Chapter

Main Factors of *Staphylococcus aureus* Associated with the Interaction to the Cells for their Colonization and Persistence

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Abstract

Staphylococcus aureus is a microorganism that can colonize the nose, pharynx, and other regions of the body. It has also been observed that it can cause persistence. Successful colonization of *S. aureus* depends in the factors that favor the interaction of the bacteria with host cells. The bacterial determinants of *S. aureus* that have the capacity to adhere to human tissues involve adhesion factors such as teichoic acids and cell-wall-anchored proteins (CWA) such as ClfA, IcaA, SdrC, FnBPA, among others. The colonization and persistence process first involve adhesion to the tissue, followed by its reproduction and the possible formation of a biofilm. This review will describe the main virulence factors that allow bacterial adhesion and biofilm formation, including the accessory gene regulator genes (*agr*), related to colonization and persistence of *S. aureus*.

Keywords: *S. aureus*, colonization, persistence, adhesins, biofilm, virulence factors, regulation, *agr*

1. Introduction

Staphylococcus aureus is a versatile pathogen that can cause infections in several mammal species including human. This is possible because several genetic variants have been associated with the host and the type of infection [1]. *S. aureus* can form a normal part of the human microbiota or act as an opportunistic pathogenic bacterium that produces a wide range of diseases that can be acquired in the hospital or in the community [2].

Several studies of colonization of *S. aureus* in the nose show that it can persist, following three patterns of carriers in the population. Around 20% of people are persistent carriers, around 30% are intermittent carriers, and non-carriers are on average 50% [3]. It has been reported that persistent carriers usually present a single

strain over time, shed the bacteria in the environment, and they can be infected more than intermittent carriers and non-carriers. Intermittent carriers may have different strains over time and less colonization [4].

In the adults *S. aureus* can be found apart from the nose at other sites in the body: pharynx (4–64%), abdomen (15%), armpits (8%), intestines (17–31%), perineum (22%), and vagina (5%) [5–7].

Bacterial adhesion to the skin or mucous membranes is usually the initial and fundamental step in colonization and persistence, with the subsequent possibility of producing infections and pathological processes in the host. By attaching, bacteria can also bypass the innate response, allowing access to nutrients, colonization, and possibly subsequent persistence, which is favored by biofilm formation, toxin production, cell invasion, and evasion mechanisms of the immune response [8].

2. Colonization factors of *S. aureus*

Colonization with *S. aureus* requires direct human contact or contact with contaminated fomites. But this does not guarantee colonization, and some people remain as non-carriers [9]. Once colonizing, permanence is an important trail in persistent infections. Therefore, it is required to study the factors involved in colonization and persistence.

2.1 Initial *S. aureus* interaction

Colonization begins by the interaction of the bacteria with the cells of the host. *S. aureus* has many adhesins that allow it to first adhere to the human cell, multiply, and even persist in the tissue. Next, several components of the bacterium that intervene in the interaction with the host are reviewed.

2.1.1 Wall teichoic acids (WTA)

Reversible binding of *S. aureus* to host cells is through wall teichoic acids (WTAs) and/or receptor-mediated protein interactions [10]. The surface of *S. aureus* is composed of polysaccharides, such as capsular polysaccharides (PC) and also by WTA. Two types of acids have been described: lipoteichoic acids (LTA), which are found in the cytoplasmic membrane, and teichoic acids (WTAs), which are bound to peptidoglycan in the cell wall [11, 12]. WTAs are found on the surface of the cell wall, which are polyanionic cell wall glycopolymers (CWGs). They are made up of approximately 40 repeat units of ribitolphosphate linked with D-alanine and N-acetylglucosamine, which are covalently linked to peptidoglycan [13, 14].

WTAs have been shown to participate in the adhesion and colonization of staphylococci [14, 15], also participate in cell division, as well as in the formation of biofilms, an elevated expression increases the virulence of *S. aureus* [16]. It has also been seen that the D-alanine residues of the WTA participate in resistance against antimicrobial peptides (defensins or cathelicidins), in addition to participating in the resistance of some antibiotics such as teicoplanin or vancomycin [11, 17]. The biosynthesis of these biopolymers in *S. aureus* is mediated by N-acetylglucosaminyltransferase (Tar) enzymes [18].

Weidenmaier et al. [19], using a *S. aureus* model for nasal colonization in cotton rats, found that the proteinaceous adhesins of the bacterium act mainly during the

later stages of colonization, while the non-proteinaceous WTA-type adhesin acts in the early stages. This is due to the high expression of the WTA *tagO* and *tarK* genes during the first and last stages of colonization, while the expression of adhesin proteins such as clumping factor B (ClfB) increases in the early stages and decreases in the later stages of colonization [14, 20–22]. Therefore, WTA is not required for the *in vitro* growth of *S. aureus*; however, it is required for establishing infection in animals [18].

The action of WTA in the initial interaction of *S. aureus* to a surface is through non-covalent surface charge interactions (WTA is a polyanionic molecule), with various associated polymeric proteins in the cell membrane (recently its interaction with the Scavenger receptor SREC-1) having been demonstrated, which allows its adhesion to the structural molecules of the cell matrix such as fibronectin, fibrinogen, collagen, etc. [23, 24].

2.1.2 S. aureus cell wall-anchored (CWA) proteins

S. aureus has been shown to produce some 25 different cell-wall-anchored (CWA) proteins, linked to peptidoglycan via transpeptidases. These CWA may function in adhesion, biofilm formation, invasion, and evasion of host immune responses [25].

Five groups have been proposed to classify *S. aureus* CWA proteins (**Table 1**). Where there are many microbial surface components recognizing adhesive matrix molecules (MSCRAMM), including fibronectin-binding proteins (FnBPA and FnBPB), proteins of the Serine-Aspartate repeat family (SdrC, SdrD, and SdrE), clumping factors (ClfA and ClfB), Protein A (Spa), iron-regulated surface determinants (IsdA, IsdB, IsdC, and IsdH), plasmin-responsive protein (Pls), *S. aureus* surface protein G (SasG), and bone sialoprotein-binding protein (Bbp). All of these proteins participate in the initial interaction with the host cell through cell adhesion and/or biofilm formation [26].

2.1.2.1 Microbial surface components recognizing adhesive matrix molecules (MSCRAMM) used to attach to cells

S. aureus reversibly or irreversibly binds to the cell surface via MSCRAMM proteins [25, 27]. During infection, these proteins allow bacteria to bind to host receptors. These proteins are made up of three parts: a binding domain, a domain that spans the entire cell wall, and a third part on the bacterial surface that serves for non-covalent binding of MSCRAMM proteins to the host cell [25, 26].

The main binding factors of *S. aureus* (**Table 1**) are reviewed below.

