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Staphylococcus aureus virulence factors and their role in biofilm-associated infections

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Abstract

Although *Staphylococcus aureus* colonises the skin and mucous membranes in approximately 30% of healthy individuals, it is also an important pathogen, primarily due to its arsenal of virulence factors that contribute significantly to its ability to cause a variety of infections. These factors include surface proteins that promote adhesion to host tissues, as well as enzymes and toxins that damage host cells and tissue. Important virulence factors such as protein A, which binds to antibodies and evades recognition by the immune system, and various exotoxins such as alpha-toxin and Panton-Valentine leukocidin, which cause cell lysis and tissue destruction, play a crucial role in pathogenesis. The ability of *S. aureus* to form biofilms on medical devices further increases its persistence and resistance to therapy. Biofilms are structured communities of bacterial cells that are enclosed in a self-produced polymeric matrix and that adhere to biotic or abiotic surfaces. Biofilm-related infections caused by *S. aureus*, such as infections of medical devices (catheters, prosthetic joints, heart valves, intravascular catheters) and human tissue (chronic rhinosinusitis, chronic wounds, endocarditis and osteomyelitis), are a significant concern in medical settings. Understanding these virulence mechanisms is crucial for the development of targeted therapies and preventive measures to effectively combat *S. aureus* infections.

Key words: *Staphylococcus aureus*, virulence factors, biofilm, biofilm-associated infections

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Introduction

S. aureus subspecies *aureus* is a ubiquitous bacterium that causes a broad spectrum of infections in humans. It colonises the skin (groin and axillae), the mucous membranes of the respiratory tract (nose and pharynx), the urogenital tract (vagina) and the colon, with a frequency of about one fifth to one third of healthy individuals for nasal and intestinal colonisation (1).

S. aureus possesses a large number of virulence factors that are secreted into the external environment or are bound to the cell membrane as an integral part of the bacterial cell (2). These molecules are involved in the colonisation of the host tissue, influence the mobilisation and function of leukocytes (3), inhibit complement components (4) or antimicrobial peptides such as defensin, protegrin and similar molecules, or lead to the destruction of leukocytes (5).

Staphylococcus aureus virulence factors

The cell envelope of staphylococci contains peptidoglycan, teichoic acid (covalently bound to peptidoglycan or membrane lipids – lipoteichoic acid) and membrane lipids. These virulence factors are similar in structure and function to those of other Gram-positive bacteria. Peptidoglycan and teichoic acid play a role in the attachment of the bacteria to the host cells. Most strains of *S. aureus* produce capsular polysaccharides 5 and 8, which have an antiphagocytic function (6, 7). The MSCRAMM protein family (microbial surface components recognizing adhesive matrix molecules), molecules that are covalently bound to the peptidoglycan of the cell wall, play an important role in the adhesion of staphylococci (2). This family includes the fibronectin-binding proteins A and B (FnBPA and FnBPB), the fibrinogen-binding proteins ClfA, ClfB (clumping factors A, B) and Efb (extracellular fibrinogen-binding protein), Cna (collagen-binding adhesin) and protein A (spa) (6, 7). In addition to these proteins, the polysaccharide intercellular adhesins (PIA), Ebh (extracellular matrix (ECM)-binding protein homolog), autolysin (Aea), SasG protein, AAP (accumulation-associated protein, homolog of SasG protein S) (8), Eap (extracellular adherence protein), Bap (biofilm-associated protein), and vWbp (von Willebrand factor-binding protein) (9) also play a role in *S. aureus* adhesion. Adhesion molecules enable staphylococci to bind to tissue or components of the extracellular matrix such as collagen, elastin, laminin, fibronectin, fibrinogen, immunoglobulins and prothrombin and to invade host cells or lead to the formation of biofilms. In addition, *S. aureus* produces a number of toxins that act on host cells via various mechanisms and lead to their damage or death or act as superantigens.

α-hemolysin

Alpha-hemolysin (α-toxin) belongs to a group of toxins that form pores in the cell membrane and induce osmotic lysis of target cells (10). In its mature form, α-toxin is a 33 kDa protein that oligomerises into a heptameric structure and forms a stable membrane-spanning pore (11). In addition to its pronounced affinity for erythrocytes, α-hemolysin also binds to keratinocytes, fibroblasts and lymphocytes and has a

dermonecrotic and neurotoxic effect (12). The differences in cytolytic capacity towards different cell types from different animal species are the result of a specific receptor-ligand interaction, as α-toxin utilises the ADAM10 protein as a receptor (10).

