Review paper

Staphylococcal and enterococcal virulence – a review

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Abstract

Staphylococcus aureus has the ability to cause a wide variety of human diseases: from superficial abscesses and wound infections to deep and systemic infections such as osteomyelitis, endocarditis and septicaemia. S. aureus invasion might be involved in some of the unique pathogenic manifestations of this bacterium, including long-term colonization. The ability to cause disease is a result of presence of virulence factors. Virulence factors are synthesized in response to the specific needs during the infectious process. The molecular mechanism and the precise role of invasion in S. aureus pathology are not known. S. aureus expresses many potential virulence factors: surface proteins, invasins (leukocidin, kinases hyaluronidase), inhibitors of phagocytic engulfment (capsule, Protein A), factors enhancing bacterial survival in phagocytes (carotenoids, catalase production), immunological disguises (Protein A, coagulase, clotting factor), membrane- damaging toxins (hemolysins, leukotoxin, leukocidin) and exotoxins (SEA-G, TSST, ET).

The pathogenicity of S. aureus infections is related to surface components including those recognizing adhesive matrix molecules (e.g., clumping factor and fibronectin binding protein) and to extracellular proteins [e.g., coagulase, hemolysins, enterotoxins, toxic-shock syndrome (TSS) toxin, exfoliatins, and Panton-Valentine leukocidin (PVL)]. In general, the precise roles of individual staphylococcal factors in invasive infections are difficult to assess.

Key words: *staphylococcus, Enterococcus, virulence, infection.*

(Centr Eur J Immunol 2011; 36 (1): 56-64)

The microbial adherence to cells and extracellular matrix is considered as an essential first step in the process of colonization and infection. A well-characterized family of staphylococcal surface adhesins, called MSCRAMMs ("microbial surface components recognizing adhesive matrix molecules") are known to mediate adherence to host extracellular matrix components, such as fibrinogen, fibronectin and collagen [1].

The virulence mechanisms that characterize *Escherichia coli* are genetically coded by chromosomal, plasmid, and bacteriophage DNAs and include heat-labile (LTI, LTIIa, and LTIIb) and heat-stable (STI and STII) toxins, verotoxin types 1, 2, and 2e (VT1, VT2, and VT2e, respectively), cytotoxic necrotizing factors (CNF1 and CNF2), attaching and effacing mechanisms (*eaeA*), enteroaggregative mechanisms (Eagg), and enteroinvasive mechanisms (Einv) [2].

Adhesins

Human staphylococcal infections are frequent. There are numerous portals of entry to the human's body: hair follicle, break in the skin, surgical wound, foreign bodies, respiratory tract. Serious consequences of staphylococcal infections occur when the bacteria invade the blood stream. Infections caused by staphylococcus can lead to serious diseases: pneumonia, osteomyelitis, endocarditis, furuncles, impetigo, food poisoning, toxic shock syndrome, boils, styes, mastitis, phlebitis, meningitis and urinary tract infections. Adhesin systems have been described for several pathogens, including *Escherichia coli*, *Klebsiella pneumoniae*, *Haemophilus influenzae*, *Yersinia pseudotuberculosis*, *Bordetella pertussis*, *Streptococcus pyogenes* and *Streptococcus sanguis*. Only recently have enterococcal adhesins been investigated [3].

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Adhesion and colonization of host tissues is a common initial step in the pathogenic process of many infectious diseases. Bacterial adherence is mediated primarily by proteins on the bacterial surface (adhesins) which bind specifically to complimentary ligands [4]. Surface proteins promote attachment to host proteins (fibronectin, collagen, laminin, bone sialoprotein, vitronectin, elastin,) and colonization of host tissues. Most strains express a fibrin/fibrinogen binding protein (clumping factor) which promotes attachment to blood clots and traumatized tissue. Both fibronectin and fibrinogen-binding proteins are expressed by most strains of *S. aureus*. Mutants defective in binding to fibronectin and to fibrinogen have reduced virulence in a rat model for endocarditis, and mutants lacking the collagen-binding protein have reduced virulence in a mouse model for septic arthritis, suggesting that bacterial colonization is ineffective.

Elastin-binding protein

Elastin is a major component of the elastic fiber. Elastin and elastic fibers are present in abundance in tissues that require elasticity such as the lung, skin and major blood vessels.

Fibronectin-binding protein

Most strains of *S. aureus* express two related Fn-binding proteins FnBPA and FnBPB. These proteins belong to the group of MSCRAMMs and are anchored to the cell wall by LPXTG motif and facilitate attachment of the staphylococcus to the host cells. Fibronectin is bound to the $\alpha_5\beta_1$ integrin on the surface of the host cell and forms a bridge between the bacterial FnBP adhesion and the mammalian cell integrin [5].