2.1.2.1.1 Clumping factor B (ClfB)

S. aureus binds to nostrils during colonization via clumping factor B (ClfB) by highly affine binding to the cornified cell envelope, mainly due to the fibrinogen binding mechanism, which is an important step in colonization by *S. aureus* [28, 29], as well as *in vitro* biofilm formation [30]. Therefore, the union of ClfB with fibrinogen promotes nasal colonization. ClfB expression occurs mainly in the early phase of bacterial exponential growth and is de-expressed in the late growth phase and stationary phase [31]. Most strains of *S. aureus* have the *clfB* gene [21, 32, 33]. The ClfB protein exhibits sequence variations depending on *S. aureus* clonal complexes, but protein variants have about 94% amino acid identity with each other [34].

Protein group	Ligand	Function
1. MSCRAMM		
Clumping factor A (ClfA)	Fibrinogen Complement factor I	Fibrinogen binding, evades immune response by binding to soluble fibrinogen Evasion of the immune response; C3b degradation
Clumping factor B (ClfB)	Fibrinogen, loricrin, keratin 10; DLL	Adherence to desquamated epithelial cells. Participates in nasal colonization
Protein C with Serine-aspartate repeats (SdrC)	B-neurexin; DLL Desquamated epithelial cells	Unknown Possible nasal colonization
Protein D with Serine-aspartate repeats (SdrD)	Desquamated epithelial cells	Possible nasal colonization
Serine-aspartate repeat-containing Protein E (SdrE)	Complement factor H	Evasion of the immune response; C3b degradation
Bone sialoprotein-binding protein (SdrE isoform)	Fibrinogen; DLL	Adhesion to the extracellular matrix (ECM)
Fibronectin binding proteins A (FnBPA) and B (FnBPB)	Fibrinogen and elastin, DLL. FnBPA domain A also binds fibronectin, but not by DLL Fibronectin	Adhesion to ECM Adhesion to ECM; invasion
Collagen adhesin (Cna)	Collagen	Adhesion to collagen-rich tissues
2. NEAT (near iron transporter) motif family		
Iron-regulated surface protein A (IsdA)	Heme, fibronectin, fibrinogen, loricrin, cytokeratin 10, Unknown ligand (NEAT motif region of C-terminal domain)	Heme absorption and iron acquisition; adhesion to desquamated epithelial cells; lactoferrin resistance Resistance to antimicrobial peptides and bactericidal lipids; neutrophil infection
Iron-regulated surface protein B (IsdB)	Hemoglobin, Heme β3 integrins	Heme absorption and iron acquisition Invasion of non-phagocytic cells
Iron-regulated surface protein H (IsdH)	Heme, hemoglobin Unknown ligand (NEAT motif region of the N-terminal domain	Heme absorption and iron acquisition Accelerated degradation of C3b
3. Three helix packaging		
Protein A	IgG Fc, IgM Fab subclass VH3, TNFR1 von Willebrand factor Unknown ligand (Xr region)	Inhibition of phagocytosis; B cell superantigen; inflammation Endovascular infection; endocarditis Inflammation
4. G5-E repeat family		
S. aureus surface protein G (SasG) and plasmin-sensitive surface protein (Pls) (a homolog of SasG in MRSA)	Unknown ligand (A domain) Unknown ligand (G5-E repeats)	Adhesion of desquamated epithelial cells Biofilm formation
5. Structurally uncharacterized proteins		

Protein group	Ligand	Function
Adenosine synthase A (AdsA)	Non-link-mediated function	Survival in neutrophils by inhibiting oxidative processes
S. aureus surface protein X (SasX)	Unknown ligand	Biofilm formation, cell aggregation, and squamous cell adhesion
Serine-rich adhesin for platelets (SraP)	Salivary agglutinin gp340 and an unidentified ligand on platelets	Endocarditis; and endovascular infection
S. aureus surface protein C (SasC)	Unknown ligand	Induces the primary attachment of cells and their accumulation in the formation of biofilms
SasB, SasF, SasF, SasJ, SasK and SasL	Unknown ligands	Possible LPXTG proteins. Unknown structure or function
Biofilm-associated protein (Bap)	gp96	It stimulates the formation of biofilms and aggregation on the surfaces of epithelial cells, prevents the invasion of epithelial cells of the mammary glands. It is only found in bovine strains.

ECM: extracellular matrix; DLL: dock, lock and latch. The shaded rows belong to the main ligands of S. aureus to the host (modified from Foster et al. [25]).

Table 1.
Main cell-wall-anchored (CWA) proteins of Staphylococcus aureus.

ClfB also binds to cytokeratin 10, in addition to binding fibrinogen, cytokeratin 10 is one of the main components of the interior of squamous cells. ClfB also binds loricrin, one of the most abundant protein in the cornified envelope of squamous cells, and is key in the colonization of *S. aureus* in the nose and human skin [28, 29, 31, 34, 35]. Initially, it was found that the ClfB protein binds to fibrinogen, it can undergo the proteolytic action of the *S. aureus* metalloprotease aureolysin [36]. ClfB protein in digested form cannot bind fibrinogen, but can bind cytokeratin 10 with good affinity. At the ligand level, ClfB interacts with the amino acid sequence Y[GS]nY found in the carboxyl-terminal of cytokeratin 10 [37].

The ClfB binding is carried out using the so-called dock, lock, and latch (DLL) mechanism, where a short peptide of cytokeratin 10 or loricrin binds the N2 and N3 domains of the ClfB protein [38, 39].

2.1.2.1.2 Serine-aspartate repeats (SdrC and SdrD) proteins

Within the MSCRAMM is the subfamily of serine-aspartate repeat (Sdr) proteins, which have an R region that presents repeats of the serine-aspartate dipeptide and is located in the sdr locus [38, 40]. In *S. aureus*, three members of Sdr are known, and they are SdrC, SdrD, and SdrE, which share a conserved structure [38].

Askarian et al. [41] reported that SdrD is required for survival of *S. aureus* within the host, giving it the ability to evade some processes of the innate immune response, particularly by inactivating the complement system through the lectin pathway. On the other hand, SdrE functions in the recognition of complement binding protein C4b.(C4BP) [42, 43]. On the other hand, SdrC is important for the formation of bacterial biofilms [44]. SdrC can also bind specifically and with high affinity to

β-neurexin [45]. *S. aureus* has at least two of the sdr genes, with the sdrC gene always being found, the other two may or may not be in the *S. aureus* genome [40]. Strains that possess only the sdrC gene are less likely to cause bone infections, because it is related to one of the SdrE variants that has been identified as a bone sialoprotein-binding protein [46]. SdrD is crucial for abscess formation and can interact with desmoglein (desmosomal protein that maintains the structure of the epidermis through its adhesive function) [41, 47, 48].