β-hemolysin (sphingomyelinase C)

Beta-hemolysin is a sphingomyelinase that hydrolyses the sphingomyelin of the cell membrane to ceramide and phosphorylcholine (13). The exact mechanism by which cell damage and cell death occur after exposure to this toxin is unknown. Cell damage is most likely due to a disruption of cell membrane fluidity and destabilization of the lipid bilayer or accumulation of ceramide in the cell membrane (14). Due to the different proportion of sphingomyelin in the composition of the cell membrane of erythrocytes, the sensitivity of erythrocytes to hemolysis under the influence of β-hemolysin is not the same in members of different mammalian species (15).

γ-hemolysin and Panton-Valentin leukocidin (PVL)

The two-component leukocidins include gamma-hemolysin and PVL, which form pores in the membrane of the target cell. They consist of two non-linked secretory proteins, the S- and F- components, named after the speed of movement during electrophoresis (S-slow, F-fast) (16). Almost all strains of *S. aureus* produce γ-hemolysin, while PVL is produced by 2-3% of strains (12). These toxins have a similar structure: γ-hemolysin consists of two combinations of the S-component (HigA and HigC) with the F-component (HigB), and PVL consists of LukS-PV and LukF-PV (2). Each monomeric component binds to the cell membrane surface and oligomerises to form a pre-pore complex consisting of four S-components and four F-components. The mature heterooctamer forms a transmembrane pore that leads to osmotic lysis of the target cell (14). Both toxins have the affinity for neutrophils and macrophages, leading to the release of proinflammatory mediators and an inflammatory response, and γ-hemolysin also leads to the hemolysis of erythrocytes in various mammals.

The toxins LukED and LukGH (or LukAB) have structural homology and the same mechanism of action as γ-hemolysin and PVL. LukGH can occur as a secreted protein, but it is also the major surface protein of the late exponential growth phase of *S. aureus* (17).

δ-hemolysin and Phenol-soluble modulins (PSMs)

Delta-hemolysin is produced by 97% of *S. aureus* strains and 50-70% of coagulase-negative staphylococci (CNS) (12). This toxin exerts a wide range of cytotoxic effects on erythrocytes and a large number of other mammalian cells, as well as on subcellular structures – membrane-bound cell organelles, spheroplasts and protoplasts. δ-hemolysin is an amphipathic molecule consisting of an α-helix with a hydrophilic and a hydrophobic domain at two different ends. The mechanism of the hemolytic effect of this toxin is not yet fully understood. Due to its specific structure, which enables it to penetrate the

membrane of the target cell, hemolysis can occur through pore formation, destabilisation of the cell membrane, or dissolution of the membrane (18).

PSMs belong to the family of small cytotoxic peptides that have the same structure as δ-hemolysin. So far, two families of these cytotoxins have been described: PSMα (consisting of 20-26 amino acids), which includes δ-hemolysin, PSMα1-4 and PSMmec, and PSMβ (PSMβ1 and PSMβ2) (consisting of 44 amino acids). The possible mechanisms of action of these toxins are the same as those of δ-hemolysin (19).

Toxic shock syndrome toxin-1 (TSST-1) and enterotoxins

Toxic shock syndrome toxin-1 (TSST-1) and enterotoxins are atypical protein exotoxins resistant to high temperatures and proteases. These toxins are secreted during the post-exponential phase of staphylococcal growth. They belong to the family of superantigens as they cause polyclonal activation of T lymphocytes with the same Vβ chain of the T cell receptor (12). The genes encoding TSST-1 and enterotoxin synthesis are located on plasmids, bacteriophages, or specific heterologous genetic elements called pathogenicity islands (2, 20).

TSST-1 (formerly known as staphylococcal pyrogenic exotoxin C or staphylococcal enterotoxin F) is a homodimer consisting of domain A and domain B (21). It is extremely resistant to the effects of high temperatures and proteolytic degradation by trypsin. This toxin causes staphylococcal toxic shock syndrome due to its ability to penetrate mucous membranes, resulting in the activation of macrophages and T lymphocytes and the release of large amounts of proinflammatory lymphokines and monokines (2).

