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Staphylococcus epidermidis biofilms: functional molecules; relation to virulence and the host immune response

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Abstract

***Staphylococcus epidermidis* is the most significant nosocomial pathogen related to people with vulnerable frameworks such as malignant growth patients, neonates, and foreign body embedded materials such as heart valves. A few virulence factors in *S. epidermidis* can cause host damage in comparison to *Staphylococcus aureus*. In spite of that, the key roles of *S. epidermidis* virulency rely on biofilm formation, bacterial biofilm is essential for the pathogenesis by encouraging microorganisms to consist shape networks of assurance rather than free planktonic cells, hence resistance to antibacterial agents, and medically uninsured problems by colonizing medical indwelling, making the disease long span, and difficult to treat. The National Institute of Health (NIH) reported 65-80% of bacterial illnesses are biofilm formed, thus making numerous passing wellbeing additional costs. Therefore, the biofilms establishing on the susceptible hosts' tissues demonstrate; preventing antibiotics efficient treatment, protecting against host defense mechanisms, and announce the bacteria virulence determinants manifesting.**

Keywords: *Staphylococcus epidermidis, Biofilm, Virulence, Immune defense*
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I. INTRODUCTION

Staphylococci are gram-positive bacteria (cocci) nonmotile, non-spore-forming, facultative anaerobic which routinely remain gathered in grape-like gatherings; The establishment of biofilm in creatures, individuals, and on therapeutic contraptions are the fundamental factor of Staphylococci related severe infections (Hago, 2018). In addition to the biofilm being one of the major destructiveness elements of microorganisms, most nosocomial contamination is Staphylococci, thus the biofilm capacity to develop in medical strains is more significant and the diseases of staphylococcus offer protection from a wide range of antibiotics (Fisher *et al.*, 2017). *S. epidermidis* is an adaptable agent, most nosocomial and commensal pathogen by the opportunistic part relationship, perceived as a significant reason of disease in the world and largely hospital-acquired, as well a capacity to arrangement strong biofilms on attachment surfaces, in this manner giving approach to infections of connected catheter and valve of heart related infections as equipment of medicine can develop handily by dirty skin of visitors and hospital workers (Costa *et al.*, 2018). *Staphylococci* groups are divided into two collections depending on the capability to produce coagulase, the enzyme that coagulation of blood plasma: positive *staphylococci* coagulase and negative staphylococci coagulase, *S. aureus* collected through *S. epidermidis* have vigorous another as causative infection of medical places (Bastos *et al.*, 2010). *S. epidermidis* is the main microbe among the negative staphylococci coagulase. It's an individual of human skin microbiota and misty mucosa, nevertheless it might perform, for example, the agent of disease which might have an important occurrence particularly in patients with immune deficiency (Fontana and Favaro 2018; Foster *et al.*, 2014).

II.VIRULENCE FACTORS

The virulence mechanisms of *S. epidermidis* are remain unclear due to the high genetic variation which may be a new marker and drug target against *S. epidermidis* invasiveness (Wang *et al.*,2020). Several virulence factors have been defined in this review; we mostly focused on biofilm formation as a significant virulence factor (Cheung *et al.*, 2010). Moreover, the invasion of human immune defense by the bacteria becomes a non-harmful function as soon as the life of bacteria as a commensal on, such as the human skin (Lood *et al*., 2015), and this ability is still not completely understood; consequently, *S. epidermidis* is occasionally referred to as "the accidental pathogen" (Bottagisio *et al.*, 2020).

S. epidermidis dissimilar many bacteria that produce toxins, the amphipathic peptide, phenol-soluble modulins (PSMs) found in *S. epidermidis*, and held several roles; interacts with biofilm formation, regulate by quorum sensing, and causes sepsis by influencing the human innate immune system response (Henderson *et al.*, 1996). PSMs consist of three parts, PSMα, PSMβ, and PSMγ (Mehlin *et al.*, 1999). *S. epidermidis* delta-toxin is identical to PSMγ and similar to PSMα, as antimicrobial activity, cytolytic capacity, and support of provocative (Oliveira *et al*., 2018; Tam and Torres 2019), under brutal guideline (Table1). The delta-poison (hld) quality is under the control of the accessory gene regulator (*agr*) quorum sensing system and translated by RNAIII delta-poison. Only one quorum sensing system is known in *S. epidermidis*, which also encodes virulence factors. Furthermore, delta-poison associated with methicillin-resistant strains, the conflict of methicillin recognized through the existence of the mecA gene, which often has a low affinity for β-lactam antibiotics (PBP2A) and encode a penicillin-binding protein (Pruneau, 2008). Delta-poison helps out human coetaneous insusceptible guards (Surewaard, 2013).

Hemagglutinin is a glycoprotein, causes the red blood cells (RBCs) to coagulate, consisting of several types that act at different temperatures, *S. epidermidis* hemagglutinin is not influenced by the pH, temperature, concentration of proteases, serum proteins, cleanser detergent, or sub-inhibitory anti-infection agents. Their importance in *S. epidermidis* pathogenesis plays a direct role in adherence to polymers, the first step in biofilm formation along with that is biomaterial-associated infections (Essa *et al.*, 2015). Furthermore, hemagglutination has been eliminated via oxidation and absorption with glycosidases and significantly repressed by beta-lactose with the simple saccharide components in a focus dependent on design. Examination of the cells that do not hemagglutinate appears supernatant compounds containing next to zero protein and minor amounts of reducing sugars, ketose, pentose, hexosamine, phosphate, and uronic corrosive. Henceforth, *S. epidermidis* hemagglutinin contains different types of polysaccharides to adhesins (Bermudes, 2019).