2.1.2.2 Iron-regulated surface proteins (Isd)

Iron-regulated surface proteins (Isd) are responsible for transporting the heme group, the system is made up of nine proteins (IsdA-IsdI) and are activated if the bacterium has iron-limited conditions [21, 49, 50]. The heme group binds to a membrane, and from there it passes to the cytoplasm, once at this site, the heme oxygenases release the iron atoms [25]. *S. aureus* requires these hemoproteins for growth and virulence [51, 52].

Isd proteins present domains of the nearby iron transporter (NEAr iron Transporter, NEAT), which participate in the capture of the heme group of hemoglobin, favoring the development of bacteria in the host in places where there is low iron concentration. Isd proteins have NEAT domains, which vary according to the type of Isd, since IsdA only has one, IsdB has two, and IsdH has three, with which it can bind to the heme group, IsdA also has a hydrophilic end C-terminal, which is responsible for decreasing the hydrophobicity of the cell surface, making the bacteria resistant to lipid bactericides and other antimicrobial peptides [25].

Isd proteins are important during bacterial pathogenesis. IsdA can bind to various host proteins in addition to the heme group (fibrinogen, fibronectin, cytokeratin 10, etc.), promoting adherence to cell lines and tissues, and acts together with IsdB to provide resistance to neutrophil killing [53].

2.1.2.3 S. aureus surface proteins (SasG and SasX)

There is a broad association between *S. aureus* surface protein G (SasG) and accumulation-associated protein (Aap), the latter being required by *Staphylococcus epidermidis* for biofilm formation and promoting intercellular adhesion [54, 55].

SasG binds covalently to the cell wall via homophilic protein-protein interactions through Zn^{2+}-dependent cleaved SasG B domains, resulting in cell-cell adhesion. However, the host cell binding ligand is still unknown [56–59].

S. aureus colonizes the nasal epithelium mainly due to ClfB and IsdA proteins, which allow adhesion to desquamated epithelial cells [25]. However, adhesion to epithelial cells is also promoted by SasG and may contribute to colonization [60]. In addition, overexpression of the *sasG* gene can inhibit clumping proteins (ClfA and ClfB) to increase biofilm formation [61, 62].

SasX protein, another CWA protein, seems to have been important in the epidemics caused by MRSA in hospitals on the Asian continent [63]. The *sasX* gene is known to be encoded by a bacteriophage that is in lysogenic form [34], SasX protein increases the formation of biofilms, by increasing cell aggregation it leads to a decrease in phagocytosis of neutrophils [63, 64] and adhesion to desquamated cells [25]. Therefore, the *sasX* gene not only encodes a colonization factor but also helps virulence of *S. aureus* by evading immune response [65]. SasX has also been associated with disease severity in skin and lung infections [63].

2.1.3 Adhesins regulation

The regulation of the virulence factors of *S. aureus* is carried out by a system that integrates signals derived from the host and the environment in a coordinated manner. Two-Component Systems (TCSs) are processes that identify environmental changes and produce regulation. Generally, membrane-associated histidine kinase is activated by an external signal, this induces its autophosphorylation and then phosphorylates a regulatory protein. This phosphorylated protein can bind to a specific DNA sequence, causing altered expression of the target gene. The majority of *S. aureus* strains have 16 different TCSs [66], the WalR/WalS system involved in regulating cell wall metabolism is essential, and some of the other 15 may be inactivated in various strains [67, 68]. Other TCSs such as arlRS, agrAC, and saeRS are implicated in *S. aureus* virulence by regulating many secreted proteins that affect the host [69].

2.1.3.1 Accessory gene regulator (Agr) system

Among the most studied regulatory systems is the accessory gene regulator (Agr), which is responsible for encoding a *quorum sensing* system that serves as the master regulator of virulence [69].

The Agr system detects a signal given by an autoinducer peptide (AIP), composed of 7–9 amino acids. There are four different alleles for the *agr* locus, each strain presenting only one of them. All four known *S. aureus* AIPs contain a cysteine residue that forms a cyclic thiolactone ring with the carboxylate at the C-terminal end of the peptide, which seems to be essential for its function [70]. Once the peptide AIP reaches the critical concentration or depletion of glucose in the extracellular medium, the system is activated in the quorum cells of the population [71]. This mechanism can induce the production of virulence factors and mechanisms of resistance to antibiotics [72]. Interestingly, AIP with a structure different from that produced by the same strain may excerpt an inhibitory effect on the Agr system, instead of the cognate autoinducing function [70]. *S. aureus* requires the Agr system to be able to adapt to changes in the environment during growth to regulate the bacteria's virulence factors [70]. The Agr system has two adjacent transcriptional regions, named RNAII and RNAIII, its expression is regulated by P2 and P3 promoters. Regarding the RNAII region, it is known that it is an operon of four genes (*agrBDCA*), which is responsible for encoding the mechanism of the *quorum sensing* system [73]. The RNAIII transcript is the main effector molecule, and its function is to regulate the expression of most of the target genes that depend on the Agr system (**Figure 1**) [69].

AgrB is a membrane endopeptidase whose function is to cleave the mature AIP from the AIP precursor (AgrD), to form the macrocyclic thiolactone structure and release it into the cytoplasm [70]. AIP interacts with AgrC, a membrane-bound histidine receptor kinase, which subsequently phosphorylates AgrA in the cytoplasm [74]; once phosphorylated, AgrA joins P2 and P3, regulating RNAII and III transcription [73].

AgrA also acts by inducing the expression of phenol-soluble modulins (PSMs). The RNAIII gene encodes a small RNA molecule that is the main effector molecule of the quorum sensing system that is responsible for increasing the expression of cell surface proteins. Four groups of Agr are known in *S. aureus* called agr I-IV each producing a distinctive AIP structure [73]. The Agr system produces increased expression of enzymes and toxins such as serine proteases, DNase, toxic shock toxin-1 (TSST-1),