S. aureus produces more than twenty antigenically distinct enterotoxins (SEA, SEB, SEC, SED, SEE, SEG, SEH and SEI), of which SEA and SEB are the major causes of food poisoning (22). Enterotoxins consist of a larger A subunit and a smaller B subunit (23). The mechanism of action of these toxins is not fully understood. The main symptoms of the disease, vomiting and diarrhoea, occur as a result of mast cell degranulation and the release of proinflammatory mediators: prostaglandin E2, leukotriene B4, 5-hydroxyeicosatetraenoic acid (24) and leukotriene E4 (25), which act directly on the neural centres in the medulla oblongata. It is assumed that the degranulation of the mast cells is caused by the direct binding of the enterotoxin to specific receptors on the surface of the target cells, and not by the interaction of the enterotoxin with IgE (24).

Exfoliative toxins

The exfoliative toxins (ETs) bind to the surface layer of the epidermis and cause Staphylococcal Scalded Skin Syndrome (SSSS). These toxins bind with high affinity to the main glycoprotein of the desmosome, desmoglein 1, which is located in the *stratum corneum* of the epidermis. After binding, the ETs exhibit serine protease activity and lead to proteolytic cleavage of the glutamic acid peptide bond at position 381, which is located between the extracellular domains 3 and 4 of the adhesion molecule desmoglein 1 (26).

In this way, the stratum corneum of the epidermis is damaged, leading to its detachment and the further spread of bacteria. Although the exfoliative toxins circulate freely in the body during infection, no further toxic manifestations of the disease occur due to the high specificity towards the surface cells of the epidermis.

Table I gives a brief overview of the role of the *S. aureus* virulence factors with the coding genes and the clinical syndromes mediated by these factors.

Table I *S. aureus* virulence factors and associated infections

Tabela I Faktori virulencije *S. aureus* i udružene infekcije

Function	Virulence factors	Genes	Clinical manifestation
Adhesion to host tissue	MSCRAMM (clumping factors, fibronectin-binding proteins, collagen and sialoprotein-binding proteins)	*clfA, clfB, fnbA, fnbB, cna, sdr, bbp*	Endocarditis, osteomyelitis, septic arthritis, infections associated with medical implants
Tissue persistence	Biofilm, intracellular persistence	*ica* locus, *hemB*	Recurrent infections, cystic fibrosis, infections associated with medical implants
Evasion of the host's immune response	Leukocidins (PVL and γ-toxin), capsular polysaccharide 5 and 8, protein A, Eap, PSM	*lukS-PV, lukF-PV, hlg, cap5* and 8 gene cluster, *spa, chp, eap, psm*-α gene cluster	Invasive skin infections and necrotizing pneumonia (PVL), abscesses (capsular polysaccharide)
Invasion of host tissue	Proteases, lipases, nucleases, hyaluronidase, phospholipase C, metalloproteinases (elastase)	*V8, hysA, hla, plc, sepA*	Tissue damage and metastatic spread of infection
Toxin-mediated diseases and sepsis	Enterotoxins, TSST-1, exfoliative toxin, α-toxin, peptidoglycan, lipoteichoic acid	*sea-q, tstH, eta, etb, hla*	Food poisoning, toxic shock syndrome, scalded skin syndrome, bullous impetigo and sepsis

Biofilm

One of the most important factors in the virulence of staphylococci, which has become increasingly important in the pathogenesis of recurrent and protracted infections in recent years, is the ability to form biofilms. Biofilms have been implicated in the development of more than 60% of chronic, recurrent and device-related human infections. The biofilm is a complex multicellular structure that forms on various biotic and abiotic surfaces (19). Compared to bacteria in planktonic form, bacteria in biofilms exhibit

