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Research Paper

Staphylococcus aureus BIOFILM DEVELOPMENT: THE URGENT NEED FOR TREATMENT ALTERNATIVES

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Abstract

Biofilm formation is a serious chronic condition that varies depending on the environment, nutrients, characteristic of the pathogen and the host. It may allow bacteria to resistthe host immune system andthe antimicrobial agents used in the therapeutic treatment. There are different factors responsible for bacterial biofilm formation, including genes such as *agr* and *ica*ADBC, as well as proteins including the MSCRAMM complex (Microbial Surface Components that recognize molecules of adhesion of the matrix), involved in some of biofilm formation specific steps (attachment, cell-cell adhesion, proliferation and detachment). Biofilm formation is one of the causative factors of hospital infection by *S. aureus*, an important pathogen from the hospital. *S.aureus*is usually found in the normal flora of a healthy individual, however, this colonization is a risk factor for developing diseases related to biofilm formation, as infections of medical devices, endocarditis and others. Since the knowledge regarding mechanisms of biofilm formation facilitates the selection of a proper treatment, the rational use of antimicrobial agents, and the development of new therapies for infections related to biofilm-forming microorganisms is a special subject to be addressed. This review intends to explore about the main characteristics of *S. aureus*, including history, virulence factors, disease processes involved and treatment options for the biofilm related infections in the last few years to contribute for generating new treatment alternatives.

Key words: biofilm formation, *Staphylococcus aureus*, virulence factors, treatment.

INTRODUCTION

The genus *Staphylococcus* is formed by Gram-positive cocci with irregular shapes (e.g. bunch of grapes) releasing hydrogen peroxide in the presence of oxygen, characterized as catalase positive (Santoset al. 2007; Kateeteet al. 2010). This genus has 33 species, in which 17 can be isolatedfrom human biological specimens(Santoset al. 2007). *S.aureus* is the most pathogenic species of this genus. Its identification is performed by a combination of phenotypic tests

including catalase, coagulase and mannitol fermentation tests (Murrayet al. 2003; Kateeteet al. 2010).

The incidence of *S. aureus* found asymptomatically in humans is about 27% (Younget al. 2012)while the most prevalent sites are nostrils, armpits and perineum (Giarolaet al. 2012).

Staphylococcus aureus has specific virulence factors and adaptive capacity that allows it to survive at different environmental conditions. This bacterium is able to resist the human innate immunity and to acquire resistance mechanisms against several antimicrobials (Falordet al. 2011).

Some *S. aureus* strains are capable of forming biofilm, which is characterized as highly organized bacterial community embedded in a self-produced polymeric matrix(Wuet al. 2011). The ability to form biofilm confers resistance mechanisms to the bacteria and it is related to many chronic diseases. More than 80% of bacterial infections involve the formation of biofilm, when combined with biofilm bacteria increased in the 1000 times endurance and survival when compared to bacterial strains are not associated with biofilms(Karaolisetal. 2005; Falordet al. 2011; Yeagleyet al. 2012). Thus, it is of great importance to know the main features of biofilm formation, the pathological processes and virulence factors involved, as well as the treatment for *S. aureus* biofilm-related infections (Donelliet al. 2007; Falordet al. 2011).

The biofilm formation involves different steps including adhesion, colonization and development. At a certain density/growth level, the cells start to detach (planktonic state) and may cause a disseminated infection (Donelliet al. 2007) (Figure 1).

The treatment of biofilm-related infections is of great importance but mostly unsuccessful. The difficulty in penetrating into this complex bacterial community matrix and its evasion of the host innate immune system is associated with about 25% of deaths and is an aggravating factor in the length of stay and hospital costs worldwide (Donelliet al. 2007; Archeret al. 2011; Kiedrowskiet al. 2011).

In this review, it is reported the latest data about the main characteristics of *S. aureus*, including history, virulence factors, disease processes involved and treatment options for staphylococcal infections related to biofilm formation.

Staphylococcus aureus: **general aspects**

Staphylococcus aureus is one of the most prevalent pathogens in infections related to health care environment.Therefore, several studies have been conducted to increase the knowledge about the mechanisms of transmission and control dissemination. *S. aureus* transmission can occur via fomites, nasal secretions, contact with colonized health professionals and/or open lesions among infected patients (Archeret al. 2011; Koch et al. 2014).

In the early 1940, the use of penicillin in the antimicrobial therapy was promising but in 1942 the resistance was already detected at the hospitals (Hartman and Tomasz 1981;Deresinski 2005; Koch et al. 2014).