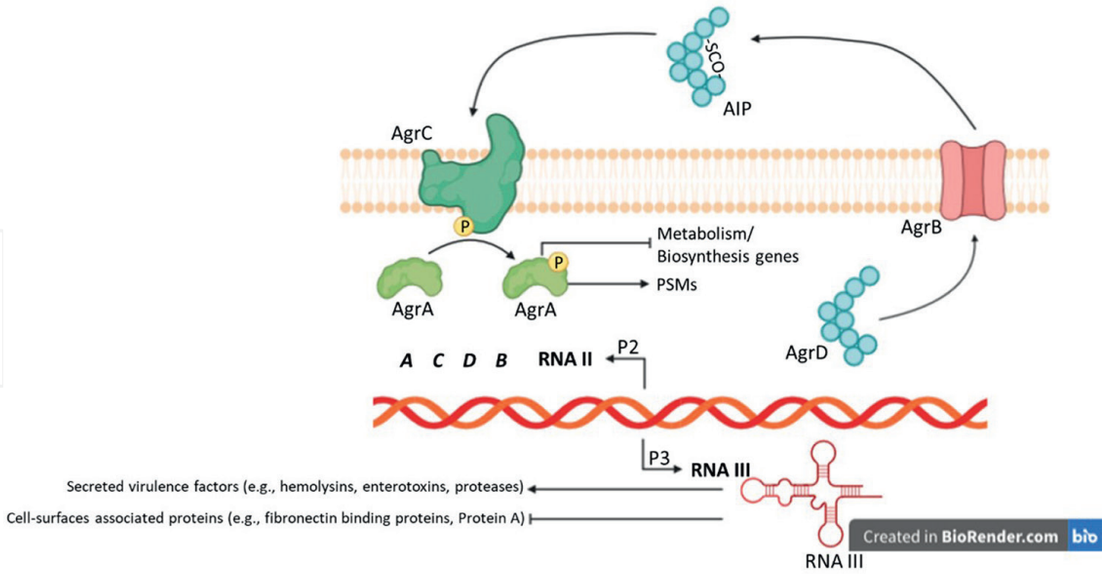

Figure 1.
The Agr system can regulate the virulence of S. aureus. The activation of the system is carried out by an autoinducing peptide (AIP), which accumulates extracellularly when reaching a critical concentration or depletion of glucose. Agr system has two adjacent transcriptional regions (RNAII and RNAIII), and its expression is mediated by the promoters P2 and P3. The RNAII transcript is encoded by the agrBDCA operon, which is the main part of the system, while the RNAIII transcript is the main effector molecule and is responsible for regulating the expression of most Agr-dependent target genes. RNAIII contains the hld (hemolysin δ) genes and leads to the expression and secretion of virulence factors (hemolysins, proteases, enterotoxins, etc.), it is also responsible for inhibiting the expression of cell surface proteins. (modified from Salam and Quave [73].

fibrinolysin, and enterotoxin B and also regulates the expression of colonization and biofilm formation factors [75].

During infectious processes, *S. aureus* produces a large number of enzymes, including lipases, proteases, and elastases, which serve to invade and damage host tissues. This bacterium can produce septic shock, and some strains produce superantigens, causing various intoxications, such as toxic shock syndrome and food poisoning. Some strains produce exfoliative toxins and epidermolysins that can cause bullous impetigo or scalded skin syndrome [76].

During the pathogenesis of *S. aureus*, it is essential to carry out the regulation of the expression of virulence factors. This regulation occurs in a coordinated manner during the bacterial infection. MSCRAMM expression generally occurs during the logarithmic phase of growth, whereas toxins are synthesized during the stationary phase. For the infectious process, the early expression of the MSCRAMM proteins is required, which promotes the initial colonization of the tissues, while later the synthesis of toxins that are secreted by the bacteria and that can cause direct damage to the host, this facilitates the spread and persistence of bacteria in the host [76, 77].

Although Agr system is one of the most important studied virulence factor regulation mechanisms, there are several other global regulators of virulence gene transcription that function in a complex network to regulate virulence. Some of these regulatory systems are *sar*, *sae*, *srr*, *sigB*, *rot*, and *mgr loci*, among others, and form a complex regulatory network controlling virulence [78]. With the advent of whole genome sequencing techniques in addition to the accumulating knowledge of virulence gene regulation and functions, attempts have been proposed to construct system biology tools to predict virulence of *S. aureus* strains from genomic sequence [79]. Although there is the great amount of information on *S. aureus* pathotypes and

genomic sequence, this goal is still far to be reached due to the complexity of the virulence regulatory network in *S. aureus*.

2.2 Biofilms

2.2.1 Polysaccharide intercellular adhesion (PIA)

Polysaccharide of intercellular adhesion (PIA) or poly-N-acetylglucosamine (PNAG) is a fundamental biofilm exopolysaccharide and constitutes most of the extracellular matrix of staphylococcal biofilms [71].

The PIA is constituted by the linear polysaccharide of poly-β(1-6)-N-acetylglucosamine and allows the mediation of bacterial intercellular adhesion; in addition, it forms the structure of the biofilm and bacterial adhesion on surfaces, in addition to protection against host defenses [75]. This is because PIA generates positive charges around the surface of bacteria (which are negatively charged by WTA), triggering electrostatic interactions that allow them to adhere to cells and tissues [71]. PIA is synthesized by the *icaADBC* locus, which is part of the accessory genes on plasmids, and therefore not all *S. aureus* strains have it [75]. However, PIA is so far the only important element involved in biofilm generation in vivo [80], but it does not appear in all isolates from biofilm-associated *S. aureus* infections, so other proteins are involved in its formation (SasG, SpA, Fnbp, among others) [26].

Figure 2 shows that the structure of the *icaADBC* locus, *icaA* (N-acetylglucosaminyl-transferase) encodes a very important transmembrane protein in the synthesis of the poly-N-acetylglucosamine polymer, being more efficient with polymer residues of more than twenty, and is only synthesized together with the *icaD* gene protein. Both proteins (*icaA* and *icaD*) are essential in the synthesis of exopolysaccharides. The third gene, *icaC*, translocates the poly-N-acetylglucosamine polymer to the cell surface, and the product of the *icaB* gene produces its deacetylation; this is very important for the structural maturation of the exopolysaccharide biofilm and allows the adhesion of the polymer with the surface of the bacteria [75, 82]. *icaR* is the fifth gene of the *icaADBC* locus, and it is transcribed in the opposite direction to the aforementioned genes, the start codon between *icaR* and *icaA* is separated by 163 bp (**Figure 2**). The role of *icaR* is to be a negative regulator of the *icaADBC* locus of *S. aureus*, and it encodes a 22 kDa protein of the TetR family. Otherwise, *icaZ* has only been found in strains of *S. epidermidis*, and its expression depends on the conditions of the medium and the incubation temperature [82, 83].

The *icaR* gene is responsible for the expression of the *ica* locus and in turn is regulated by the SarA and σB stress sigma systems (**Figure 3**). SarA belongs to the family of staphylococcal regular accessory proteins (Sar) and functions as an activator or repressor of the transcription of various *S. aureus* genes involved in its pathogenicity, so SarA is a virulence factor of great importance. The *agr* locus is regulated by SarA [78]. The Agr system regulates the change in expression of cell surface proteins in the early phases of bacterial growth (latency and exponential phase), to the synthesis of degrading proteins and toxins (post-exponential and stationary phase). The ability of *S. aureus* to form biofilms can be reduced by expression of the *agr* locus [75, 80, 82].