FnBP-A and FnBP-B afford the pathogen the ability to connect to cellular integrins and to trigger internalization into host cells. Interestingly, and despite the presence of numerous adhesins in this microbe, the fibronectin- binding proteins A and B (FnBP-A and FnBP-B) were identified as major factors in initiating the internalization of *S. aureus.*

Both FnBPs are crucial for invasion of eukaryotic cell types by *S. aureus* and mutants lacking FnBP-A and FnBP-B are severely impaired in host-cell. Fn serves as a bridging molecule that links FnBP, expressed by *S. aureus*, with integrin $\alpha_5\beta_1$, the principal Fn receptor on the surface of host cells The crucial role of integrin $\alpha_5\beta_1$ for the uptake process is highlighted by the fact that cells lacking the integrin β1 subunit do not internalize *S. aureus* in significant numbers [6].

Integrin $\alpha_5\beta_1$ served as host cell receptor, which interacted with staphylococcal FnBPs through cellular or soluble fibronectin. FnBP-deficient mutants lost invasiveness for epithelial cells, endothelial cells and fibroblasts. Thus, fibronectin-dependent bridging between *S. aureus* FnBPs and host cell integrin $\alpha_5\beta_1$ is a conserved mechanism for *S. aureus* invasion of human cells. This may prove useful in developing new therapeutic and vaccine strategies for *S. aureus* infections [7].

Collagen-binding protein

The collagen binding protein CNA, as second important adhesive molecule is encoded by gene *cna* [8]. The collagen binding adhesin is a mosaic protein which is composed of an N terminal 55-kDa A domain containing a unique sequence; a B domain, which is composed of 1, 2, 3, or 4 repeats of a 25-kDa unit; and a C-terminal domain containing a cell wall attachment site, a hydrophobic transmembrane segment, and a short, cytoplasmic segment rich in positively charged residues. The collagen binding activity has been localized to a 19-kDa subfragment (M19) within the A domain [4]. The A region of CNA –(30-531) was found to be fully responsible for the collagen binding activity of CNA. The presence of this gene is associated with pathogenesis of osteomyelitis and septic arthritis [5].

Aggregation substance

Aggregation substance is a surface-bound protein encoded by pheromone-responsive plasmids of *E. faecalis* and expressed in response to pheromone induction. Aggregation substance converts the surface of the donor bacterium into one adherent to potential recipient cells, causing aggregation or clumping and facilitating transfer of plasmids [3].

Aggregation substance appears to be anchored in the bacterial membrane by its C terminus in a manner common to cell wall-associated proteins of gram-positive bacteria. Genes encoding aggregation substance from a variety of cytolysin-specifying and non-cytolysin-specifying, pheromone-responsive plasmids are highly conserved [3]. Kreft *et al*. demonstrated a potential role for aggregation substance in mediating adherence of enterococci to renal epithelial cells. Aggregation protein involved in adherence to eukaryotic cells; cell aggregation and conjugation. Aggregation substance mediates adhesion to cultured renal tubular cells and augments the internalization of *E. faecalis* by cultured human intestinal epithelial cells. Recent studies suggest a potential role for extracellular superoxide as a virulence factor.

In an effort to identify new factors that may contribute to enterococcal pathogenesis, we derived and systematically panned a database of nucleotide sequences compiled from random sequencing of the genome of *E. faecalis* MMH594, which caused multiple infections within a hospital ward, for sequences that appeared to encode surface proteins. A chromosomal gene with localized sequence identity to Streptococcus agalactiae *rib* and *bca* (encoding C α antigen) was identified from partial sequence information, and its preliminary characterization was reported. The Rib and C α proteins of group B streptococci are structurally related and consist of highly repetitive structures. These group B

streptococcal surface proteins have been shown to be virulence determinants and to confer protective immunity, and they appear to contribute to immune system evasion. We have named the enterococcal gene *esp* (since it encodes enterococcal surface protein) [9].

Invasins

Invasins promote bacterial spread in tissues as they damage the membranes of cells. Some invasions lyse erythrocytes, causing hemolysis. Leukocidins cause membrane damage but they are not hemolytic.

Leukocidin

Leukocidin is a multicomponent protein toxin produced as separate components which act together to damage membranes. Leukocidin forms a hetero-oligomeric transmembrane pore composed of four LukF and four LukS subunits, thereby forming an octameric pore in the affected membrane. Leukocidin is hemolytic, but less so than alpha hemolysin. Only 2% of all of *S. aureus* isolates express leukocidin, but nearly 90% of the strains isolated from severe dermonecrotic lesions express this toxin, which suggests that it is an important factor in necrotizing skin infections.