The gene that confers *S. aureus* resistance to penicillin and codes for the synthesis of beta-lactamase is *blaZ*. Beta-lactamase is an extracellular enzyme responsible for hydrolyzing penicillin beta-lactam ring, which is an essential part of this antibiotic biological activity(Rammelkamp and Maxon 1942; Koch et al. 2014).

Differently,*S. aureus* resistance to methicillin is defined by *mec*A gene that is inserted into the Staphylococcal Cassette Chromosome mec (SCC*mec*), present in all Methicillin Resistant *Staphylococcus aureus* (MRSA) strains (Mehndiratta and Bhalla 2012). There are eleven types of SCC*mec* described so far, and the SCC*mec* Type III and IV are the most prevalent in human infections in Brazil (Cabocloet al. 2012), currently five CA-MRSA clonal lineages were identified associated with epidemics: Pandemic (USA300, CC8), Midwest clone (USA400, CC1), European clone (CC80), clone Southwest-Pacific Oceania (CC30), the clone Pacific (CC59) (DIEP and OTTO, 2008; KARAUZUM et al, 2013).The classification of different types of SCCmec is made from the internal regions of the cassette, and molecular classification, these movable elements may carry other genes for resistance to various drugs, including sulfametoxazol-trimetoprim, doxycycline and rifampin, as well as others (Deresinski 2005; Rudkinet al. 2012).

The mecA gene encodes a protein with low affinity to beta-lactam called PBP2a, structurally modifying the protein responsible for membrane penicillin (PBP2) making Methicillin resistant *S. aureus*. The PBPs are membrane bound enzymes that catalyze the transpeptidation and trans glycosylation of peptidoglycans, the major bacterial wall component. Current literature describes MRSA resistance as a result of the lower affinity of PBP2a for beta-lactam, allowing the survival of these bacterial strains even in the presence of these antimicrobials (Rudkinet al. 2012). Thus, MRSA infections cause increased mortality (Hagihara et al. 2012), longer hospital stay and increased costs compared with infections caused by Methicillin Sensitive *S. aureus* (MSSA).

Risk factors associated with the bacteremia caused by MRSA include: age, where patients aged 61 or older are more susceptible (Souza and Figueired 2008), prolonged hospitalization, previous treatment with antibiotics, catheterization and nasogastric tube use(Butterlyet al. 2010; Dhand and Sakoulas 2012; Hagiharaet al. 2012; Rudkin et al. 2012).

A prospective study showed that colonization is a risk factor for post operative complications(Butterly et al. 2010). MRSA strains have spread around the world since the 1960 and resistant strains are no longer exclusive to the hospital environment (Hospital-Acquired Methicillin-Resistant *S. aureus* - HA-MRSA), being also isolated from community infections (Community-Acquired Methicillin-Resistant *S. aureus* - CA-MRSA) (Hagihara et al. 2012; Rudkinet al. 2012).

Genetic analysis of HA-MRSA and CA-MRSA revealed that HA-MRSA SCCmec take a larger (e. g. types I, II and III) allowing a greater amount of resistance genes as CA-MRSA usually carries a lower structure SCCmec type (e. g. types IV and V) with a smaller amount of antimicrobial resistance genes (Rudkin et al. 2012)(Rudkin et al. 2012). CA-MRSA strains are known to cause severe invasive infections (e.g. necrotizing fasciitis) and to be involved in chronic diseases such as endocarditis, osteomyelitis and foreign-body infections (Kiedrowskiet al. 2011).

Staphylococcus aureus and virulence factors

To establish an infection, the bacteria needs to express a variety of molecules that determine the pathogenicity, known as virulence factors (Konget al. 2006). The high difficulty in successfully treating MRSA infections classifies it as highly pathogenic or virulent (Rudkinet al. 2012). Studies that compared the virulence strains of staphylococci showed that HA-MRSA has more antimicrobial resistance genes whereas CA-MRSA and MSSA are more virulent than HA-MRSA (Rudkinet al. 2012).

There are toxins and factors that increase the virulence of *S. aureus* strains and are of great importance including: a) toxin A, b) esfoliatin, causing the scalded skin syndrome, c) enterotoxins that cause food poisoning and d) toxin Panton-Valentine Leucocidin (PVL), responsible for altering the permeability of membranes of leukocytes and macrophages(Otto 2010).

Staphylococcus aureus and biofilm formation

Biofilm formation is also a factor involved in the development of numerous staphylococcal infections (Karaolis et al. 2005; Koch et al. 2014). *S. aureus* and *S. epidermidis* are primarily related to biofilm associated staphylococcal infections (Otto 2010). *S. aureus* are capable of resisting to the mechanical stress, antimicrobial therapies and the innate immune system of the host, becoming a serious chronic condition when protected by the biofilm (Kiedrowski and Horswill 2011; Kiedrowski et al. 2011).