The formation of biofilms is generated from a complex production of extracellular polymeric molecules, such as amyloid fibrils, extracellular DNA, and phenol-soluble modulins (PSM), and this is due to the synthesis of nucleases, proteases, and PSM peptides [84]. The presence of PSM is highly regulated by Agr, this could indicate that

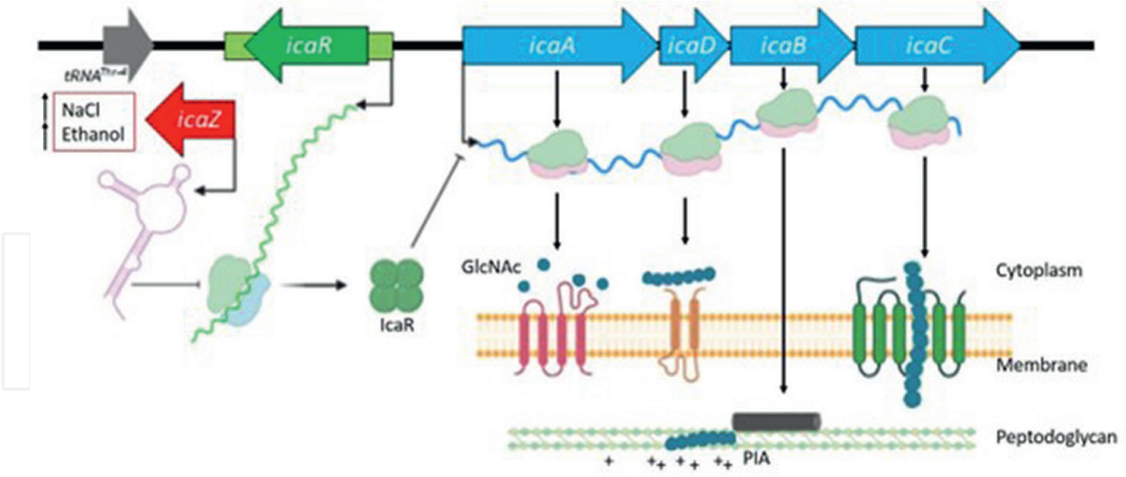

Figure 2.
Structure of the icaADBC locus. Organization of the locus in S. epidermidis RP62A. Colored arrows indicate the coding regions. icaA encodes the enzyme N-acetylglucosaminyl-transferase (membrane protein), icaC is responsible for the translocation of the poly-N-acetylglucosamine polymer to the surface of the bacteria, in the case of icaB, it deacetylates the polymer. icaR is transcribed in the opposite direction with respect to the mentioned genes, and its function is to regulate the operon and therefore the biofilm. icaZ has only been reported in S. epidermidis under some environmental and temperature conditions (modified from Lerch et al. [81]).

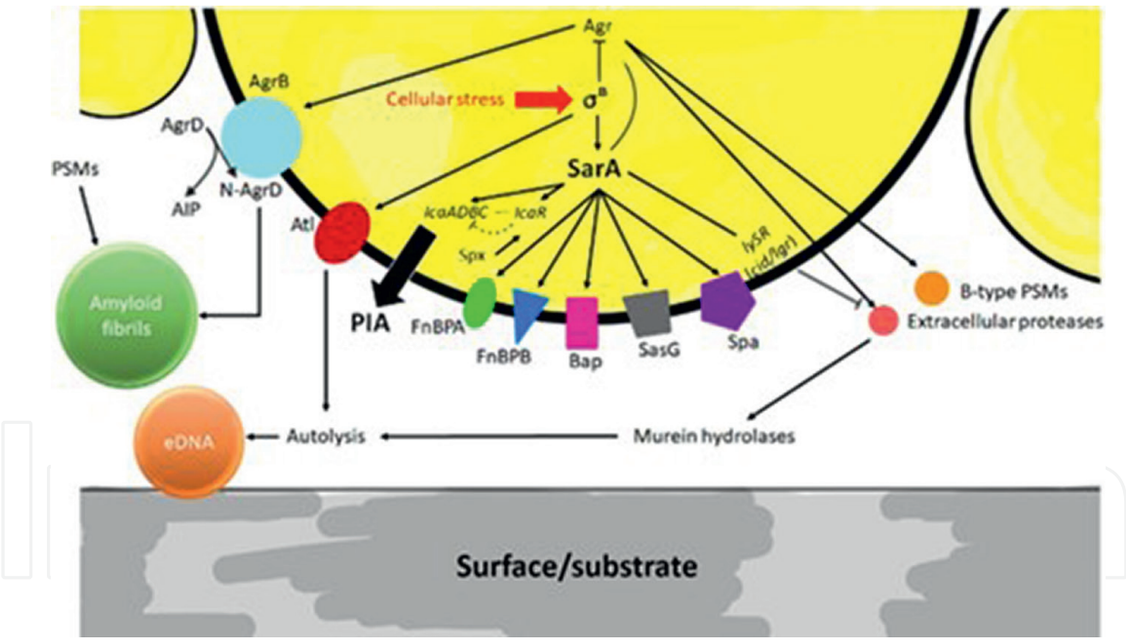

Figure 3.
Diagram of the interactions that favor the formation and degradation of biofilms. The anabolic phase of biofilm formation is shown on the left side of the figure, where several critical extracellular polymeric substances (EPS): PIA, amyloid fibrils, and eDNA, are present. Also shown is the lytSR operon with its lrg/cid target genes. Membrane protein components involved in biofilm formation are shown in the center of the figure, including FnBP adhesins, biofilm-associated protein (Bap), Spa, and SasG. On the right side, the molecules of the catabolic processes of the biofilm are shown, including extracellular proteins and PSM. The Agr system, the σB factor, and SarA are the main regulators, modifying bacterial behavior in response to various environmental stimuli (modified from Arciola et al. [75]).

the biofilm formation processes that depend on the Agr system are due to the expression of PSM [77]. The mechanisms of sessile and planktonic phenotypes require sensitive coordinated and efficient control during the invasive phase of bacteria [75].

There is evidence that *S. epidermidis icaA*(+) overexpresses the biofilm formation phenotype under *in vitro* conditions. However, *S. aureus* makes it different since the positive strains of the *ica* locus are not always expressed *in vitro* and do not need anaerobiosis or medium supplementation with other nutrients to express it. In contrast, *S. aureus* strains have higher biofilm production under *in vivo* conditions. Some stress-induced conditions *in vitro*, such as starvation, iron limitation, non-inhibitory concentrations of ethanol, heat stress, NaCl, and various antibiotics, have been reported to increase biofilm production [75].