Hyaluronidase

Bacterial hyaluronidases, enzymes capable of breaking down hyaluronate, are produced by a number of pathogenic Gram-positive bacteria that initiate infections at the skin or mucosal surfaces. Since reports of the hyaluronidases first appeared, there have been numerous suggestions as to the role of the enzyme in the disease process. Unlike some of the other more well studied virulence factors, much of the information on the role of hyaluronidase is speculative, with little or no data to substantiate proposed roles. Over the last 5 years, a number of these enzymes from Gram-positive organisms have been cloned, and the nucleotide sequence determined. Phylogenetic analysis, using the deduced amino acid sequences of the Gram-positive hyaluronidases, suggests a relatedness among some of the enzymes. Molecular advances may lead to a more thorough understanding of the role of hyaluronidases in bacterial physiology and pathogenesis.

Many pathogenic bacteria produce extracellular products that have tissue-damaging efects. Some of the diverse products from pathogenic bacteria serve as virulence factors in the pathogenesis of disease by facilitating the spread of bacteria or toxins through tissues; these are commonly referred to as spreading factors. For many years, the term hyaluronidase has been synonymous with spreading factors. Although all extracellular hyaluronidases are probably spreading factors, not all spreading factors are hyaluronidases. Hyaluronidase is a general term used to describe enzymes that are able to breakdown the substrate hyaluronate (hyaluronic acid, hyaluronan), however, some of these enzymes are also ableto cleave chondroitin sulfate, albeit at a slower rate. The hyaluronidases can be subdivided into three types. A wide variety of microorganisms produce enzymes capable of degrading hyaluronate. Enzymes produced by the Gram-negative organisms are periplasmic rather than being excreted to the extracellular milieu, and so are less likely to play a role in pathogenesis. Some of these enzymes are chondroitin lyases, as they are also capable of degrading substrates such as chondroitin sulfate [10].

Gelatinase

Gelatinase is 28- to 32-kDa metalloproteinase (zinc-endopeptidase) from *E. faecalis* capable of hydrolyzing gelatin, collagen, casein, hemoglobin, and other small biologically active peptides and bioactive compounds [5].

AS-48

AS-48 is a 7.4-kDa peptide produced by *E. faecalis* that inhibits and lyses a wide spectrum of gram-negative and gram-positive bacteria, including enterococci. This basic peptide is lytic via the generation of pores in cytoplasmic membranes of target cells that lead to depolarization. It also appears to induce lysis of selected enterococci through activation of an autolysin. The significance of this bacteriocin remains uncertain, however, since the prevalence of AS-48-producing strains among human commensal and infection isolates has yet to be defined. Peptide AS-48 induces ion permeation, which is accompanied by the collapse of the cytoplasmic membrane potential, in sensitive bacteria. Active transport by cytoplasmic membrane vesicles is also impaired by AS-48. Electrical measurements suggest that AS-48 can form channels in lipid bilayers.

Coagulase and clumping factor

Coagulase is an extracellular protein which binds to prothrombin in the host to form a complex called staphylothrombin. The protease activity characteristic of thrombin is activated in the complex, resulting in the conversion of fibrinogen to fibrin. Coagulase is a traditional marker for identifying *S. aureus* in the clinical microbiology laboratory. However, there is no overwhelming evidence that it is a virulence factor, although it is reasonable to speculate that the bacteria could protect themselves from phagocytic and immune defenses by causing localized clotting. There is some confusion in the literature concerning coagulase and clumping factor, the fibrinogen-binding determinant on the *S. aureus* cell surface. Partly the confusion results from the fact that a small amount of coagulase is tightly bound on the bacterial cell surface where it can react with prothrombin leading to fibrin clotting. However, genetic studies have shown unequivocally that coagulase and clumping factor are distinct entities. Specific mutants lacking coagulase retain

clumping factor activity, while clumping factor mutants express coagulase normally [5].

Staphylokinase

Many strains of *S. aureus* express a plasminogen activator called staphylokinase. This factor lyses fibrin. The genetic determinant is associated with lysogenic bacteriophages. A complex formed between staphylokinase and plasminogen activates plasmin-like proteolytic activity which causes dissolution of fibrin clots. The mechanism is identical to streptokinase, which is used in medicine to treat patients suffering from coronary thrombosis. As with coagulase, there is no strong evidence that staphylokinase is a virulence factor, although it seems reasonable to imagine that localized fibrinolysis might aid in bacterial spreading.