Staphylococcus aureus is the main causative agent of biofilm-forming infections on medical devices such as orthopedic implants, ventilators, intravenous catheters, heart valves, pacemakers and vacuums. Periodontitis and peri-implant diseases that cause wounds (e.g. foot and pressure ulcers, diabetes), chronic endocarditis, eye infections (conjunctivitis, keratitis), osteomyelitis sepsis syndromes and metastatic infections are mostly derived from biofilm-forming strains (Otto 2010; Archer et al. 2011; Yeagley et al. 2012; Koch et al. 2014).

The biofilm is initially formed by a monolayer of bacteria that attaches and accumulates, generating a bacteria-containing polymeric matrix and extracellular slime on a solid surface

(Atshan et al. 2012; Linet al. 2012; Revdiwalaet al. 2012). When a microorganism infects a medical device, numerous variables determine biofilm formation. First, the microorganism must adhere to the exposed device surface long enough to make an irreversible binding. Then, the microorganism binding rate is determined by both number and types of the cells present in the fluid that pass through the device. The characteristics of surface physical chemistry is also an important factor for the biofilm formation (Revdiwalaet al. 2012).

The biofilm formation includes 1) determination, 2) cell-cell adhesion and 3) post regulated formation of biofilm mediated by genes such as a*gr, ica*ADBC, and adhesin proteins(O'Gara 2007).

The *ica*ADBC genes on the genome of the cell and the hoisting gene (regulator genes) form a single operon(Cramtonet al. 1999; Gotz 2002; Archeret al. 2011). The presence and expression of operon *ica* encoding enzymes is necessary for the production of Intercellular Polysaccharide Adhesin (PIA) also calledPoly-N-Acetyl Glucosamine (PNAG). Those are essential for cell-cell adhesion and biofilm formation in some strains (O'Gara 2007; Atshanet al. 2012). Additional surface proteins are also necessary for biofilm formation and fixing (Atshanet al. 2012; Linet al. 2012; Pozziet al. 2012).

Expression of gene *icaR* encodes a transcriptional repressor that negatively regulates the production of PIA (Cramtonet al. 1999; Gotz 2002). Importantly, the anoxia increases the transcription of operon *ica* consequently increasing PIA production by *S. aureus* and *S. epidermidis* strains (Archeret al. 2011). Proteins IcaA, C and D are membrane proteins whereas icaB is located in the extracellular matrix. The isolated icaA shows less activity when co-expressed with *icaD.* The *icaAD*acts as a new combination of enzymes that can facilitate the connection between icaC and icaA. Co-expression of icaAC reacts with specific PIA present in the medium whereas icaAC may be related to the translocation of plasma membrane polysaccharides as this protein activation is essential for biofilm formation (Gotz 2002) (Figure 2A).

Intercellular Polysaccharide Adhesin is an essential component for the formation of biofilm in some strains (Kiedrowskiet al. 2011). Some authors reported that MSSA strains depend on PIAorPNAG while others suggested that MRSA strains are more favorable to biofilm formation, independent of PIA (O'Neillet al. 2007). These authors reported the expression of the phenotype FnBPA / B e Alt, suggesting a relationship of beta-lactams and biofilm (Archeret al. 2011; Pozziet al. 2012). MRSA strains depend on fibronectin-binding proteins A and B (FnBPA and FnBPB) and autolisin (Atl). The Atl presents an autolytic activity and releases extracellular DNA. It can also adhere to the polymer surface, which is related to the phase of initial adherence of biofilm development (Cramtonet al. 1999; Gotz 2002; Pozziet al. 2012) and also binds to fibronectin demonstrating a relationship with later stages of biofilm formation and adhesion (Cramtonet al. 1999).

Staphylococcus aureus strains are particularly capable of adhering to, not just medical devices, but also a wide variety of extracellular matrix components, host components in infectious diseases, heart tissue, cartilage and chronic wounds for initial colonization. When a medical device is implanted, it is quickly covered by the host matrix proteins such as fibronectin, fibrinogen and collagen. *S. aureus* presents adhesins that are frequently covalently linked to peptidoglicans, present in the cell wall. These proteins belong to the family of Microbial Surface Components that recognize molecules of adhesion of the matrix (MSCRAMM) and bind to host proteins, colonizing it (Gotz 2002; Archeret al. 2011; Kiedrowskiet al. 2011). Thefibrinogen proteins A and B (FnBPA and FnBPB), collagen binding protein (Cna), binding and agglomeration fibrinogen proteins A and B (ClfA and ClfB) belong to this MSCRAMM family (Gotz 2002). *S. aureus* produces multiple extracellular proteases with self-cleavage activity that can separate the cells from surfaces, reinforcing the hypothesis of a protein based matrix existence (Kiedrowskiet al. 2011). Other proteins are also important and participate in the synthesis of staphylococcal biofilms, including surface proteins (SSP1) and biofilm-associated proteins (BAP) (Gotz 2002).