2.2.2 Amyloid proteins

The stability of the biofilm is due to the presence of amyloid proteins [85]. The amyloid structure is composed of three packed β-fibers that are resistant to denaturing conditions and are not degraded by proteases [86].

Amyloid proteins can bind to eDNA and function as inters fibrils in the biofilm, functioning as a solid bond, which allows the bacteria to wait for the environmental conditions to improve to favor their dissociation and allow the dispersion of the biofilm [85]. PSMs are necessary to increase the volume, roughness, thickness, and channel formation in the biofilm [87]. These surfactant peptides (PSM) play a fundamental function in the three-dimensional structure of the biofilm, in addition to favoring its detachment [87], and are determinants of biofilm maturation in vivo [71, 82]. **Figure 4** shows a diagram of the main components expressed by *S. aureus* in the formation of biofilms.

2.2.3 Fibrin biofilm

S. aureus can survive in the blood due to the production of the enzyme coagulase (CoA), which is regulated by the SaeRS two-component system. Detection of enzyme activity (Coa or staphylocoagulase) is very common in the clinical laboratory to identify strains of *S. aureus* from other staphylococci [89]. Highly relevant in the development of biofilms is *coa* gene, under natural conditions and is present in 100% of *S. aureus* strains. After maturation, fibrin-coated biofilms have increased defense and resistance against antibiotics [88].

Coa function is activated by binding to prothrombin from the blood, allowing the formation of the active staphylothrombin complex that converts soluble fibrinogen to insoluble fibrin, which is used by *S. aureus* to reinforce the biofilm. Whether S. *aureus* can form biofilms mediated by the *coa* gene depends on contact of bacterial cells with the host cell surface, and an important protein for this binding is ClfA [90].

There are indications that the colonization of medical devices by *S. aureus* is due to the production of fibrin biofilms mediated by the *coa* gene; however, over longer periods of time, other adhesins that also form biofilms play a more important role in their maturation [88]. Zapotoczna et al. [91] observe that after 24 h of fibrin biofilm formation, they became weaker in the presence of antibiotics compared with biofilms of another protein composition (e.g., FnBP) in the same period of development; however, with the passage of time, the fibrin biofilms became more resistant.

2.3 Biofilm formation

Upon initial contact, a planktonic cell can reversibly associate with a surface, and if the cell does not detach, then it will irreversibly bind to it [25, 27].

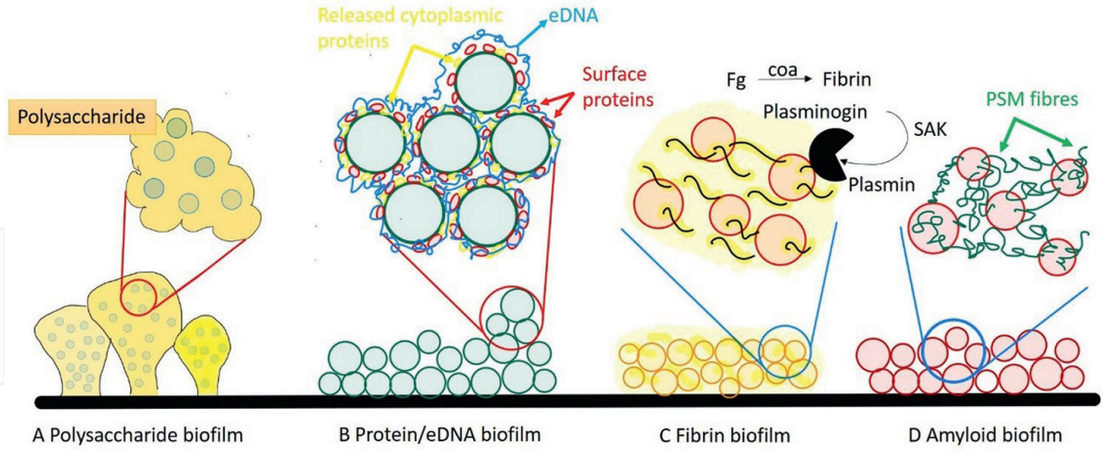

Figure 4.
Main types of biofilms. A: PIA/PNAG polysaccharide biofilm by strains with the icaADBC operon (common in MRSA), B: surface proteins (BAP, FnBP, and SasG), interact between cells during biofilm formation. eDNA and cytoplasmic proteins diffused after lysis participate as elements of the biofilm matrix, C: coagulase-mediated activation of fibrinogen (Fg) into fibrin, which is activated to strengthen the biofilm, which can be dissociated by the plasmin produced post-staphylokinase (SAK) (plasminogen-mediated), D: PSMs have surface-active properties that promote biofilm breakdown and, in turn, can accumulate as amyloid aggregates (modified from Zapotoczna et al. [88]).

When *S. aureus* adheres to host cells and tissues or to the surface of prosthetic materials, it can reproduce, colonize, and persist in these sites, in a variety of ways [76]. The first of the mechanisms used by bacteria is the formation of biofilms, *S. aureus* can form them on the surface of tissues, thereby colonizing and persisting in tissues, in addition to evading some of the host's immune mechanisms, also to blocking the role of antibiotics [92].

The biofilm is defined as a set of aggregated bacteria and is made up of cells adhered to each other (sessile cells). The cells are located within a matrix with extracellular polymeric substances (proteins, exopolysaccharides, adhesins, eDNA, etc.), which present an altered phenotype of growth, genetic expression, and protein production [92, 93], with respect to normal cells, normal planktonic (free life) [90]. Biofilms can form on biotic and abiotic surfaces, and those bacteria that are coated within the biofilm are 10–1000 times less sensitive to antibiotics than planktonic bacterial cells [71, 94, 95].

The formation of biofilms has been described through a cycle from the study of different bacterial species and is composed of (1) reversible adhesion, (2) irreversible union (formation of microcolonies), (3) maturation, and (4) dispersion [71, 96]. **Figure 5** shows a schematic of the biofilm formation cycle. However, in 2014, Moormeier et al. [23] proposed five stages in the formation of biofilms for *S. aureus*: (1) fixation, (2) multiplication, (3) exodus, (4) maturation, and (5) dispersion. The first stage of biofilm formation was mentioned in the section on adhesins.