Staphylokinase is bacteria plasminogen activator. It contributes to spread of the bacteria. Staphylocinase activates plasminogen to dissolve clots, destroyes fibrin fibers that hold cells together. Staphylocinase is not an enzyme itself but form complexes with plasminogen that coverts other plasminogen molecules to plasmin, a potent enzyme that degrades proteins of the extracellular matrix.

α-hemolysin

α-hemolysin forms pores in cell membrane to kill or limit the ability of neutrophils. β- hemolysin is associated with tissue invasion. Sphingomyelinase, specifically cleaves sphingomyelin into phosphocholine and ceramide.

Extracellular enzymes

Proteases, lipases and deoxyribonucleases provide nutrients (aminoacids) for bacteria.

Toxins – enterotoxins, toxic shock syndrome toxins, exfoliative toxins

S. aureus is both a commensal and an extremely versatile pathogen in humans, causing three basic syndromes: superficial lesions such as skin abscesses and wound infections and deep-seated and systemic infections such as osteomyelitis, endocarditis, pneumonia, and bacteremia; and toxemic syndromes such as toxic shock syndrome (TSS) and staphylococcal scarlet fever [both due to toxic shock syndrome toxin 1 (TSST-1) and staphylococcal enterotoxins (SEs)], staphylococcal scalded-skin syndrome (SSSS; due to exfoliatins), and staphylococcal food poisoning (due to SEs). With the exception of toxemia, the molecular basis of *S. aureus* pathogenicity is multifactorial, depending on the expression of a large class of accessory gene products that comprise cell wall-associated and extracellular proteins. Expression of most virulence factors in *S. aureus* is controlled by the *agr* locus, which encodes a two-component signaling pathway whose activating ligand is a bacterial-density-sensing peptide (autoinducing peptide) also encoded by *agr.* A polymorphism in the amino acid sequence of the autoinducing peptide and of its corresponding receptor (AgrC) has been described. *S. aureus* strains can be divided into four major groups on this basis: within a given group, each strain produces a peptide that can activate the *agr* response in the other member strains, whereas the autoinducing peptides produced by the different groups are usually mutually inhibitory [11].

Enterotoxins and toxic shock syndrome toxin

Staphylococcus aureus produces a wide variety of toxic proteins, including the staphylococcal enterotoxins (SEs)2 A-E and G-J, the toxic shock syndrome toxin-1 (TSST-1), and the exfoliative toxins (ETs) A and B. These toxins were initially described as being responsible for specific acute staphylococcal toxemia syndromes, such as toxic shock syndrome (TSS) and staphylococcal scarlet fever (SSF) (both due to TSST-1, SEB, and SEC), scalded skin syndrome (SSSS, due to the ETs), and staphylococcal food poisoning (due to the SEs). SEs and TSST-1 share common structural and biological properties, suggesting that they derived from a common ancestor.

They display significant homology in their primary sequence and secondary and tertiary structure. Based on amino acid sequence comparisons, SEs have been divided into several groups; one includes SEA, SEE, SEJ, SED, and SEH, and another SEB and SEC, whereas SEG and SEI could not be clearly attributed to a specific group. Biologically, SEs and TSST-1 exhibit superantigen activity, stimulating polyclonal T cell proliferation through coligation between MHC class II molecules on APCs and the variable portion of the T cell Ag receptor β-chain (TCR Vb). The pattern of Vb activation is specific for each of these superantigens. T cell/APC activation by these toxins leads to the release of various cytokines/lymphokines and IFN, enhances endotoxic shock, and causes T and B cell immunosuppression, all of which may hinder the immune response against bacterial infection [12].

Staphylococcus aureus secretes two types of toxins with superantigen activity, enterotoxins, of which there are six antigenic types (named SE-A, B, C, D, E and G), and toxic shock syndrome toxin (TSST-1). Enterotoxins cause diarrhea and vomiting when ingested and are responsible for staphylococcal food poisoning. TSST-1 is expressed systemically and is the cause of toxic shock syndrome (TSS). When expressed systemically, enterotoxins can also cause toxic shock syndrome. In fact, enterotoxins B and C cause 50% of non-menstrual cases of TSS. TSST-1 is weakly related to enterotoxins, but it does not have emetic activity. TSST-1 is responsible for 75% of TSS, including all menstrual cases. TSS can occur as a sequel to any staphylococcal infection if an enterotoxin or TSST-1 is released systemically, and the host lacks appropriate neutralizing antibodies [5].