Table 1. Extracellular enzymes and exotoxins of *S.epidermidis* (Otto, 2009).

Enzyme name	Category	Biological activity
Staphylococcal cysteine B	Proteases of cysteine	Damage of tissue
Cysteine protease of extracellular	Proteases of cysteine	Damage of tissue
Efflux pump of Staphylococcal	Elastase or metallo-protease	Tissue damage, AMP resistance and Lipase maturation.
S. epidermidis of Glutamyl endopeptidase,	Glutamic endo-peptidase	Destruction of fibrinogen
Serine enzyme of Staphylococcal	Protease of serine	Complement factor C5 and fibrinogen degradation
Glycerol ester hydrolase	Lipases enzymes	Determination fatty acid, Discharges
Altering enzymes of Fatty acid	Un known	Bactericidal fatty acids detoxification
Urease enzymes	Urease enzymes	Bacterial invasiveness, ureolysis, and pH changes.
Phenol-soluble modulins, for instance, Delta (δ) toxin	Toxins of tissue-damaging	Cytolysins of proinflammatory, for example, affecting necrotizing enterocolitis
Teichoic acids D-alanylation	Proteins related -AMP	teichoic acid D-alanylation
Multiple peptide resistance factor protein	Proteins related -AMP	Phospholipids Lysylation
Vancomycin resistance	Proteins related -AMP	AMP exporters
Antimicrobial peptide-sensing system (APS)	Proteins related -AMP	controls AMP, resistance mechanisms, and AMPs of senses.
Staphylo ferrins	Importer of Iron	Acquisition of Bacterial iron
Siderophores	Importer of Iron	Acquisition of Bacterial iron
Iron transporter ABC of Staphylococcal	Importer of Iron	Acquisition of Bacterial iron

On the external surface of the bacterium the proteins are tied decisively to buildups of sugar on the erythrocyte surface and different cells of eukaryotic (Goneau, 2014), this protein revealed to bind fibronectin, and mediate adherence to the surface protein of sheep erythrocytes (Miyoshi *et al.*, 2006). Often, a wide variety of erythrocytes can agglutinate because nonspecific and carbohydrate nature of *S. epidermidis* hemagglutinin. While hemagglutination can be inhibited by purified polysaccharide intercellular adhesin (PIA), a specific receptor of hemagglutinin that might be interacting with PIA on the erythrocyte surface (Duffus *et al.*, 2017), PIA is essential for a specific action of *S. epidermidis*

hemagglutinin, for example, icaADBC operon contained glycosyltransferase which is a single homologous gene and without any synthetic genes associated with the biosynthesis of sugar precursor (Somerville *et al.*, 2009), The disruption of icaADBC operon could result in polysaccharide synthesis impaired from PIA. In addition, erythrocyte hemagglutination is a characteristic of *S. epidermidis* that correlates with the formation of biofilms and is necessary for biomaterial-associated infections pathogenesis which is explained by *S. epidermidis* (Ong *et al.*, 2019).

Poly-γ-glutamic acid (PGA) and Polysaccharide intercellular adhesion (PIA) play an important role by protecting the bacteria against innate host defense; phagocytosis of neutrophils, antimicrobials peptides and is essential for survival, such as promoting growth during high salt concentrations as commensal on the skin and increased osmotolerance of biofilms in infectious agents (Sabaté Brescó *et al.*, 2017). *S. epidermidis* exopolymers with biofilm formation have analogous functions such as poly-γ-glutamic acid defense against immunoglobulins and deposition of complement (Bocian *et al.*, 2020). The PIA produced by *S. epidermidis* adhesins is measured to be the chief efficient component interceding intercellular linkage in biofilms by adhesion of cell-to-cell on non-living surfaces, furthermore act as the main virulence factor in experimental biomaterial–associated infections (Kucinskas, 2017).

PNAG plays a role in the pathogenesis of S. *epidermidis* (Otto, 2014), the immunological homology between the staphylococcal PNAG and poly-glucosamine on gram-negative pathogens could protect against numerous major human pathogens because PNAG appears to be of great importance through putting vaccine against PNAG. The proteins encoded by the *icaADCB* locus are used to synthesize PNAG (Zecconi and Scali, 2013), PNAG has several functions: it's responsible for biofilm maturation and cell actions such as intercellular adhesion, which encourages cell-to-cell collection (Otto, 2018). *S. epidermidis* biofilm polysaccharide intercellular adhesin deacetylase (IcaB) encouraging PNAG deacetylation which is essential for biofilm formation and connotation by the surface of bacterial cells (Otto, 2009) (fig.1). Poly-N-Acetyl-Glucosamine plays a critical role in the protection of planktonic *S. epidermidis* from antibodies (Rao, 2020). Biofilms of *S. epidermidis* widen to about 100 μm, although the famous rise in PNAG production is associated with biofilm development, the antibody continues to transmit during the biofilm (Gonçalves, 2016).