2.3.1 Components of the biofilm matrix

2.3.1.1 Extracellular DNA (eDNA)

When the biofilm is formed, the extracellular matrix (ECM) is produced, made up of polysaccharides, proteins, and/or extracellular DNA, which confers the three-dimensional structure that stabilizes and matures the biofilm [97]. The hypothetical

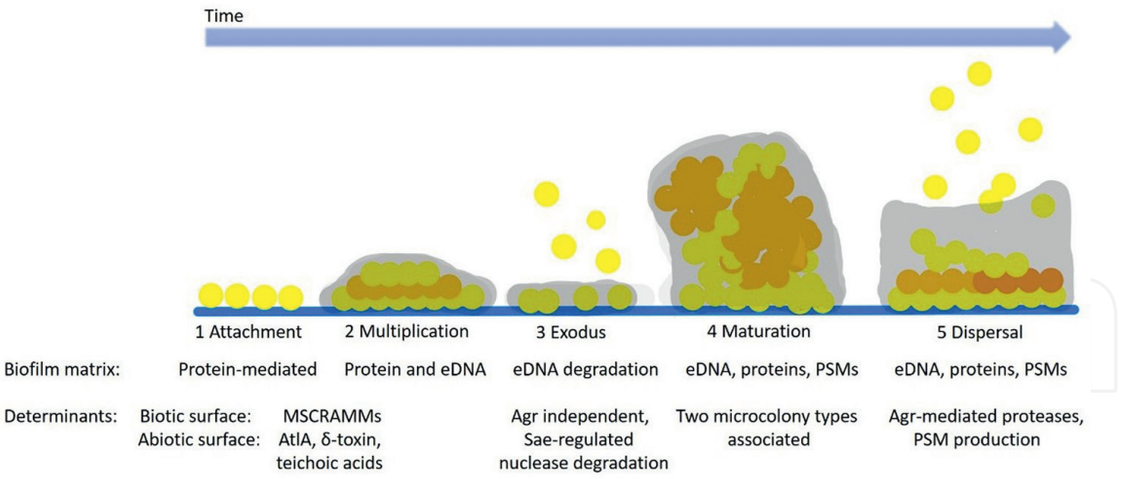

Figure 5.
The five parts of S. aureus biofilm formation. The process of biofilm formation can be explained in five main stages: (1) initial attachment or binding, (2) multiplication, (3) exodus or primary migration, (4) maturation, and (5) dispersal. 1. S. aureus Binds to a surface (abiotic or biotic) via MSCRAMM or nonpolar interactions. 2. Once cells adhere, a biofilm is formed, which is a confluent layer of cells, eDNA, and protein matrix. 3. When confluence is reached, cell exodus occurs, releasing a small number of cells from the biofilm by degradation of nuclease enzymes to eDNA (regulated by Sae), which allows the development of microcolonies in the biofilm space. 4. These microcolonies are formed from cellular sources that remain attached in the exodus stage. This stage consists of accelerated cell division that forms protein aggregates, including eDNA and PSM. 5. Quorum sensing by the Agr system initiates regulation of the biofilm matrix and cell dispersal through activation of proteases and/or PSM (modified from Moormier et al. [26]).

mechanism of eDNA adhesion postulates that eDNA is adsorbed on the membrane of individual bacteria in long loop structures measuring up to 300 nm [98]. It has also been described that DNA loops interact with rough surfaces at the nanoscale, which increases the bacterial adhesion surface to this type of surface (**Figure 5**) [99].

eDNA favors the hydrophobicity of the bacterial surface, single-stranded DNA has amphiphilic properties, the hydrophilic part for deoxyribose, and the hydrophobic part for nitrogenous bases. Otherwise, double-stranded DNA hybridizes with each other by hydrogen bonds (Watson-Crick bonds) and hydrophobic interactions. Various studies have reported that eDNA increases the hydrophobicity of bacteria. Das et al. [100] reported that the presence of eDNA increases the adhesion of bacterial cells on hydrophobic surfaces (**Figure 6**) [99].

eDNA also favors resistance to antimicrobial drugs by inducing the expression of resistance genes. eDNA can form complexes with divalent metal cations (Mg^{2+}, Ca^{2+}, Mn^{2+}, and Zn^{2+}), which neutralizes the negative charge on the outer part of the bacterial membrane and increases its resistance to host antimicrobial peptides and cationic antibiotics such as aminoglycosides. However, eDNA can induce immune system activation, although the biofilm protects bacteria from some processes such as phagocytosis [99].

How components of the biofilm matrix are externalized is still not fully understood. Mutant strains defective in autolysis have been reported to have poor biofilm-forming capacity compared with strains that do not produce PIA biofilms [94]. Phagocytosis-mediated cell death is another mechanism of eDNA release and lysis-independent methods such as specialized secretion or vesicle formation [101, 102].

2.3.2 Biofilm multiplication stage

After bacterial attachment to a surface and under sufficient nutritional conditions, adherent *S. aureus* cells can multiply and accumulate. However, newly divided cells

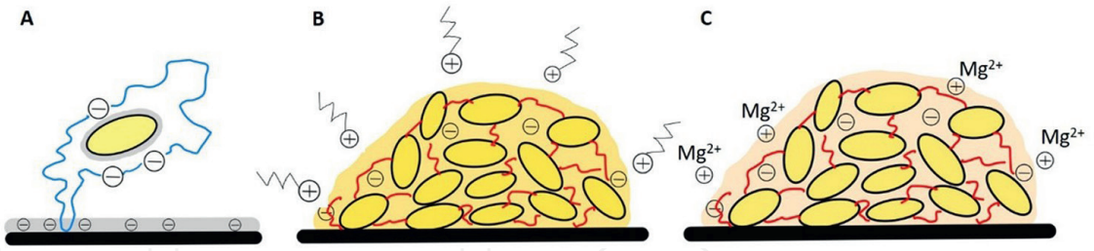

Figure 6.
Functions of extracellular DNA. (A) eDNA aids adhesion on surfaces by penetrating the electrically repulsive double layer. Acid-base interactions lead to bacterial adhesion. (B) eDNA generates chelating complexes with cationic antimicrobial peptides of the host's innate immune system. (C) eDNA generates complexes with divalent cations, triggering a response in the bacteria that increases pathogenicity and antimicrobial resistance (modified from Okshevsky et al. [99]).

are very susceptible to detachment, primarily from fluid flow. To maintain immature biofilm stability, *S. aureus* can produce a wide range of molecules that stabilize intracellular interactions. This process is called the multiplication stage [26].

Staphylococci strains can produce a wide range of extracellular proteins (CWA, FnBP, SdrC, and ClfB), which promote biofilm formation by favoring intercellular binding, once they are attached to the surface through a dual role in the stage's union and accumulation. But there is evidence that they are also involved during the multiplication stage of biofilm development [23]. PIA functions as a component of ECM in the early stages of *S. aureus* biofilm formation [26].

Foulston et al. [103] showed that the enzymes enolase and glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (which is not a biofilm-forming protein) can be activated as a component of the ECM in response to a decrease in pH, around the biofilm (**Figure** 7). This would imply that under acidic conditions, enolase and GAPDH can bind to eDNA [104]. Otherwise, it has been reported that other extracellular proteins such as PSM, β-hemolysin (Hlb), and IsaB (immunodominant surface antigen B) bind to eDNA to stabilize the ECM [26].