Toxic shock syndrome (TSS) is a life-threatening multisystem disorder caused by strains of *Staphylococcus*

aureus. It is characterized by rapid onset of fever, arterial hypotension, scarlatiniform rash, and multiorgan failure.

Staphylococcus aureus TSS toxin 1 (TSST-1) was the first toxin shown to be involved in TSS, in both menstrual and nonmenstrual cases. Staphylococcal enterotoxins A to D and H (SEA to SED and SEH) also appear to have caused some cases of nonmenstrual TSS. TSST-1 and SEA to SED have been linked to other staphylococcal syndromes such as staphylococcal scarlet fever (SSF) and recalcitrant erythematous desquamating disorder (REDD), both of which were suggested to be variants of TSS on the basis of toxin production and certain clinical similarities. Another staphylococcal enterotoxin (SEE) was isolated from chicken and food specimens but has not been associated with TSS. All of these toxins exhibit superantigen activity, stimulating polyclonal [13].

Exfoliative toxin

The exfoliative toxin (ET), associated with scalded skin syndrome, causes separation within the epidermis, between the living layers and the superficial dead layers. The separation is through the stratum granulosum of the epidermis. This is probably why healing occurs with little scarring although the risks of fluid loss and secondary infections are increased. Staphylococcal exfoliative toxin B has been shown to specifically cleave desmoglein 1, a cadherin that is found in desmosomes in the epidermis. Most exfoliatin-producing strains responsible for SSSS belong to *agr* group IV, but the clonality of these strains has not been investigated [11].

α-toxin damage membrane of *S. aureus.* It binds to the membrane of susceptible cells. Subunits form heptameric rings with a central pore through which cellular contents leak. In humans, platelets and monocytes are particularly sensitive to α-toxin. Susceptible cells have a specific receptor for α-toxin which allows the toxin to bind causing small pores through which monovalent cations can pass. The mode of action of α-hemolysin is likely by osmotic lysis.

β-toxin is a sphingomyelinase which damages membranes rich in this lipid. The classical test for β-toxin is lysis of sheep erythrocytes. The majority of human isolates of S. aureus do not express β-toxin. A lysogenic bacteriophage is known to encode the toxin.

δ-toxin is a very small peptide toxin produced by most strains of *S. aureus*. It is also produced by *S. epidermidis*. The role of delta toxin in disease is unknown.

Exfoliative toxin D

Exfoliative toxin D (ETD) was identified recently as a new exfoliative toxin serotype. Like other exfoliative toxins, ETD induces intra-epidermal cleavage through the granular layer of the epidermis of neonatal mice. Isolates responsible for bullous impetigo and generalised staphylococcal scalded skin syndrome did not harbour *etd*, but *etd* was significantly more frequent in isolates causing cutaneous abscesses and furuncles. Most *etd*- and Panton-Valentine leukocidin-positive strains belonged to the clone of community-acquired methicillin-resistant *S. aureus* [14].

Staphylococcus aureus produces a variety of extracellular toxins, including enterotoxins, toxic shock syndrome toxin (TSST1) and exfoliative toxins (ETs). ETs cause the epidermal cleavage seen in staphylococcal scalded skin syndrome (SSSS) and bullous impetigo. Two major serological forms of ET, designated ETA and ETB, have been linked to human infections. A third ET (ETC) has been characterised and purified from a *S. aureus* isolate obtained from a horse. ETs SHETA-B and ExhA-C are responsible for *Staphylococcus hyicus* exudative epidermitis in pigs. An ET-like toxin has also been detected in *Staphylococcus intermedius* (SIET), and may have a pathogenetic role in canine pyoderma.

A new ET, designated ETD, is a 27-kDa protein with sequence similarities of 40% with ETA, 59% with ETB, 13% with ETC, 16% with SHET-A, and 63% with SHET-B. SSSS is associated classically with ETA or ETB.

The *etd* gene was detected mainly in isolates from patients with skin and soft-tissue infections, such as furuncles, abscesses and finger pulp infections, which are caused rarely by *S. aureus* strains harbouring *eta* or *etb*, but are associated strongly with production of PVL, and was detected only rarely in isolates from patients with other suppurative diseases. It is unclear whether ETD contributes directly to the pathogenesis of these diseases, but the present findings support the hypothesis that ETD may disrupt the cutaneous epithelial barrier and thereby contribute to bacterial spread.

Indeed, purified ETD induces intra-epidermal cleavage through the granular layer of the epidermis of neonatal mice in a manner similar to ETA and ETB. As for other ETs, a possible substrate for ETD is desmoglein 1, a desmosome trans-membrane glycoprotein belonging to the cadherin gene superfamily [14].