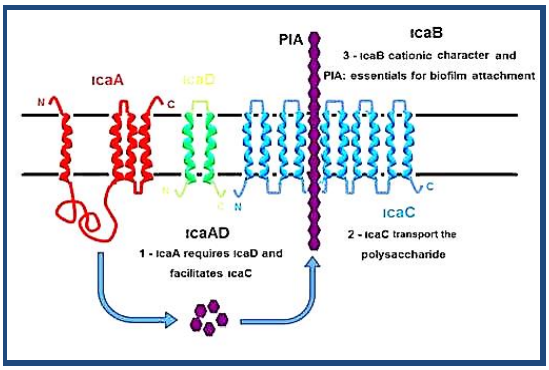

Figure 1. Outlining the activity of proteins icaADBC on biofilm formation process (A) and mechanisms of agr quorum sensing (B). (A) Proteins involved in exopolysaccharide synthesis of PIA are complex genomic icaADBC. Proteins icaA, icaC and icaD are transmembrane proteins and protein icaAB is located 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 (Silva-Santana *et al.*, 2015).

This possible resistance strategy to opsonic destruction is unlikely to explain the diminished cell murdering. Moderately, the expansion of PNAG inside the biofilm appeared to defeat the additional counteracting agent and equipped to repress the slaughter of planktonic cells when the network of biofilm blended with neutralizer which was used as a measure of phagocytosis (Smith, 2016) (Fig.2). PNAG particles have been utilized as an objective for the discovery of immunizations based on antibodies targeting staphylococcal PNAG particles, the development of antibodies based on PNAG may be utilized against different specialists of irresistible sicknesses (Sause *et al.*, 2016), which is the furthermost significant harmful property found in biofilm formation and in *S. epidermidis*; thus, the immune system of the host contributes to the persistence of biofilm infections and protection the bacteria from antimicrobial agents (Gomes *et al.*, 2014).

A. Biofilm

Typically, assessment of the proposed destructiveness factors reveals that *S. epidermidis* has developed different frameworks to protect itself against elements of the intrinsic insusceptible framework, including antimicrobial peptides and phagocytosis, rather than those elements that help with intervening obtrusive contaminations (Jyoti *et al.*, 2020). Otto in 2009 has as of late distributed a few fantastic surveys zeroing in the science of *S. epidermidis*, as of late depicted the phenol-solvent modulins (PSMs) consist of three-part antimicrobial peptide framework and different variables help the intervention protection from the inborn resistant framework (Singh *et al.*, 2017).

surface component recognizing adhesive matrix molecule (Otto, 2009).

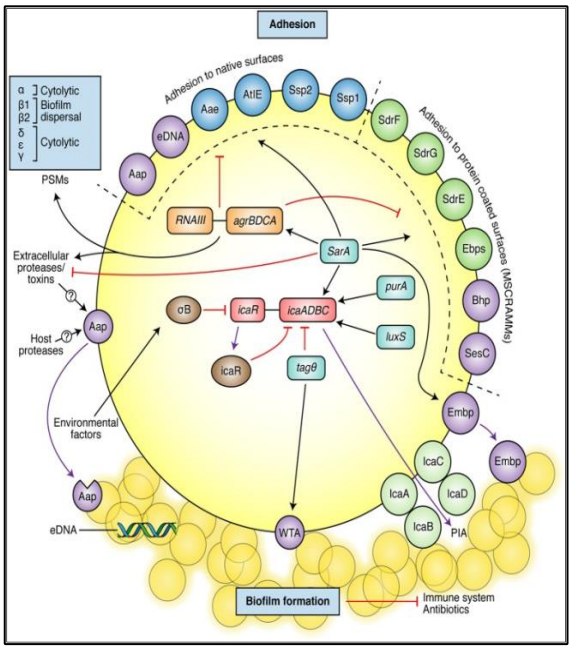

Figure 2. *S.epidermidis* main mechanisms of biofilm formation and adhesion molecules (Sabaté Brescó *et al*., 2017),).

Table 2. Major EPSs Components and Their Function in Biofilms of Bacteria (Flemming *et al.*, 2016) Functions of EPS Components in the Biofilms Context.

Polysaccharides	Adhesion, aggregation of bacterial cells, cohesion of biofilms, protective barrier, retention of water (by hydrophilic polysaccharide), sorption of organic and inorganic compounds sinks for excess energy.
Proteins	Enzymatic activity, electron donor or acceptor, adhesion, aggregation of bacterial cells, cohesion of biofilms, protective barrier, sorption of organic and inorganic compounds sinks for excess energy, export of cell component.
DNA	exchange of genetic information, adhesion, aggregation of bacterial cells, cohesion of biofilms.
Divalent ions	Mechanical stability, regulation of bap, regulation of EPS production.
Surfactants and lipids	Bacterial attachment and detachment, hydrophobicity.
Water	Provides hydrated environment, medium for movements for nutrients.