2.3.3 Biofilm exodus stage

In time-lapse microscopic observations of biofilms, a phase was found that was termed "Exodus," due to a clear coordinated cell release around 6 h after the start of the multiplication stage, which is an early dispersal event that occurs at the same time as the formation of the microcolony and produces the restructuring of the biofilm (**Figure** 7). The exodus phase is determined by the degradation of eDNA by nucleases and does not depend on the Agr system, which is produced after the development of the microcolony. The degradation of eDNA in the ECM by endogenous nucleases decreases the total biomass of the biofilm [23, 24, 83, 105]. The exodus phase is highly regulated, since only a part of the bacterial cells in the biofilm presents the expression of the *nuc* gene (which encodes a thermonuclease, used as an identification criterion for *S. aureus*), which favors the shedding of most of the cell population of the biofilm formed [36]. Also, Moormeier et al. [23] noted changes important in ECM as the biofilm structure advances, initially only consisting of membrane protein components (binding and multiplication phase), to relying on eDNA and proteins released to the outside (exodus stage). Therefore, a biofilm is only composed of PIA, protein, and eDNA must be replaced by a more complex model of biofilm development and ECM composition over time as the biofilm forms [26]. Therefore, the reduction of the

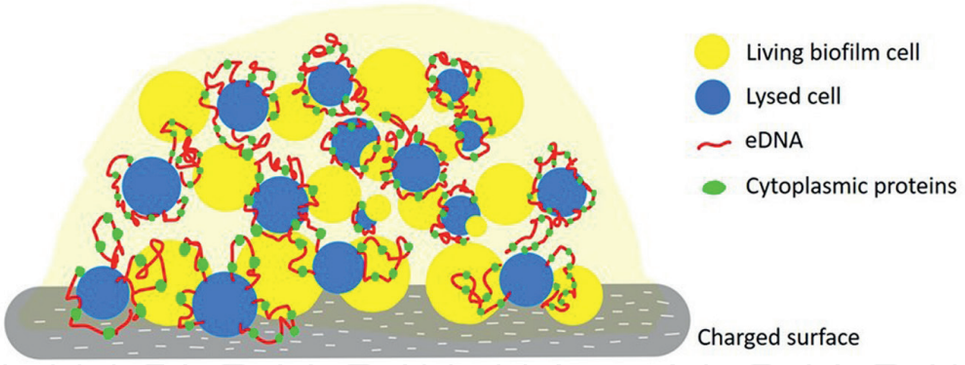

Figure 7.
Scheme of intercellular interactions in the biofilm multiplication stage. Early in biofilm development, free-living (planktonic) cells adhere to surfaces through electrostatic interactions mediated by teichoic acids, PSM, autolysin A, etc. As the multiplication stage progresses, some cells die or lyse (blue circles) releasing cytoplasmic proteins (green circles) and eDNA (red lines) into the extracellular medium, enveloping living bacteria (yellow circles) in a mixture of DNA and proteins cytoplasmic (modified from Moormeier et al. [23]).

bacterial population at the beginning of biofilm formation (by death or exodus) is an important requirement for its maturation. It has been observed that when there is no exodus phase, as is the case with *S. aureus* strains that have mutations in the *nuc* gene, the formation of microcolonies does not occur [23].

2.3.4 Biofilm maturation stage

The formation of microcolony structures is essential in the biofilm maturation process, since they provide a larger contact surface for obtaining nutrients and eliminating waste, in addition to favoring the dispersion of bacterial cells within the biofilm. Research carried out on other species of bacteria has reported the development of microcolony-like structures during the biofilm formation stages of *S. aureus* [23, 87, 106], the mechanism of its formation is not known.

A previously described model [87] mentions that the formation of microcolonies in the development of the biofilm is a subtractive process, in which channels are formed in it due to the dispersion caused by the PSM. However, in microscopy observations at different times, it has been described that microcolonies are formed from different cell foci of the basal layer once the exodus phase begins (**Figure 5**).

After the maturation stage, the release of bacteria from the interior of the biofilm occurs through dispersion, which reactivates the free-living state of the bacterial cell (planktonic state) [93, 107]. DNase I has been reported to be an inhibitor of PIA-independent biofilm development in MRSA strains of clinical isolates; however, it does not inhibit PIA-dependent MSSA strains [104]. In the same investigation, DNase I effectively inhibited biofilm development of MRSA strains, but failed to destroy already formed biofilms [108, 109].

2.3.5 Biofilm dispersion stage

Dispersion processes are fundamental in the composition of the biofilm, since through these the cells are released from the biofilm individually or in large groups of bacteria, if there are favorable environmental conditions. This is very important in biofilm-associated infections, as they facilitate systemic spread, and it has been shown that cells shed from biofilms from medical devices and catheters can cause endocarditis or sepsis [71, 80].

Mechanisms influencing the control of biofilm scattering have been studied and reported to be mediated by Agr quorum sensing control [84]. In the dispersion stage, the bacteria of the outermost layers of the biofilm are responsible for the expression of the *agr* genes, which leads to the detachment of the cells, and at the same time the renewal of the biofilm; however, *agr* genes are also expressed by bacteria in the inner part of the biofilm, where it is used for channel formation [70, 87, 110].

Some toxins influence the development of biofilms. For example, α-hemolysin (Hla) and leukocidin AB (LukAB) are involved in biofilm persistence [111]; Hla and LukAB are also synergistically involved in promoting macrophage dysfunction and death. Dastgheyb et al. [112] showed that PSMs block biofilm formation by disrupting interactions between ECM molecules with the bacterial surface. Perasamy et al. [87] reported similar results regarding the influence of the PSMs of *S. aureus* with the development of the biofilm, and that PSM degraded it, which produced its early dispersion due to the surfactant properties of the toxin [113].

The importance of the Agr system is essential for cell communication within the biofilm formed, to form and establish the three-dimensional structure by controlling cell dispersion. However, Agr system does not regulate other adhesive molecules of biofilm formation, as is the case with PIA [75].

3. Conclusions

S. aureus is a highly relevant pathogenic bacterium for humans and other mammals, since it can bind very intensely to different components of the extracellular matrix and thus infect cells. It also has mechanisms that allow it to colonize, persist, and survive in unfavorable environmental conditions for growth, such is the case of the formation of biofilms, which allows it to evade various human immune mechanisms very efficiently. The complex and dynamic composition of *S. aureus* biofilms, as well as the existence of a complex genetic regulatory network driving biofilm formation and maturation, offer a wide variety of potential pharmacological targets for the control of *S. aureus* infections.

Conflict of interest

The authors declare no conflict of interest.

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