different phenotypic characteristics, sensitivity to antibiotics and chemotherapeutics, and mechanisms of innate and acquired immunity (27). The phases of biofilm formation are attachment, proliferation, growth and maturation of the biofilm, and biofilm dispersion. The initial attachment of planktonic bacteria to various tissue cells or abiotic surfaces is mediated via van der Waals forces and is weak and reversible. The irreversible attachment of bacteria occurs through a receptor-ligand interaction mediated by the MSCRAMM family of adhesion proteins. Within the biofilm, the bacteria begin to multiply and communicate via quorum sensing systems. They form a thick extracellular polymeric substance (EPS) that is resistant to many physical factors that inhibit biofilm formation, to the response of the host immune system, to oxidative damage and metal cations, and to antibiotics. Bacterial microcolonies grow and form various forms of biofilms (e.g. “fungal" or “tower” form) that extend beyond the tissue surface or into the lumen of artificial devices or tubular organs. As the biofilm matures, the matrix breaks down and parts of the biofilm detach and spread into the adjacent tissue, creating a new focus of metastatic biofilm (28). During biofilm formation, staphylococci adhere to the surface of living cells or to plastic polymer surfaces of various medical devices and implants. Attachment to abiotic surfaces occurs directly to the plastic polymer or indirectly by attachment of the staphylococci to ECM components and host proteins lining the implant (19). Within the biofilm, there is a developed network of channels with active water and nutrient flow, which creates the conditions for cell growth and division (2). Staphylococci in the biofilm are in four different metabolic states *in vitro* – there are cells that grow under aerobic conditions, cells that provide energy through fermentation processes, metabolically inactive cells (so-called persisters and cells that grow very slowly), and dead cells. The most metabolically active cells are located in the oxygen-rich surface layer of the biofilm and in the deeper layers of the biofilm, which are rich in nutrients from the liquid phase. Other cells are in a metabolically inactive state in an anoxic environment (29). Different nutrient concentrations in the biofilm layers lead to different gene expression and protein synthesis between cell groups within the same biofilm. A specific type of cell organisation enables staphylococci to evade the host's immune response (resistance to phagocytosis or the action of proteases and free radicals) and to be resistant to antimicrobial agents (2, 30). The compactness of the biofilm and the density of the ECM represent a physical barrier that prevents the diffusion of antibiotics through the layers of the biofilm, and cells that are in a metabolically inactive state have a lower degree of division and therefore a lower number of target molecules that would be acted upon by antibiotics. Persisters are tolerant even to high concentrations of bactericidal antibiotics and spontaneously revert to a metabolically active state after antibiotic therapy is discontinued, making them a constant source of bacteria for recurrent infections (31). In addition to the thick EPS, which physically hinders the diffusion of antibiotics, extracellular DNA (eDNA) also contributes significantly to the antimicrobial resistance of the biofilm. eDNA is actively secreted into the biofilm or released by dying cells and has chemical properties that induce resistance via several mechanisms. It lowers the pH of the biofilm and acidifies the local environment, promoting the antibiotic resistance phenotype in the bacteria, and as an anion, eDNA chelates with

cationic antimicrobials (such as vancomycin), which reduces their diffusion through the biofilm. Other eDNA-mediated mechanisms involved in antimicrobial resistance include promotion of horizontal gene transfer by conjugation of plasmids between cells in biofilms and neutralisation of innate immunity effector molecules (32, 33).

Phases of biofilm formation and virulence factors associated with staphylococcal attachment and invasion to host tissue are presented at Figure 1.