Staphylococcus aureus and quorum-sensing (QS)

Gram-positive and Gram-negative bacteria evolve and become capable of synthesize signaling molecules called auto inducers peptides (AIP). During the bacteria growth, signaling molecules accumulate in the extracellular medium and reach a specific cell density or "quorum" able to activate a regulatory cascade that controls a particular type of cellular process. This phenomenon named *quorum sensing* is a regulatory system capable of inducing these AIP for communication among cells(Konget al. 2006; O'Gara 2007; Thoendelet al. 2011). *S. aureus* has two *quorum sensing* systems, *Lux*S and *Agr*, involved in biofilm formation (Konget al. 2006). The *Agr* from *S. aureus* and *S. epidermidis* controls the expression of several toxins and virulence factors and interacts with the innate immune system (Konget al. 2006).

The function of locus *agr* during the infection by Gram positive microorganism is controversial. *Agr* activation is not favorable for biofilm formation and virulence. Instead, it reduces the virulence and the ability to form biofilm (Shirtliffet al. 2002; Konget al. 2006). The locus *agr* negatively regulates genes associated with cell wall adhesion factors, leading to a lower adhesion, indirectly reducing biofilm formation and favoring the deployment of these cells, so that they return to the planktonic state (Santoset al. 2007). The quorum-sensing *agr* system is related to the ability of *S. aureus* to colonize other sites in the host. The *agr* gene increases the detachment of the biofilm, while the *lux*S gene reduces the ability of cell-cell interaction via down-regulation and expression of polysaccharides essential for biofilm development (Konget al. 2006; Kuehlet al. 2009; Tsujiet al. 2011).

The gene *agr* is located in *S. aureus* chromosome and its locus has two different transcripts, RNAII and RNAIII. The transcription of RNAII is an operon of four genes (*agr*B, *agr*D, *agr*C and *agr*A) that encodes factors for the synthesis of AIP (Konget al. 2006; Thoendelet al. 2011) (Figure 3).

The gene is the *agrD* promotes the production of PAI *agrB* while the developer is essential in the biosynthesis of AIP (Thoendel et al. 2011). Inducing self molecules bind to membrane through agrC protein, activating it. The agrC kinase acts as a sensor for adjustment of the two component system. The active agrC regulates activation agrAwhich in turn induces transcription of RNAII and RNAIII. The agrA is a DNA binding protein RNAIII while the effector molecule is in regulating the AGR system (Kong et al., 2006).

Since a) biofilm-forming bacteria strains are protected against the host immune system and antimicrobial treatment and b) the detachment of biofilm cells can generate colonization at other sites and an acute infection, the understanding of biofilm formation and proper treatment to avoid it is essential. The *quorum sensing* control systems in staphylococci are important and represent promising targets for the development of new antimicrobial that should be more explored by further research (Gotz 2002; Davies 2003; Konget al. 2006).

Staphylococcus aureus: infection treatment

The treatment for bacterial infections should be as rational as possible. Thus, it is necessary to perform a pharmaceutical care whereas the therapy selection should be based on laboratorial tests involving pathogen identification and phenotypic resistance assays (Lunaet al. 2010). Errors in antimicrobials prescription are associated with increased mortality and morbidity. The unnecessary use of the antibiotics and the long course periods are factors that increase the cost of hospitalization and the prevalence of resistant organisms. Thus the rational use of antimicrobials allows improving prognosis of patients, generating benefits and reducing costs (Lunaet al. 2010).For the treatment of infections with HA-MRSA and CA-MRSA has been glycopeptidevancomycin common approach for the treatment of staphylococcal infections. However, the use of incorrect antibiotic medium led to the appearance of various strains with reduced susceptibility to vancomycin (Koch et al. 2014). These strains are referred to as vancomycin intermediate *S. aureus* (VISA), although its level is moderate resistance (MIC = 4-8 µg / ml) compared to the vancomycin-resistant strains (VRSA) (MIC ≥ 16 mg / ml), *S. aureus* resistant strains pose a great threat for the treatment of staphylococcal infections (Howden et al, 2010;. Koch et al. 2014)

Treatment biofilm-associated infections

The most effective treatment for biofilm related diseases is a surgical debridement, drainage and cleaning of infected site as well as the medical implant removal (Archeret al. 2011). However, in many cases, the removal is not feasible as in joint prosthetics or among patients that do not withstand a surgical process (Donelliet al. 2007).