By the specific instrument needed to frame utilitarian, developing of staphylococcal biofilms looked obscure. Be that as it may, it has been traditionally seen as a four-venture measure: adherence, aggregation, development, and separation (Josse *et al.*, 2019). A developed *S. epidermidis* biofilm comprises an assortment of cement atoms, including polysaccharide intercellular adhesin (PIA), proteinaceous elements (Bhp, Aap, and Embp), teichoic acids, and extracellular DNA (Eftekhar and Mirmohamadi, 2009). Nonetheless, confusing trial examination of *S. epidermidis* biofilm development is the way that not all disengages encode factors that are thought to enlarge the biofilm arrangement. For example, not all segregate encodes icaADBC; the operon answerable for incorporating PIA. Even though many *S. epidermidis* disconnects are obtained from a characterized biomaterial disease encoding icaADBC, numerous investigations have shown that most commensal *S. epidermidis* isolates obtained from the skin of sound people don't encode icaADBC (Rowson and Townsend, 2016).

1. Structure of biofilm

The basic structural units of biofilms are microcolonies, a separate group of bacterial cells implanted into extracellular polysaccharides, microcolonies are the greatest shell rod-like or mushroom formed and they are able to comprise other microbes (van Gestel *et al.*, 2015), reliant on the type of bacteria, microcolonies comprise (10–25) % cells and (79–90) % matrix of EPS (Maier and Wong, 2015); The quantity of extra polymeric substances increases with age, as well as polysaccharides and ions of metal; the bacterial biofilms consist of proteins, biomolecules like DNA, organic substances and lipids (Decho and Gutierrez, 2017) (Table 2), low rates of nitrogen and carbon, with a very large amount of potassium and phosphates inhibit biofilm production. In contrast, slow growth of bacteria improves biofilm formation (Flemming *et al.*, 2016). The diverse constituents show the integrity of the biofilm and protect the cells from; (i) harsh environmental factors, such as ultraviolet radioactivity, pH change, osmotic pressure, and desiccation (Delcaru *et al.*, 2016). (ii) A physical barrier counter to defense substances, antibiotic diffusion, or other significant compounds from the host (Matthews *et al.*, 2019), in addition to, the nutrients access to microorganisms surrounding. Bacterial cell microcolonies are separated via channels of water that permit the nutrients, O_2, and microbes to flow from a single place to new by rotation of the fluid (Clutterbuck *et al.*, 2007). Biofilms vary in flora, consisting of tiny deposits alternating from simple layer cells to numerous layers comprising aquatic channels (Kumar *et al.*, 2017) moreover, biofilms do not only consist of polymeric matrices and microbial cells but also comprise a diversity of bioparticles containing enzymes, proteins, and ions (Fig. 3).

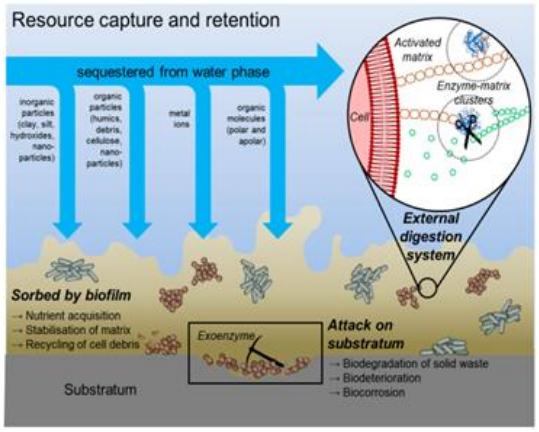

Figure 3. Illustrative demonstration of microbial biofilms consortium components (Vasudevan, 2014).

2. Biofilm Formation

Biofilm development reflects a three-step process including several genes, the free-living planktonic state (motile) showed the growth of most bacteria nevertheless, several are capable of showing diverse phenotypes, which vary in physiological features including the structure and metabolic alterations (Levipan *et al.*, 2019). Biofilm develops through multiple routes shared to produce a diversity of adaptive responses and interactions of the organisms inside the biofilms (Green, 2010), and biofilm formation is a difficult process that requires activities of corresponding, and improvement of biofilm occurs in numerous stages. The main stage is attachment, connecting to a surface either external body substantial or environment of human and attempts to adhere to it (Petrova *et al.*, 2016). The original molecular procedures were established by using PCR for the diagnosis of dangerous biofilm-developing strains such as the recognition of the genes that create extracellular polysaccharides; icaD and icaA genes (de Castro Melo *et al.*, 2013). The bacteria communicate by making chemotactic elements otherwise quorum sensing which reflects such as, a main behavior matching mechanism to order gene appearance in agreement with populace density using sign molecules, recognized as autoinducers (Wh *et al.*, 2016), the paths of are made out on a few principal parts, counting microorganism inhabitants, sign molecules, goal genes and activators of protein, which influence biofilm formation (Hu *et al.*, 2018) (Fig. 4). *S. epidermidis* uses diverse cell envelop related to adhesions named components of bacterial surface identifying molecules of the adhesive environment (Speziale *et al.*, 2014), by distinguishing specific proteins or polymers. for instance, the fibronectin-binding protein (Embp) (Esteban *et al.*, 2014) and GehD of lipase fix on collagen. Additionally, the autolysin AtlE is the cell external position protein included in the establishment of *S. epidermidis* biofilm, acting as a promoter in the generation of extracellular DNA for the second stage of development before mature biofilm formation (Xu and Siedlecki, 2014). The formation of biofilms is further expected to rely on cell-to-cell adhesion instead of on the cell quantity primarily committed to the surface (Van Houdt and Michiels, 2010; McCall *et al.*, 2019) cell-to-cell adhesion is possibly encouraged by specific connections and not prejudiced by the physicochemical connections of the germs and substrate of attachment (Okshevsky and Meyer, 2015).