Community-acquired (CA) methicillin-resistant *Staphylococcus aureus* (MRSA) infection among individuals without healthcare-associated (HA) risk factors was first recognized about a decade ago. It has now emerged as an epidemic that is responsible for rapidly progressive, fatal diseases including necrotizing pneumonia, severe sepsis and necrotizing fasciitis. Unlike HA-MRSA, CA-MRSA are usually pan-susceptible to non-b-lactam antimicrobials. In addition to novel methicillin resistance genetic cassettes, many CA-MRSA harbor a phage harboring Panton-Valentine Leukocidin (PVL) genes and some data support the idea that PVL is responsible at least in part for the increased virulence of CA-MRSA. The tight association between the novel methicillin resistance cassettes and PVL phage cannot be explained, as they integrate into distinct sites on the *S. aureus* chromosome. This paper presents the evidence that CA-MRSA isolates are distinct

strains emerging de novo from CA-methicillin susceptible isolates rather than from HA-MRSA isolates that have escaped from the hospital setting and that these novel CA-MRSA isolates may be more virulent than HA-MRSA [16].

Biofilm production

Staphylococcus epidermidis has become the most important cause of nosocomial infections in recent years. Its pathogenicity is mainly due to the ability to form biofilms on indwelling medical devices. The production of a slime resulting in biofilm formation. The slime is predominantly a secreted teichoic acid, normally found in the cell wall of the staphylococci. This ability to form a biofilm on the surface of a prosthetic device is probably a significant determinant of virulence for these bacteria [5]. Formation of thick biofilms on biomaterials is a very efficient way to escape from recognition and inactivation by antimicrobial host molecules, phagocytes, and antibiotics. Defensin-like cationic antimicrobial peptides disrupt bacterial membranes and play key roles in innate immunity [17].

In *S. epidermidis* infections on indwelling devices, biofilm formation is considered to be the main virulence factor. The extracellular substance surrounding the multilayered cell clusters is composed of bacterial and host products of different chemical composition [18].

In the course of a foreign body-related infection, two different stages have been distinguished: the primary attachment of the bacteria to the material and the formation of multi-layered cell clusters with cell-cell adherence depending on the production of a "slimy" extracellular substance [18].

The genetic and molecular basis of biofilm formation in staphylococci is multifaceted. A trademark is the production of the slime substance PIA, a polysaccharide composed of β-1,6-linked *N*-acetylglucosamines with partly deacetylated residues, in which the cells are embedded and protected against the host's immune defence and antibiotic treatment. Mutations in the corresponding biosynthesis genes (*ica* operon) lead to a pleiotropic phenotype; the cells are biofilm and haemagglutination negative, less virulent and less adhesive on hydrophilic surfaces. *ica* expression is modulated by various environmental conditions, appears to be controlled by SigB and can be turned on and off by insertion sequence (IS) elements. A number of biofilm-negative mutants have been isolated in which polysaccharide intercellular adhesion (PIA) production appears to be unaffected. The accumulation-defective mutants are unable to form a biofilm on both polystyrene and glass surfaces, display no intercellular aggregation and do not produce PIA. The transposon had integrated at various sites in an operon, which we named ica for intercellular adhesion. The operon is composed of the icaR (regulatory) gene and *icaADBC* (biosynthesis) genes [19].

Primary attachment

The first stage of staphylococcal colonization of the polymer material of foreign bodies may either proceed as direct attachment of the bacteria to the plastic surface or binding to host-derived matrix proteins which have previously coated the polymer material [18].

Primary attachment to uncoated plastic material

The attachment of *S. epidermidis* to uncoated plastic material is dependent on the physico-chemical properties of the plastic and the bacterial surface. As the plastic surface is hydrophobic, and the primary adhesion of *S. epidermidis* has been shown to be similar on many investigated biomaterials, the main parameter determining bacterial adhesion is the hydrophobicity of the bacterial surface [18].

S. epidermidis major autolysin AtlE

The mutant did not produce AtlE and its surface hydrophobicity was reduced, which represents the likely cause for the impaired attachment. AtlE and its degradation products make up the main fraction of *S. epidermidis* surface proteins that are non-covalently attached. Therefore, it is not clear if AtlE represents a protein with specific ability to interact with hydrophobic surfaces or if the difference in mutant and wild-type strain is just due to the fact that most surface proteins are removed in the mutant [18].