Currently, the antimicrobial therapy alone is not successful, but associated with surgery, when possible. Medical doctors should also take into account drug interactions and adverse events in this process. Beta-lactam agents (e.g. penicillin G, oxacillin) are often used for treatment of susceptible strains (plasma concentration= 4x MIC). In cases of drug resistance or missing options, the aminoglycosides (vancomycin) are used, being considered their nephrotoxic effects (Archeret al. 2011; Kiedrowskiet al. 2011).

Linezolid or daptomycin are current alternatives for the treatment of biofilm-associated infections by MRSA (Archeret al. 2011; Kiedrowskiet al. 2011). Tetracyclines perform efficiency when used as pretreatment. Patients that used catheters coated with minocycline rifampin did not develop associated bloodstream infections when compared to the non-pretreated group. In a murine model of endocarditis associated with biofilm, tigecycline was more effective than vancomycin. However, despite the promising effects in the treatment of endocarditis and osteomyelitis, tigecycline has not been approved for the treatment of biofilm-associated infection (Kiedrowskiet al. 2011).

Due to the difficulty on treating infections and on limiting biofilm forming strains, new studies are being conducted to launch new prototypes antimicrobials to treat such infections and increase the current therapeutic options.The c-di-GMP activity was observed as to prevent cell-cell adhesion among *S. aureus*, one of the basic stages of biofilm formation (Karaolis et al. 2005).

Donelli et al. (2007) reported that Actinobacillusactinomycetemcomitans produces β-N-acetylglucosaminidase soluble call dispersin B (DspB) capable of dispersing mature biofilm produced by Staphylococcus epidermidis and other bacterial species, such as an alternative for interfering with quorum sensing devices analyzing phenomena doctors. They showed that dispersin B reduced the number of colony forming units (CFU) on devices previously treated when combined with an antimicrobial agent. However, the polymers treated with the antibiotic for prophylactic showed no activity.

Steven et al.(Rogerset al. 2010) described the antibiofilm activity of carbamate derivatives clinically relevant for different strains including MRSA. Novel N-ethyl-carbamate were synthesized varying the aromatic grouping, by adding triazol groups, indane, tetrahydroquinoline, indoline, pyridine, *para*-amino, *para*-methoxi methyl carbamate and bromine substitution in the ring.

Interestingly, the compounds *para*-amino, pyridine and indole (200mM) showed 90% inhibition of MRSA biofilm formation, whereas triazoles, pyridine and substituted indole inhibited *E. coli* biofilm formation (80%). The most potent inhibitor containing methyl-carbamate was promising to inhibit biofilm formation but not to destroy it. Thus it can be used in non-therapeutic and prophylactic treatments (Rogerset al. 2010).

In another study, Steven et al.(Rogerset al. 2010) evaluated the synergistic effect of a new 2-aminoimidazol/triazol compound and Novobiocin, an antibiotic used to treat infections of medical devices by *S. aureus* and *S. epidermidis*. The study suggested that the new agent retained the bacterial cells at the planktonic state while the antibiotic eliminated the bacterial population, including MRSA strains. The association presented favorable results compared to the compound alone.

Another study with the compound 2-aminoimidazol/triazol (2-AI) also demonstrated that the radical groups containing 2-AI were also capable of dispersing the bacterial biofilm of MRSA strains also suppressing the resistance against penicillin G, methicillin and oxacillin, acting similarly to those derived from the study of Steven et al.(Rogerset al. 2010; Yeagleyet al. 2012).

Some new classes of compounds including lactones, brominated furanones, phenethyl, 2-carbamate have been evaluated, showing activity against biofilm formation. Structural analogs of 2-aminopyrimidine inhibited biofilm formation by MRSA and MSSA strains (80.1 to 88.5% reduction in biofilms) also suppressing the resistance to antimicrobials. These derivatives may

be promising when used in association with existing drugs in the market such assulbactam(Lindseyet al. 2012).

Recently,13thiol bismuth derivatives were evaluated and three reached higher antibiofilm activity against MRSA and *P. aeruginosa*. In comparison, ampicillin, menocicline, vancomycin, rifampicin and daptomycin were tested for MRSA and mostly were completely ineffective, except for rifampicin (MIC = 100µg/mL) (Folsomet al. 2011).