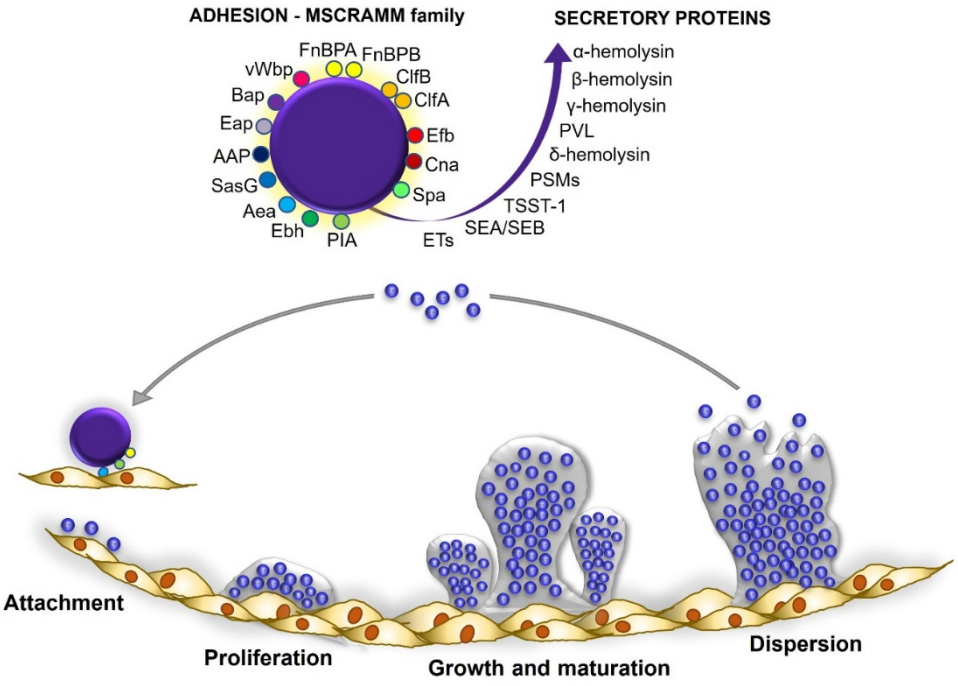

Figure 1. Phases of biofilm formation and virulence factors associated with staphylococcal attachment and invasion to host tissue

Slika 1. Faze formiranja biofilma i faktori virulencije udruženi sa vezivanjem stafilokoka i invazijom u tkivo domaćina

Planktonic cells irreversibly adhere to various tissue cells or abiotic surfaces through receptor-ligand interaction mediated by the MSCRAMM family of adhesion proteins. The bacteria multiply and proliferate, produce an extracellular polysaccharide matrix and communicate via quorum-sensing systems. As the biofilm grows and matures, the matrix breaks down and parts of the biofilm detach and disperse to the adjacent tissue, forming a metastatic biofilm.

Planktonske ćelije se ireverzibilno vezuju za različite ćelije tkiva ili abiotske površine putem receptor-ligand interakcije posredovane MSCRAMM familijom adhezionih proteina. Bakterije se razmnožavaju, proizvode ekstracelularni polisaharidni matriks i komuniciraju putem quorum-sensing sistema. Kako biofilm raste i sazreva, dolazi do razgradnje matrika i odvajanja delova biofilma koji se disperguju u susedna tkiva formirajući metastatski biofilm.

Regulation of *S. aureus* virulence factors and biofilm formation

The regulation of virulence factor expression in *S. aureus* occurs via several regulatory systems (i.e., quorum sensing system, QS), of which two general regulators are best studied: *agr* (accessory gene regulator) and SarA (staphylococcal accessory regulator) (34). In addition, the regulatory function is performed by several other QS systems whose activity depends primarily on the environmental factors in which staphylococci grow. The *agr* quorum sensing system is considered to be one of the main regulators of gene expression in *S. aureus*. This regulatory system is activated when the cell population reaches a critical density and regulates the expression of genes coding for the synthesis of toxins and other virulence factors. The *agr* gene locus encodes two primary RNA transcripts (RNAII and RNAIII) that play a role in the synthesis of signalling and effector proteins. The P2 operon of the *agr* locus contains four structural genes: *agr*B, *agr*D, *agr*C and *agr*A, which encode the *agr* signalling mechanism via the RNKII transcript (35, 36). AgrB is a transmembrane protein that plays a role in the posttranslational modification of the primary AgrD transcript into an octapeptide (37, 38) and its modification into the active ring form (39). The resulting autoinducer peptide (AIP) activates the two-component AgrC-AgrA system by binding to the transmembrane protein AgrC, which has histidine kinase activity and phosphorylates the AgrA regulator. Activated AgrA leads to a significant increase in the transcription of operons P2 and P3 during staphylococcal growth in the late logarithmic phase. Transcription of the P3 operon via the RNAIII transcript leads to the synthesis of δ-toxin and other secretory virulence factors such as TSST-1 and α-hemolysin (35) and inhibits protein A synthesis by interfering with *spa* (protein A) gene expression at the posttranscriptional level (34, 40). During the exponential phase of staphylococcal growth, the synthesis of enterotoxins A and K is not affected by RNAIII, and the synthesis of enterotoxins B, C and D is only partially regulated by RNAIII transcripts. Based on the sequence differences of the *agr*B, *agr*D and *agr*C genes, four *agr* types were identified in *S. aureus*. The AIPs produced by different *agr* types inhibit each other – AIP of one *agr* type inhibits the expression of the other *agr* types (36, 41).

In addition to the *agr*-QS system, which plays a central regulatory role in the virulence of staphylococci, several other regulators have been described that exert their function independently or in conjunction with the *agr* regulatory system (2, 34). SaeRS, ArlS-ArlR and SrrAB are two-component systems whose regulatory activity depends on the environmental factors under which staphylococci grow.