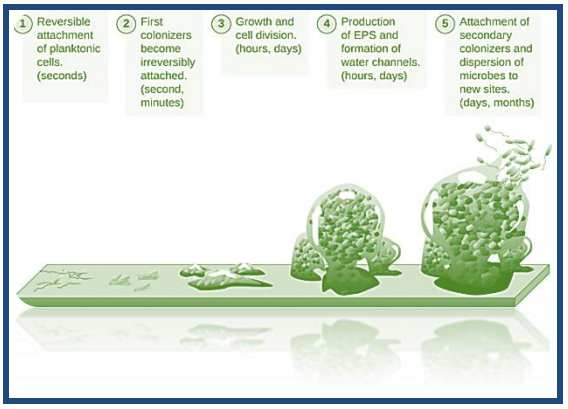

Figure 4. Different phases of biofilm arrangement and advancement including 1. planktonic cell-attached to a surface, 2. cell to cell adhesion, 3. proliferation, 4. cell maturation, and 5. cell releasing to another surface (Hu *et al.*, 2018).

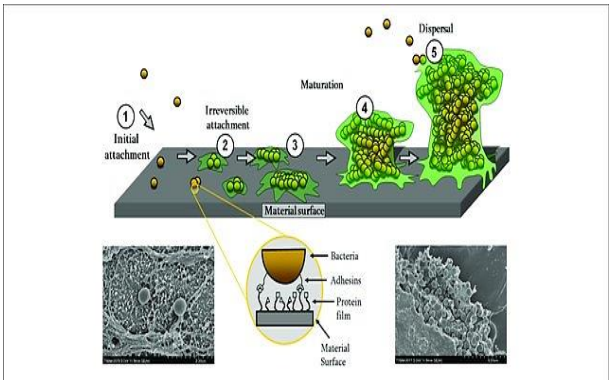

Figure 5. Biofilm formation scheme with scanning electron micrographs *of S. epidermidis* single cells (lower left) or in biofilm community surrounded by EPS (lower right) on a titanium surface (Moriarly *et al.*, 2011).

The three-dimensional structure of the mature biofilm in (Fig.5) shows that extracellular substances are built with an irregular shape of mushrooms, frequently in the top linked to each other (Chow, 2014). Channels permit a fluid flow containing oxygen and nutrients that can enter bacteria deeper into the layers of biofilm, the biofilm detachment begins as soon as the cell density and mature biofilm reach a confident level, this step is significant in the sense that it

permits the spread of bacteria and colonies to other sites. This mechanism in staphylococci is controlled by agr quorum sensing (Q.S) system (Adedoyin, 2017).

- Connection

The free planktonic (Motile) bacteria convert to sessile form before the formation of biofilm where they stick to a favorable surface; such as host tissue or medical device, pili (fimbriae) are specialized organelles that in some cases adhesins are located on it (Hago, 2018), and there are two attachment stages, the alterable-connection occurs when the bacteria are capable of returning to the planktonic form and travel away from the attachment surface. Nonetheless, at the irreversible stage, the organisms adhere and biofilm formation is initiated (Petrova and Sauer, 2016).

- Microcolonies Development

Collective cells bond on the surface at that time divide into daughter cells, from the point of attachment they multiply upward and outward to form clusters of cells, extracellular polymeric substances (EPS) and molecules of quorum sensing are produced by the dividing cells, hence accumulating cells in biofilms and microcolonies attaches to surface on which it is formed (Giupponi and Candiani, 2017), increasing organisms number cause microcolonies become bigger and quantity of EPS increased as well increasing of signaling molecules and in this stage, EPS are produced within microcolonies (Karimi *et al.,* 2015). The structure of a fully mature biofilm consists of a polymer matrix, bacterial cells, and channels of interstitial water that help the waste and nutrient exchange due to the biofilm spreading into the neighboring location (Sauer *et al.,* 2007).

- Separation and dispersal

The height population density inside a complete biofilm encourages bacteria to automatically detach from the biofilm by secretion of chemical substances (Kaplan, 2010), once the bacteria react to biological substances and are secreted by them detachment occurs, for example, degradative enzymes, signaling molecules, proteins and oxidative or nitro tension-inducing molecules such as nitric oxide (NO) created from metabolic procedures inside a biofilm (Skariyachan *et al.,* 2018). The polymer matrix is cleaved into short oligosaccharides by the degradative enzyme produced by biofilm organisms due to increased biofilm organism's detachment (Cellini, 2010), which are prompting factors for bacterial biofilm dispersal by increasing bacterial growth and quorum sensing production, which generally help the processes of dispersal inside the biofilm (Emerenini *et al.,* 2015). Moreover, biofilm detachment is caused by nutrient starvation, hence the processes of detachment improve the biofilm sloughing and sessile organisms switching inside a biofilm, separate isolated bacteria from other locations continue to form biofilms (Lyons, 2012), biofilm dispersal causes infections to spread in the host which occasionally might cause thromboembolism and may result in death Brading *et al.,* 1995).

