus* surface-associated protein), which is present in more than 98% of clinical isolates, was discovered by culturing *S saprophyticus* on dialysis membranes placed on top of brain heart infusion agar.[77] The protein can be released from the bacteria by mild shearing. It has an apparent M_r of 95 000 when run under reducing conditions and shows a native M_r of about 500 000 under non-reducing conditions in the presence of 8 M urea. Bacteria capable of producing this protein show large amounts of surface-associated material. The appendages are similar in length, are most prominent between adjacent cells, and tend to clump. Scanning electron micrographs showed that septation areas of dividing cells are not covered with this material, a situation similar to that seen with the aggregation substance of *Enterococcus faecalis*.[78] Using an ELISA, Gatermann et al[79] noted that the protein bound to tubular epithelial cells (LLC-PK_1) in a concentration-dependent manner. This protein may, therefore, be involved in the interaction of *S saprophyticus* with uroepithelial cells.

Most recently, another cell surface-associated protein, designated Sdrl, was described. This protein is similar in sequence and structural organisation to the Sdr proteins of *S aureus* and *S epidermidis* and was seen to have adhesive properties.[80]

Once colonisation has been established and microcolonies have formed, the staphylococci elaborate a urease, which contibutes to cystopathogenicity and tissue invasiveness by inducing severe damage to the bladder tissue. By introduction of cloned urease genes into a urease-negative mutant followed by experimental infections, the urease-positive parent strain and the complemented mutant were shown to exhibit similar bacterial counts in bladder tissue. Bacterial counts for these strains differed significantly from that seen with the urease-negative mutant. These studies established that the urease functions as a major virulence factor during UTI due to *S saprophyticus*.[81,82]

In addition to urease, other enzymes such as elastase, FAME, and lipase may act as invasion factors.[5]

Diseases and virulence factors in *S lugdunensis* and *S schleiferi*

Following the description of *S lugdunensis* and *S schleiferi* in 1988, these CoNS have been reported as causative pathogens in a range of nosocomial infections. Infections include endocarditis, polymer-associated infections, osteomyelitis, septic arthritis, UTIs, and wound infections.[83–89] Because multiple cases highlighted the aggressive nature of infections, particularly of endocarditis due to *S lugdunensis*, this species has been regarded as more pathogenic than most other species of the genus *Staphylococcus*.[83,90,91]

S lugdunensis and *S schleiferi* resemble *S aureus* in that these species may express a clumping factor and/or produce a thermostable DNase.[92,93] While the pathogenic mechanisms by which *S lugdunensis* and *S schleiferi* cause infections are still unknown, these species also seem to share virulence determinants with *S aureus*.

About 25% of clinical isolates of *S lugdunensis* produce extracellular slime or glycocalyx which has a role in bacterial colonisation and interferes with the phagocytosis-associated activities of neutrophils. It was shown that glycocalyx purified from defined cultures of *S lugdunensis* is a strong stimulator of monocyte prostaglandin E2, which in turn contributes to the inhibition of T-cell proliferation. This activation of monocytes also results in modulation of two significant antimicrobial activities of macrophages, tumour necrosis factor α and the nitric oxide production.[94–96]

In addition, production of enzymes that may act as invasion factors has been detected in *S lugdunensis* strains including esterase, FAME, protease, and lipase.[93,97] Binding to collagen, fibronectin, vitronectin, laminin, fibrinogen, thrombospondin, plasminogen, and human IgG immobilised on latex beads has been shown. However, *S lugdunensis* showed only moderate attachment on fibronectin-coated polymethylmethacrylate (PMMA) coverslips.[5,98,99]

Production of a synergistic haemolysin termed SLUSH has been variably noted in *S lugdunensis*. SLUSH is phenotypically similar to the δ-haemolysin of *S aureus* and consists of three very similar 43-residue peptides highly related to the "gonococcal growth inhibitor", a bacteriocin secreted by *S haemolyticus*.[5,94]

As for *S lugdunensis*, various potential virulence factors were also described in *S schleiferi* in the past decade. These include the glycocalyx as well as enzymes such as esterase, protease, FAME, lipase, and haemolysins.[93,96,97] Studying the adhesion to polymers, most strains of *S schleiferi* showed a moderate or a strong attachment on fibronectin-coated and

Search strategy and selection criteria

Data for this review were identified by searches of Medline, references from relevant articles and book chapters, and personal reference manager files. Reference articles were identified by a Medline search that cross-referenced the terms "coagulase-negative staphylococci", "*Staphylococcus epidermidis*", "*Staphylococcus saprophyticus*", "*Staphylococcus schleiferi*", and "*Staphylococcus lugdunensis*" with "pathogenesis" and "infection". Only English language papers were included.

fibrinogen-coated PMMA coverslips, equivalent to that of *S aureus*.[5] However, fibrinogen-binding surface components homologous to the ClfA protein of *S aureus* were not identified, neither by Western immunoblotting nor by analysis of the ClfA antigen by fluorescence-activated cell sorting. Of interest, a recent study provided phenotypic and genotypic evidence for the expression of a cell-wall-anchored fibronectin-binding protein by this species.[100]

Conflict of interest

We have no conflicts of interest.

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