Locus ica

The *ica* locus is composed of the genes *icaA*, *icaD*, *icaB* and *icaC*. IcaA is an *N*-acetylglucosaminyltransferase, which only reaches low activity without the presence of IcaD. IcaA and IcaD only produce *N*-acetyl oligomers of up to 20 residues of length. IcaC is responsible for the production of PIA of full length, able to react with anti-PIA antisera. The function of IcaB remains unclear [18].

Polysaccharide intercellular adhesin

A140-kDa protein named AAP (accumulation-associated protein) has been shown to be responsible for accumulative growth on polymer surfaces. The function of this protein is not clear. Most genetic and biochemical evidence has been achieved for the involvement of an extracellular polysaccharide in intercellular adhesion, named PIA (polysaccharide intercellular adhesin). PIA is composed of a major polysaccharide I and a minor polysaccharide II [18].

Extracellular polymeric substances

The extracellular polymeric substances (EPS) of bacterial biofilms form a hydrated barrier between cells and their external environment. Better characterization of EPS could be useful in understanding biofilm physiology.

The EPS are chemically complex, changing with both bacterial strain and culture conditions. Previously, we

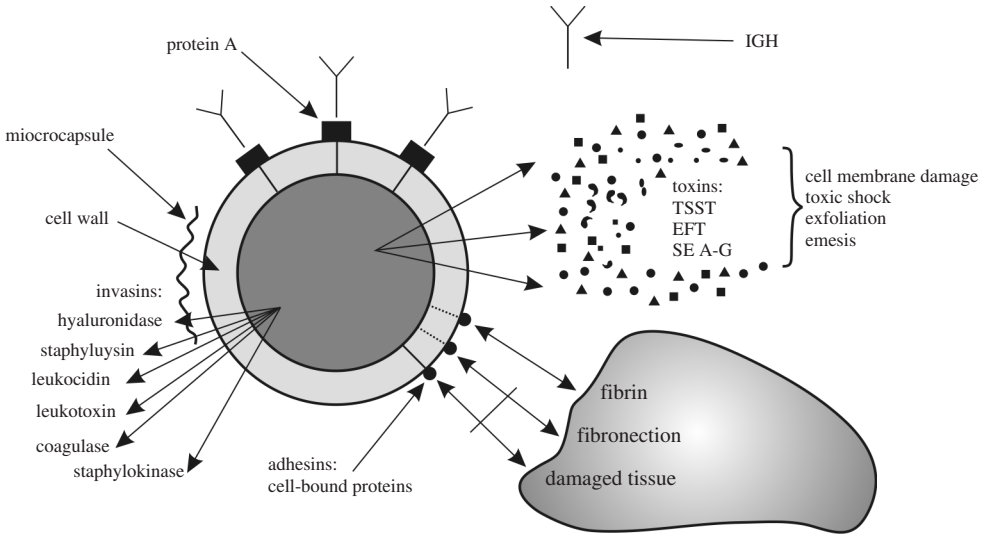

Fig. 1. Virulence determinants of *Staphylococcus aureus* [5]

reported that *Pseudomonas aeruginosa* unsaturated biofilm EPS contains large amounts of extracellular DNA (eDNA).

Biofilm extracellular polymeric substances (EPS) are a complex mixture of hydrated polymers that serve various purposes, including nutrient and water retention and protection from toxins such as antibiotics and pollutants. EPS is often described as polysaccharide, although the model organism in biofilm studies, *Pseudomonas aeruginosa*, has long been known to excrete large amounts of DNA. Extracellular DNA (eDNA) has previously been shown to be essential for saturated biofilm stability during the early stages of biofilm growth. Scientists recently reported that extracellular DNA was continuously present in unsaturated *Pseudomonas aeruginosa* biofilms and was maximally 50% more abundant than cellular DNA. Since unsaturated biofilms are not subjected to hydrodynamic shear, the structural roles for eDNA in this context are questionable. The eDNA may also enhance gene transfer and provide nutrition during oligotrophic conditions. However, little is yet known about the universality, composition, and persistence of eDNA, particularly in natural and multiple-species environments, so little more than speculation about its purpose is possible at the moment. These concerns should be addressed to improve our understanding of biofilm physiology; they also could have implications for culture-independent assessments of microbial communities [20].

ClfA, clfB

Structural organization of ClfA and ClfB is very similar. ClfA and ClfB bind to different sites in fibrinogen. ClfA binds to the γ-chain wheras ClfB binds to the α-chian. ClfA through its fibrinogen-binding function is a mediator of *S. aureus*-included platelet aggregation.