B. Quorum Sensing [QS] Cell-to-cell Communication

Quorum sensing is analogous to assessment-making method types, which performance is corresponding done a "chemical vocabulary" and used to the signaling of a cell to cell and uniform among species (Baroncini *et al.,* 2019). Communication of cell-to-cell among microbes including autoinducers (AIs) which are the small hormones like molecule, organisms in the interior environment by signaling molecules production able to full formation of biofilm, and established in lacking strains were capable of causing infections and less antimicrobial susceptibility (Lyon, 2015) (fig.6). However, several virulence factors are not related to molecules (Senturk *et al.,* 2012). Quorum sensing depends on phenotypes diverge along with the organism's isolation site. Staphylococci autoinducers (AIs) is a peptide and when AIs level reaches a convinced threshold the bacteria gene expression alters (Pérez-Velázquez *et al.,* 2016) (fig.7). Furthermore, quorum sensing investigation of *S.aureus* has been completed, but similar developments have been established in further *staphylococci* genes, such *S. epidermidis* (Reddy *et al.,* 2019).

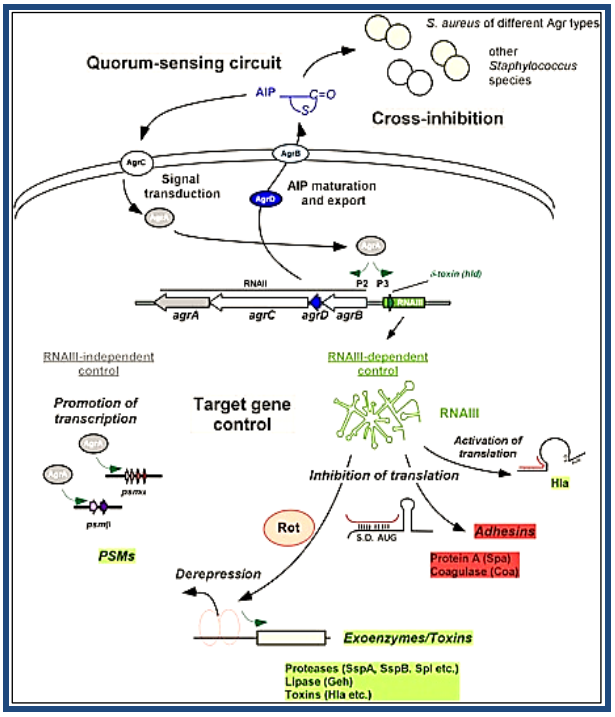

Figure 7. Diagram summary of *agr* QS arrangement in *Staphylococcus spp.* The quorum-sensing is controlled by released AIP signal from AgrD by the AgrB and then when the AIP reaches a specific concentration the AgrC-AgrA activates the transcription, Agr inhibition occurs in other bacteria Agr groups and is regulated by RNAIII. RNAIII inhibits the translation of target gene control in most states. RNAIII promote PSM transcription (Le and Otto, 2015).

Staphylococci quorum sensing system is under the control of accessory gene regulator (agr) (Kane, 2017). The upregulation of exo-protein synthesis and downregulation of surface proteins is mediated by the bacteria. Therefore, a system of decoration gene regulator controls in *S. epidermidis* is an important portion of the chromosomal genes (approximately16%) such as genes involved in virulence, separation of cells, and metabolic alterations (Kane *et al.*, 2018). The size of *agr* gene consists of four accessory genes, regulator genes (*agrB, agrD, agrC* and *agrA*) and it is a complex gene approximately 3.5kb, all regulator genes transcribed via RNAII. The expression of this autoinducing peptide is regulated by *agrC* and *agrA*, while *agrD* encodes for autoinducing peptide (AIP), which is exported and modified by *agrB*. The gene for delta-toxin (hld) is placed adjacent to the *agr* complex and transcribe by RNAIII (Marroquin *et al.*, 2019).

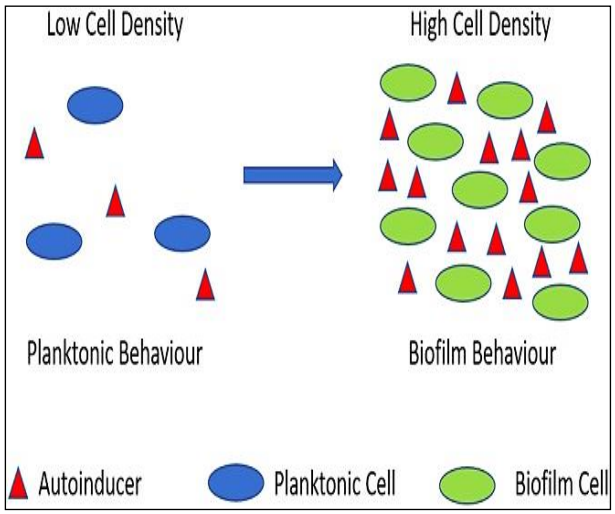

Figure 6. Quorum sensing illustration. During planktonic cell growth (blue ovals), the relative amount of autoinducers (red triangles) is proportionally low. As cells enter a densely populated mode of growth (green ovals) the relative proportion of autoinducers increases (Verderosa *et al.*, 2019).

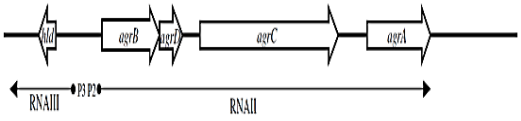

Figure 8. various genes forming the *agr* intricate and then ear by hld gene, with the transcriptions RNAII and RNAIII marked (Halebeedu *et al.*, 2014).