SaeRS is a system that enables staphylococci to recognize and respond to environmental stimuli: high salt concentrations, low pH, glucose and subinhibitory concentrations of antibiotics (41). *Sae* mutants produce lower levels of hemolysin and coagulase without affecting RNAIII transcription, and the *sae* system can also be activated downstream of the *agr* locus (42).

ArlS-ArlR regulates autolytic activity, NorA efflux pump activity and antagonises the agr system by counteracting AIP, leading to a decrease in the production of α-toxin,

β-hemolysin, exoenzymes – lipase, coagulase and serine protease (Ssp), and protein A (43, 44). Mutants for the *arlRS* gene increase the production of adhesive molecules and polysaccharide intercellular adhesins, leading to increased biofilm formation, suggesting that this regulator in its non-mutant form has an inhibitory effect on biofilm formation (45).

SrrAB (staphylococcal respiratory response regulator) is a regulatory system that inhibits the expression of RNAIII and is itself under the control of the *agr* gene that carries out its suppression (46). *SrrAB* mutants lose the ability to grow in an anaerobic environment, suggesting that the expression of *srrAB* is necessary for the regulation of genes involved in energy metabolism (47).

In addition to the above-mentioned systems that regulate the expression of virulence factors and biofilm formation, other gene loci are also involved. Their activity leads to an increased expression of virulence factors that play a role in biofilm formation, but also of other virulence factors that are not directly involved in this process. One of the most important components of the biofilm is the extracellular DNA (eDNA), which increases the adhesion of the biofilm to the biotic or abiotic surface, which is important in the first phase of biofilm formation (48). Several enzymes are involved in cell lysis and the release of eDNA, the most important being murein hydrolase, which is encoded by the *cidA* gene. The deletion or mutation of this gene leads to a decrease in biofilm adherence due to a lower amount of eDNA in the biofilm matrix (49). The products of the *lrgAB* gene negatively regulate the activity of *cidA* and murein hydrolase, as well as the release of eDNA, which also leads to a decrease in biofilm adherence (48). Staphylococcal autolysins are also involved in the release of eDNA – the Atl autolysin of *S. aureus*, encoded by the *atl* gene (50), and the AtlE autolysin of *S. epidermidis*, encoded by the *atlE* gene (51). In the early phase of biofilm formation, AtlE enables *S. epidermidis* to adhere directly to the hydrophobic surfaces of medical implants or to adhere indirectly to the extracellular matrix proteins of the host that line the implant (e.g. vitronectin) (51). Atl-autolysin plays a similar role in *S. aureus* adherence. The product of the *sigB* operon is also involved in the early phase of *S. aureus* biofilm formation. The product of this locus – the σ^B factor – increases the expression of Clf A and B, FnbpA and coagulase, proteins that are essential for the initial adherence of staphylococci (52). Conversely, σ^B activity leads to a decrease in the expression of β-hemolysin, enterotoxin B, serine and cysteine proteases (SplA and B), metalloproteinase Aur and leukotoxin D, which are abundant in the planktonic phenotype of staphylococci (53).

Pathogenicity of *S. aureus* and biofilm-associated infections

S. aureus most commonly causes mild to moderate skin and soft tissue infections, such as bullous impetigo, abscesses, boils and scalded skin syndrome or wound infections. The infections may be the result of direct invasion by microorganisms (skin and soft tissue infections, systemic infections with fatal outcome – sepsis, endocarditis and pneumonia) or production of staphylococcal toxins (toxic shock syndrome and food poisoning) (2). These infections are most common in neonates, patients with chronic lung

diseases (such as cystic fibrosis, emphysema and chronic bronchitis), leukaemia, malignancies, influenza, patients with burns, chronic skin diseases, diabetes, transplanted organs, postoperative wounds, intravenous catheters, medical implants or in immunocompromised patients (6). In recent years, great importance has been attached to the biofilm as a particular virulence factor of *S. aureus* and the diseases that arise from biofilm formation. These diseases are characterised by a protracted course, resistance to conventional antibiotic therapy, recurrent infections and the dissemination of biofilms with the development of distant foci of infection. Staphylococcal biofilms are the most common pathoanatomical substrate in osteomyelitis, infections in patients with implanted medical devices, endocarditis, chronic wounds, upper respiratory tract and ocular infections, and infections caused by a mixed flora of microorganisms.