In 2012, Cobradoet al.(Cobradoet al. 2012) evaluated the antibiofilm activity of cerium nitrate, low molecular weight chitosan and hamamelitannin. The Hamamelitannin decreased *S. aureus* biofilm formation whereas cerium nitrate reduced fungal biofilm and chitosan decreased metabolic activity of *S. epidermidis*. Indole derivatives also significantly reduced biofilm formation by *S. aureus* and *S. epidermidis* (Leeet al. 2012).

Cinnamaldehyde can inhibit the growth of MRSA biofilm in a dose-dependent way *sar*A(Jiaet al. 2011). Derivatives of antraquinones reduce *Streptococcus mutans*biofilm formation through plasma membrane disturbance (Coenyeet al. 2007). Derived thimoquinonic demonstrated bactericide activity and has inhibited *S. aureus* ATCC 25923 and *S. epidermidis* biofilm formation(Chaiebet al. 2011). Interestingly, the antibiofilm activity of triazoles has also been demonstrated in other studies(Suet al. 2012).

Broad-spectrum antimicrobial activity was found in photodynamic therapy (PDT) for the treatment of infections related to bacterial biofilm of MRSA strains, *P. aeruginosa* and fungal infections. It appears effective to kill multi-drug resistance microbes, acting faster against microorganisms than antimicrobials, and there is no reported evidence for PDT resistant mechanisms. Many different types of photosensitizers and light sources have been studied. Methylene blue (MB) and other phenothiazines have been used as photosensitizer agents. Both kinds are active as strong oxidizers and cause cellular damage, membrane lysis and protein inactivation (Bielet al. 2011; Daiet al. 2012; Parket al. 2012; Biel et al. 2013). Bacteriophages or phages are viruses that specifically infect bacteria. There are DNA and lytic enzymes bacteriophages, both act on the cell wall (Fischetti 2008). Therapies with bacteriophages are also being studied, due to the action of specific bacteriophages not reaching the normal flora and proliferating at the site of infection. Studies report action including antimicrobial resistant strains to antimicrobials and biofilm formers (Burrowes et al. 2011; Chibeuet al. 2012; Gilmore 2012; Yilmazet al. 2013). But the use of this therapy requires further study since its pharmacodynamics remains unknown because it is a replication agent (Kirby 2012). Another study shows that bacteriophages can be an effective topical therapy against *S. aureus* biofilm-infected wounds in the setting of a deficient or disrupted biofilm structure. Associated treatment in order to disrupt the extracellular biofilm matrix, allowing for increased penetration of species-specific bacteriophages, represents a new and effective approach to chronic wound care and infection biofilm formation (Sethet al. 2013).

Studies with nanoparticles vary according to the substances used, but exhibit promising antimicrobial effect, also reducing biofilm infections related to surfaces, when pretreated with nanoparticles (Jonathanet al. 2012; Obermeier et al. 2012; Regielet al. 2013).

A vaccine development has been extensively studied but the main limitation is the identification of a selective antigen that mark the biofilm forming strains (Archeret al. 2011). Glucosamine, lipoprotein ABC transport protein, hipolitic conserved and conserved lipoprotein formed a tetravalent vaccine, which was analyzed against a staphylococcal biofilm-related infection. This study has used chronic osteomyelitis rabbit model revealing that the vaccination treatment associated with vancomycin reduces by 67 to 82% biofilm formation when compared to the group treated with vancomycin alone. The vaccination alone showed no significant reduction of biofilm. The association with vancomycin therapy reduced from 87.5% the biofilm MRSA strains. Currently, there is no effective vaccine against biofilm-forming strains and the expression and large number of virulence factors makes the discovery of protective antigens particularly difficult (Bradyet al. 2011).

FINAL CONSIDERATIONS

Staphylococcus aureus is an important pathogen associated with nosocomial infections and biofilm formation, being a serious chronic condition. Biofilm formation confers resistance against not only the host immune and innate system, but also against different antimicrobials on the market. This situation turned the search for new treatment approaches necessary. More studies are needed to understand the formation of biofilm as molecular targets.

Figure 1: Biofilm formation steps.