III. IMMUNE RESPONSE

Throughout the body, a large and complex series of immune system elements are widely distributed including several functions the protection against pathogens and responses to foreign materials (Aslani and Ghobadi, 2016). Immune responses include two classes: non-specific or innate immune responses and acquired or adaptive immune responses, which are highly specific to a specific pathogen. Humoral immunity includes molecules in biological fluid solutions (Mak *et al.*, 2013; Liu *et al.*, 2019b), humoral immunity (also called the antibody-mediated system) is the part of immunity that is mediated via macromolecules present in extracellular fluids, such as secreted antibodies, complement proteins, and confident antimicrobial peptides (Magiri, 2019). When immunoglobulin binds, the infectious agents deactivate; hence, cellular immunity includes the expansion of immune cells that are capable of recognizing, binding, and killing additional cells that were previously infected via foreign infectious agents (Augustyniak *et al.*, 2017; Land, 2018). Humoral immunity involves complement and antibodies; thus, cytokines are produced chiefly through immune cells, and in almost every aspect of immunity and inflammation, cytokines are included (Parham, 2014; Brook and Dobson, 2015). IL-6 is a pro-inflammatory cytokine that plays an important role among pro-inflammatory cytokines in immunity similarly, a pro-inflammatory chemokine Interleukin-8 is secreted from a variety of cell types including leukocytes, endothelial cells, cancer cells, and fibroblasts (Molina *et al.*, 2010).

Bacteria produce a chemical substance within the biofilm that is resistant to antimicrobials and immune mechanisms of the body. Biofilm formation matures at a sluggish rate which improves their ability to resist host immune appliances and antimicrobial interference (Golob, 2016). Biofilm-produced bacteria cause infections via means of their protection from antimicrobials and the body's immune system (Liu *et al.*, 2019a). In normal human serum, biofilm bacteria are less susceptible to phagocytic killing after opsonization (Banerjee *et al.*, 2020). Several studies have been established the chief role of biofilms in overcoming the immune system and biofilm ability to resist the host immune mechanism elements (Alhede *et al.*, 2014), which is one of the causes of diseases, and biofilms importance is seldom determined through a personality's specific immune system (Bjarnsholt *et al.*, 2013). The polymeric matrix is the first defense of the pathogen, and the presence of EPS protects the microbes from the phagocytic process by preventing phagocytic and neutrophil cells from engulfing microbial cells (Arciola *et al.*, 2012). Because of the inefficiency of opsonization to the bacteria that form biofilms inside the body, the levels of killing decreased by the phagocytic cells; polymorphonuclear (PMNs) of the positive wild-type bacteria to form biofilms (Kırmusaoğlu, 2016). Destruction of *S. epidermidis* biofilms by immune defense fewer than planktonic cells which can be opsonized with immunoglobulin (Priyanka, 2014). The diffusion of Anti-PIA/PNAG antibodies into the biofilm of *S. epidermidis* is sufficient to allow opsonization (Rohde *et al.*,

2010) regardless, of the mechanism of biofilm, accumulation related proteins, polysaccharide intercellular connections, and extracellular matrix requisite proteins. *S. epidermidis* from the collected biofilm cells caused a minor inflammatory response by macrophages which was more than the scatter biofilm formed by microbes or mutant biofilms. Undesirable, which may show additional mechanisms of *S. epidermidis* in chronic infection aid under the immune system (Sparling, 2013).

The ability of biofilms to defend microorganisms against host innate immunity is essential for *S. epidermidis* pathogenesis, it protects *S. epidermidis* from phagocytosis by decreasing the opsonization of IgG, C3b connect to the surface of bacteria, and stimulation of the complement cascade intermediated via PIA biofilm (Li *et al.,* 2018), *S. epidermidis* decreasing cytokine production by animal mononuclear cells (for instance monocytes) invitro (Kuwahara *et al.,* 2005). PIA and non-PIA protect *S. epidermidis* biofilm from phagocytosis, by the disconnect between PRRs and bacteria on the leukocytes (design recognition receptors), In addition to stimulating a weak NF-κB (nuclear factor kappa-light-chain-enhancer of activated B cells) mediated macrophage inflammatory immune, the absence of connection between the bacteria and macrophages, as well happenings altering the macrophage role look to yield portion in the host miscarriage to eliminate *S. epidermidis* through infections (Granslo, 2012).

The bacterial biofilm phenotype is arranged in various features by planktonic development to improve the antibacterial resistance and variance in gene expression, in addition to the ability of biofilm to defend local bacteria from the violence of the immune system (Otto, 2014). Antibody production can be stimulated by the release of antigens through mature biofilms, but the bacteria inside the membrane will be resistant to these defense mechanisms. Mature biofilms can discharge antigens and encourage the creation of antibodies, nonetheless, the bacteria that are inhibited inside the biofilm are resistant to these attack appliances. Moreover, PNAG defends planktonic *S. epidermidis* in contrast to antibody-independent phagocytosis, in that PNAG is improved by opsonization, which includes complement and immunoglobulin mediated phagocytosis (Bhattacharya *et al.*, 2015). The mechanisms of bacterial resistance in biofilms to immunity are known by the antibodies diffusion assessed through biofilm. Antibody-mediated phagocytic destruction of the planktonic cells in *S. epidermidis* biofilm cells by the use of a rabbit antibody against PNAG (Boisvert *et al.,* 2016), these immunoglobulins are opsonic and defend against infection with planktonic cells of PNAG positive *S. epidermidis*, the immune response to PNAG promptly enters the biofilm and is connected to similar parts in the biofilm, a lectin recognized to tie biofilm parts (Cerca *et al.,* 2006). On the other hand, the biofilm cell has additional impervious to opsonic slaughtering than planktonic partners even though creating more PNAG per cell than planktonic cells, Biofilm removes repressed opsonic destroy intervened by antibody to PNAG, the PNAG antigen inside biofilm matrix inhibits antibody binding closely to surface bacteria, which required for capable opsonic killing (Shahrooei *et al.*, 2012).