The role of biofilm in the pathogenesis of osteomyelitis

Osteomyelitis is a bone infection that can be caused by various microorganisms, of which *S. aureus* is the most common cause of chronic infection and is responsible for over 90% of all forms of osteomyelitis in children. The bacteria are usually spread via the hematogenous route or by direct inoculation following traumatic injury or surgery (54). The first step in the formation of a biofilm is the increased expression of adhesion molecules through which bacteria bind to the appropriate receptors in the bone matrix, fibrinogen, fibrin, osteopontin, fibronectin, collagen, elastin, etc. (55). After adhesion to the bone surface, the bacteria multiply and an early biofilm forms. As the bacterial population grows, the expression of molecules changes, the expression of adhesion molecules decreases and the expression of secretory virulence factors such as toxins, enzymes and other immunomodulatory molecules that evade the host's immune response increases (56). At the same time, bacterial inflammatory mediators, formyl methionyl peptide, lipoteichoic acid, peptidoglycan, hemolysin and staphylococcal DNA (i.e., unmethylated cytosine-phospho-guanosine DNA sequences) are released. Although these mediators activate polymorphonuclear leukocytes, the secretion of a large amount of different virulence factors leads to their lysis and ineffective phagocytosis (57). Phagocytic activity further exacerbates tissue damage and increases the devitalised surface on which the biofilm forms. As the biofilm spreads on the bone tissue, it also matures, with parts of the biofilm breaking off and forming metastatic foci of infection. The most effective treatment for osteomyelitis in adults is a combination of surgical removal of the focus of infection and antibiotic therapy. Conversely, antibiotic therapy in children usually leads to spontaneous resorption of the necrotic bone and tissue and disappearance of the surface on which the biofilm forms, so that administration of antibiotics without surgery is sufficient (58).

Medical device-associated infections

S. aureus is one of the main causes of chronic infections caused by the formation of biofilms on the surface or inside medical devices. Infection with staphylococci occurs during a surgical procedure, by hematogenous dissemination or through a subsequent injury to the implant. Biofilms form most frequently on orthopaedic implants such as

artificial joints (arthroprostheses) and osteosynthesis material for fracture fixation (surgical wires, plates, external fixators, screws, wedges, etc.), as well as materials based on plastic polymers and silicone (central venous catheters, intravenous catheters and urinary catheters, coronary stents, artificial heart valves, neurosurgical ventricular shunts, intraocular lenses, endotracheal tubes, drains, shunts, cochlear implants, dental implants, cosmetic implants, etc.) (59). Biofilms also form on the surface of medical devices such as aspirators, ventilators, defibrillators or electrical conductors for cardiac rhythm. The bacteria bind directly to the hydrophobic surface of the implant or indirectly to proteins and components of the host extracellular matrix that envelop them (19). The dense polysaccharide matrix of the biofilm represents a physical barrier to the diffusion of antibiotics, so the only treatment for these infections is surgical removal of the implant and its replacement with a new one.

Endocarditis

Infective endocarditis (IE) is as an infection of the inner lining of the heart (i.e., the endocardium), which mainly occurs on the heart valves or implanted cardiac devices. Heart valve infection with *S. aureus* is associated with a high mortality rate, reaching almost 100% if untreated, and a high rate of postinfectious complications. Persistent or recurrent cases of IE after apparent initial infection control may be related to biofilm formation in the heart valve vegetations. The formation of the biofilm and its size are limited by the forces of blood flow. Infection is usually hematogenous, and the biofilm represents a constant source of bacteria that spread metastatically in the bloodstream and form distant foci of infection (29, 60).

Chronic wound infections

Chronic wound infections in which a staphylococcal biofilm forms are most common in patients with impaired peripheral arterial or venous circulation. The biofilm forms on the surface of venous or arterial ulcers and on the surface of wounds that occur in diabetic patients (e.g., diabetic foot) and impedes epithelialisation and wound healing (61). The impaired tissue repair and wound healing is most likely the result of a collagen-1 deficiency in the granulation tissue of the wound due to the inhibition of collagen synthesis, as well as the induction of collagenolysis by staphylococcal biofilms. Such a deficiency impairs the reepithelialisations of the wound (62).