Staphylococcal cassette chromosome *mec*

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing is essential for understanding the molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA). SCC*mec* elements are currently classified into types I, II, III, IV, and V based on the nature of the *mec* and *ccr* gene complexes and further classified into subtypes according to their junkyard region DNA segments. MRSA strains have acquired and integrated into their genome a 21- to 67-kb mobile genetic element, termed the staphylococcal cassette chromosome *mec* (SCC*mec*), which harbors the methicillin resistance (*mecA*) gene and other antibiotic resistance determinants. These newly emerging community-acquired MRSA strains possess novel, small, mobile SCC*mec* type IV or V genetic elements which contain the *mecA* gene with or without additional antibiotic resistance genes and are more easily transferred to other strains of *S. aureus* than larger SCC*mec* (types I, II, and III) elements. SCC*mec* is a mobile genetic element characterized by the presence of terminal inverted and direct repeats, two essential genetic components (the *mec* gene complex and the *ccr* gene complex), and the junkyard (J) regions. The *mec* gene complex is composed of IS431*mec*, *mecA*, and intact or truncated sets of regulatory genes, *mecR1* and *mecI*. The ccr gene complex encodes the recombinases (*ccr*) that mediate the integration of SCC*mec* into and its excision from the recipient chromosome and are, therefore, responsible for its mobility. To date, there are three classes (A, B, and C) of *mec* complex and four allotypes (types 1,

2, 3, and 5) of *ccr* complex. Different combinations of these complex classes and allotypes generate various SCC*mec* types [21].

IS256

Staphylococcus epidermidis is a normal constituent of the healthy human microflora, but it is also the most common cause of nosocomial infections associated with the use of indwelling medical devices. The genomes of clinical *S. epidermidi*s strains carry multiple copies of the insertion sequence *IS*256, while other typical staphylococcal insertion sequences, such as *IS*257 and *IS*1272, are distributed equally among saprophytic and clinical isolates. Moreover, detection of *IS*256 was found to be associated with biofilm formation and the presence of the *ica*ADBC operon as well as with gentamicin and oxacillin resistance in the clinical strains. The data suggest that *IS*256 is a characteristic element in the genome of multiresistant nosocomial *S. epidermidis* isolates that might be involved in the flexibility and adaptation of the genome in clinical isolates [16].

Previous studies have shown that staphylococcal biofilm formation is a highly variable factor which is influenced by both regulatory processes and genetic mechanisms such as phase variations, mutations, and chromosomal rearrangements. The observation that some of these genetic processes are mediated by the action of insertion sequence (IS) elements prompted us to investigate the distribution of common staphylococcal IS elements among *S. epidermidis* strains of clinical and commensal origin. Moreover, scientists analyzed the relationship between IS presence, antibiotic resistance, and biofilm formation as well as the spontaneous mutation rate in this important nosocomial pathogen [16].

Kozitskaya *et al*. [16] investigated the distribution of three typical IS elements which have been described previously as components of staphylococcal genomes, i.e., *IS*256, *IS*257, and *IS*1272. *IS*256 was initially described as the flanking region of the composite aminoglycoside resistance-mediating transposon Tn4001.

It was shown that *IS*256 can be involved in phase variation of biofilm formation in *S. epidermidis*. *IS*257 is associated with the trimethoprim resistance-mediating transposon Tn4003 and numerous other resistance genes and plasmids in staphylococci (e.g. cadmium resistance). Isoforms of the element are also detectable on the SCCmec element in *S. aureus* and *S. epidermidis*. *IS*1272 is detectable in many staphylococcal species and is prevalent in multiresistant clinical isolates. Normally, *IS*256 is part of the composite transposon Tn4001, which mediates gentamicin resistance [16].

*IS*256 integrates into the ica operon. An intact *ica* operon is more prevalent in clinical *S. epidermidis* isolates (septicaemic diseases, shuntassociated meningitis) than in skin isolates of nonhospitalized persons. Defined isolates of biofilm-negative variants occur at a frequency of Ş 10–5 and, in 30% of these variants, the insertion sequence element *IS*256 is integrated at specific hotspots within icaA or icaC. Furthermore, the transposition of *IS*256 into the ica operon is reversible. After repeated passages, the biofilmforming phenotype can be restored. In these revertants, *IS*256 is precisely excised, including the initially duplicated 8 bp target sites. These results elucidate, for the first time, a molecular mechanism for turning PIA expression on and off. The recently sequenced genomes of *S. aureus* N315 and Mu50 contain, in addition to prophages and various transposons, 10 copies of *IS*1181 and two of *IS*431, distributed over the genome. Authors have probably underestimated the contribution of IS elements and transposons to genetic flexibility and environmental adaptation in staphylococci [19].

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