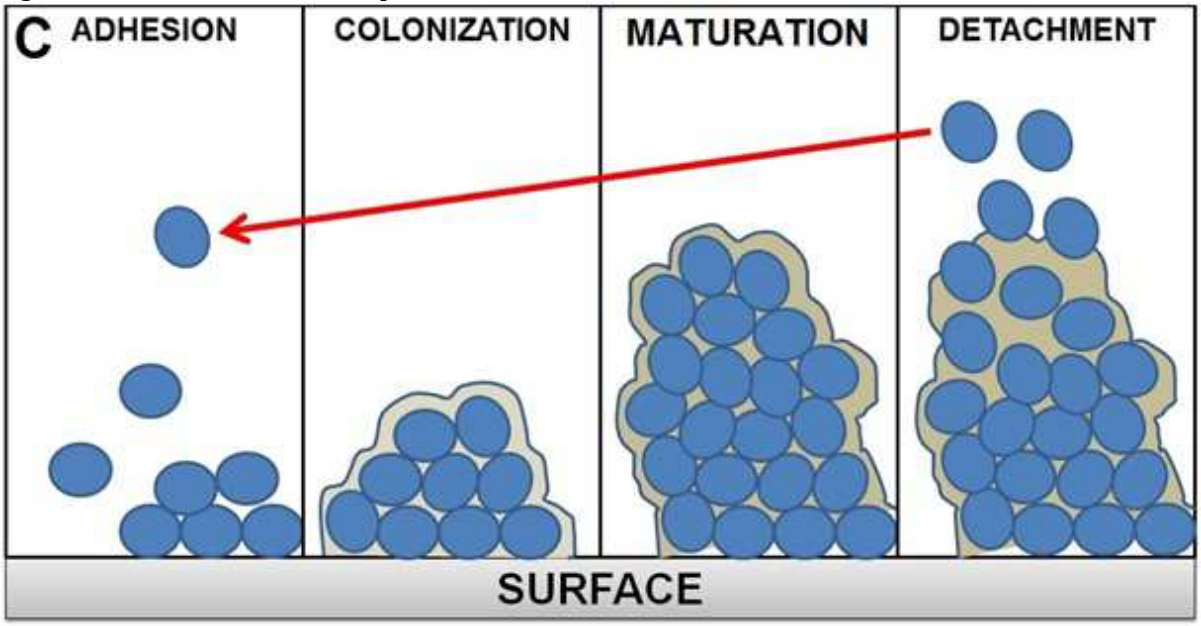


Figure 2: Outlining the activity of proteins icaADBC on biofilm-forming process (A) and mechanisms of *agr quorum sensing* (B). (A) Proteins involved in exopolysaccharide synthesis of PIA are complex genomic *ica*ADBC. Proteins icaA, icaC and icaD are transmembrane proteins and protein icaAB is located in the extracellular matrix. The first step in the synthesis of PIA is icaAD association will facilitate the export of PIA via membrane through association and activation of icaAC. Once in the extracellular matrix, PIA protein icaAB removes some N-acetyl groups, providing essential cationic character for attaching to surfaces.