Upper respiratory tract and ocular infections

Chronic rhinosinusitis is an inflammatory disease of unknown etiopathogenesis. *S. aureus* forms a biofilm in approximately 50% of cases, leading to severe forms of the disease with poor outcome and frequent postoperative complications (63). Multidrug-resistant strains of *S. aureus* form a biofilm on the mucosa of the eye and most commonly cause conjunctivitis. In more severe cases, the infection penetrates into the deeper structures of the eye and causes keratitis and endophthalmitis. Methicillin-resistant *S. aureus* is isolated more frequently than methicillin-susceptible *S. aureus* (MSSA), which further complicates the treatment of these diseases (64, 65). A biofilm often forms around

the teeth, leading to periodontitis (inflammation of the ligaments and bone around the tooth that support it) and peri-implantitis (destructive inflammation of the soft and hard tissues around dental implants). *S. aureus* not only forms a biofilm on the surface of the oral mucosa, but also binds with high affinity to the titanium surfaces of dental implants (66).

Biofilm-related polymicrobial infections

Candida albicans and *S. aureus* are most frequently isolated from oral or vaginal swabs as the causative agents of infections associated with mixed species of microorganisms. Both species have the ability to form multilayered biofilms on mucous membranes. In addition to biofilm formation on mucous membranes, *S. aureus* binds to the surface of *C. albicans* hyphae and also forms a biofilm on them. The hyphae of *C. albicans* are able to penetrate the epithelial cells of the host, leading to the transfer of staphylococci into the intracellular environment and the occurrence of a local and/or systemic infection (67, 68).

Conclusion

Staphylococcus aureus possesses a variety of virulence factors, of which the ability to form biofilms is a major contributor to persistent and resilient infections. Understanding the roles these virulence factors have during diseases can provide the knowledge necessary for designing new treatment strategies for biofilm-associated infections and identifying vaccine targets. The prevention and treatment of staphylococcal biofilms requires a comprehensive and multifaceted approach. Effective prevention strategies include strict aseptic techniques, the use of antimicrobial coatings on medical devices and prophylactic antibiotics to minimise the risk of infection during surgical procedures. In terms of therapy, a combination of high-dose, prolonged courses of antibiotics and surgical procedures to remove or replace infected devices is often required. In addition, complementary treatments aimed at disrupting biofilms and improving the efficacy of antibiotics, such as enzymes or antimicrobial peptides, are promising. Further research into novel antimicrobial agents, biofilm-disrupting therapies and potential vaccines is essential to improve outcomes and reduce the incidence of biofilm-associated staphylococcal infections.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Author contributions

DB: Conceptualization; Visualization; Roles/Writing - original draft; and Writing - review & editing.

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Faktori virulencije *Staphylococcus aureus* i njihov značaj u infekcijama povezanim sa biofilmom

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Kratak sadržaj

Staphylococcus aureus kolonizuje kožu i sluzokožu kod približno 30% zdravih osoba, ali je takođe važan patogen, prvenstveno zbog brojnih faktora virulencije koji značajno doprinose njegovoj sposobnosti da izazove različite infekcije. Ovi faktori uključuju površinske proteine, koji promovišu adheziju za tkiva domaćina i enzime i toksine koji oštećuju ćelije i tkiva domaćina. Važni faktori virulencije kao što su protein A, koji se vezuje za antitela i izbegava prepoznavanje od strane imunskog sistema, i različiti egzotoksini, kao što su alfa-toksin i Panton-Valentin leukocidin, koji izazivaju lizu ćelija i nekrozu tkiva, igraju ključnu ulogu u patogenezi bolesti. Sposobnost *S. aureus* da formira biofilm na medicinskim uređajima i implantima dovodi do perzistentnih infekcija koje su otporne na terapiju. Biofilm predstavlja organizovanu zajednicu bakterijskih ćelija koje su uronjene u ekstracelularni polimerni matriks vezan za biotsku ili abiotsku površinu. Infekcije udružene sa stvaranjem biofilma, kao što su infekcije udružene sa primenom medicinskih uređaja i implantata (kateteri, veštački zglobovi, srčani zalisci, intravaskularni kateteri) ili tkiva (hronični rinosinusitis, hronične rane, endokarditis i osteomijelitis), predstavljaju značajan terapijski problem. Razumevanje molekulskih mehanizama virulencije je ključno za razvoj ciljane terapije i preventivnih mera za efikasnu borbu protiv infekcija izazvanih bakterijom *S. aureus*.

Ključne reči: *Staphylococcus aureus*, faktori virulencije, biofilm, infekcije udružene sa stvaranjem biofilma