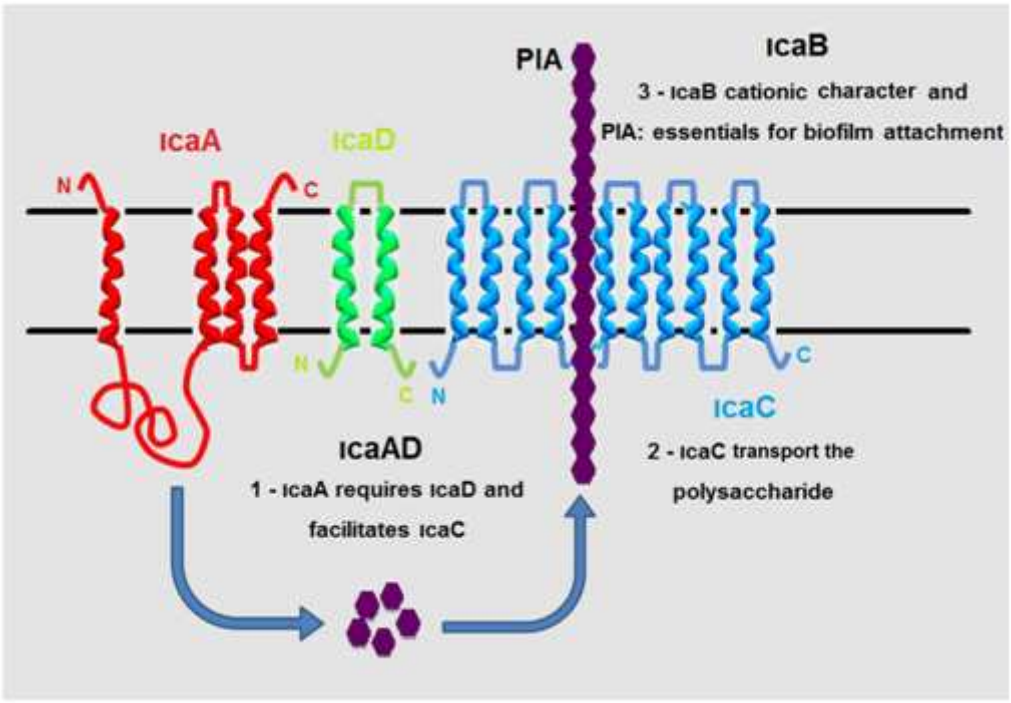
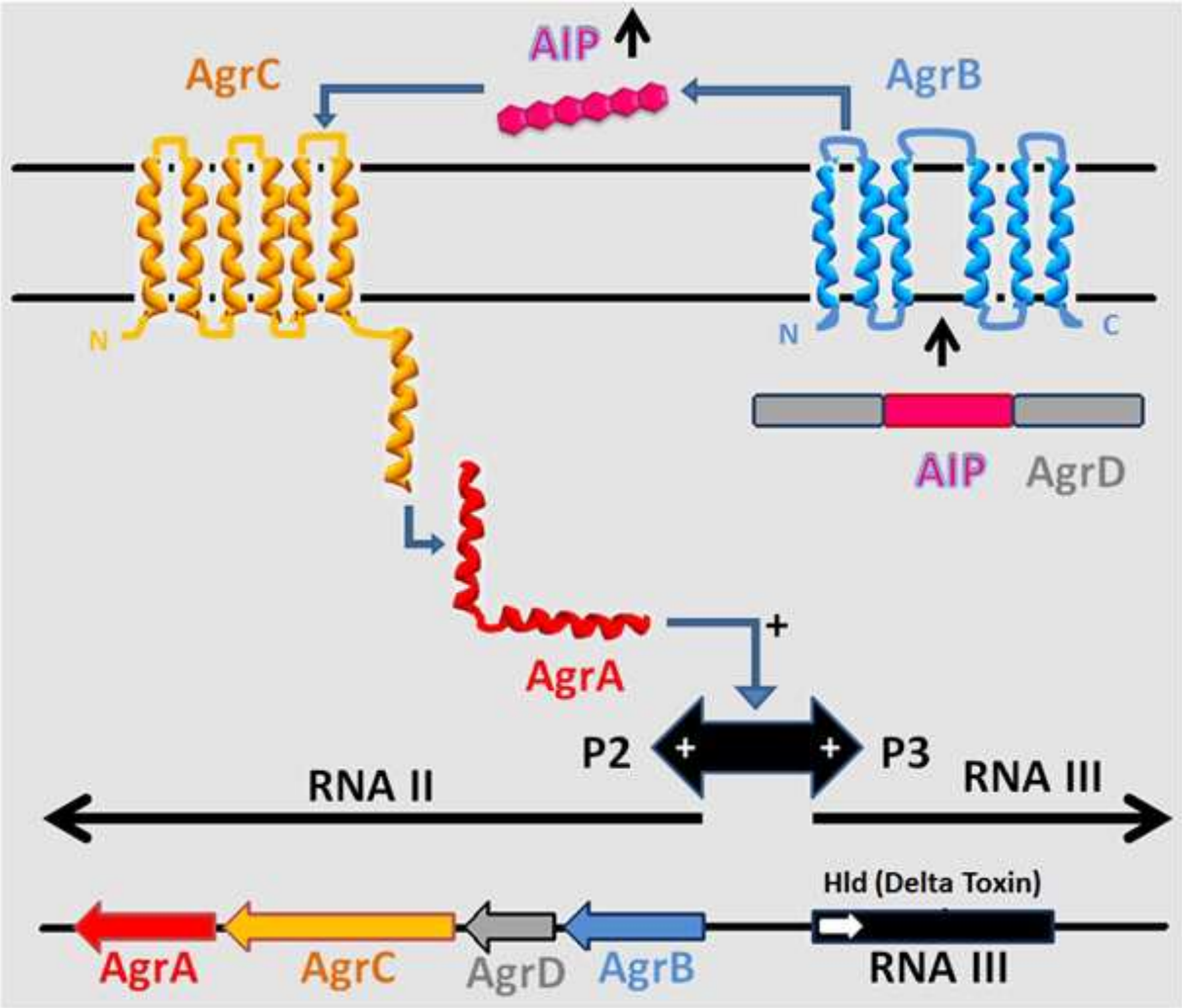


Figure 3: AgrD is a polypeptide chain and a precursor of auto inducer protein (AIP) that is exported outside the cell via AgrB. After reaching a threshold density, AIP binds to AgrC transmembrane receptors, which will phosphorylate AgrA and initiate agr transcription promoters. Active AgrA will induce transcription of RNAII and RNAIII. The transcription of RNAII contains four genes (*agrB*, *D*, *C* and *A*) important to the PIA synthesis in a positive feedback system. The RNAIII transcription is responsible for transcription of other phenotypes quorum (bacterial exotoxins).



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