Expanding obstruction of biofilm cells to opsonic murdering interceded by another defensive counteracting agent is expected not to be a biofilm-explicit phenotype, whereas significant heights of antigen inside bacterial biofilms that inhibit bacterial opsonization through the immune response, the matrix of *S. epidermidis* biofilms is essentially out of the enormous exopolysaccharide PNAG (Moser *et al.*, 2017). The creation of PNAG is essential for *S. epidermidis* biofilm arrangement and are produced via gene result of the icaADBC gene group, therefore the creation of pNAG/pIA and biofilm development are controlled by different sigma factor sB (Sabaté Brescó *et al.,* 2017), opsonic antibodies to PNAG intercede assurance against fundamental disease by *S. epidermidis* where it's called capsular polysaccharide/attachment (Kırmusaoğlu, 2016). As a result, the biofilm matrix can shield microscopic organisms from immunoglobulin-mediated phagocytosis in the proximity of antibodies opsonically dynamic versus planktonic cells. An enormous measure of PNAG antigen exists inside the matrix limiting antibodies official to the bacterial cell surface, which it requests to advance opsonic slaughtering just as more PNAG produced per cell inside the biofilm matrix (Skurnik *et al.,* 2016), this supports the conclusion that this enormous amount of antigen can inhibit antibody binding to the bacterial cell surface (Cywes-Bentley *et al.,* 2013). PNAG is a secure planktonic microbial against the autonomous phagocytic immune response and it creates the impression that even in the proximity of opsonic antibodies to PNAG, the overabundance of the objective antigen inside the biofilm can forestall productive opsonic murdering (Veerachamy *et al.,* 2014).

A. Biofilm-associated infections of S. epidermidis prevention and treatment

Biofilms protect microbial cells from serious ecological situations, such as the toxicity of metals, ultraviolet introduction, lack of hydration and saltiness, acid exposure, and anti-toxins or other antimicrobial operators just as phagocytosis (Ahmed *et al.,* 2020). The essential problem with bacterial biofilm infections is affinity to clearance of resistance by antimicrobial agents and host immune system when compared to their free (planktonic) partners. Microscopic organisms inside a biofilm (sessile) are up to 1000 times progressively resistant to anti-microbial causes (Turkina and Vikström, 2019); thus, the elements that add to overall biofilm resistance are:

- Diminishing the development ratio of microbes in a biofilm creation that is less inclined to antimicrobials by goal metabolic paths.

- Obligate diffusion of antimicrobial composites (di Biase *et al.*, 2019).

An unmistakable phenotype that presents resistance to antimicrobials, host immune resistance and encourages horizontal gene transmission, numerous systems utilized to battle the biofilm diseases can be summarized in (Grimaldi *et al.*, 2020).

The preventive methodologies target are; functional particles, systems of the gene, and administrative, which control the beginning time of biofilm improvement; different materials are utilized to inhibit initial biofilm formation and disrupt the maturation biofilm, for example, catalysts are prompting dis-regulation of particles balancing out the biofilm design and causing disintegration of the matrix of the biofilm (Mahamuni-Badiger *et al.*, 2020), Moreover, alteration of biomaterial surface which prevents the colonization of bacteria through applying biomaterials that have antimicrobial activity, wholly these methodology depends on prophylactic utilizing of antitoxin as well as submersion, covering and loading of a matrix (Darwin, 2011). Thus, to prevent microbial colonization on the surface of the clinical device is the major goal and then the bacterial establishment with biofilm formation stopped (Khan *et al*., 2020; Subhi *et al*., 2020), therefore antimicrobial agents can decrease the biofilm stratum next to sub-inhibitory concentrations. Sub inhibitory focuses of several antibiotics that stay viewing together impede and stimulate the formation of coagulase-negative staphylococci biofilm, these effects seeming to be based on antimicrobial agent type and the bacterial strain (Mohapatra *et al*., 2020).

IV. CONCLUSIONS

Biofilm is bacterial colonies aggregation, commonly of multiple species that create a protective EPS and forms a micro-environment inside, conducive to persistence and finally leads to chronic infection in the form of pulmonary infections, stones of kidney, cutaneous non-healing wounds, and endocarditis. Bacteria undergo genetic changes when exposed to environmental stressors that promote the formation of biofilm. Biofilms consist of multiple elements, for example, extracellular DNA, proteins, polysaccharides, and water/ biosurfactants, completely which have unique functional and structural characters that create the biofilm formation and its properties. Biofilms are the main cause of chronic disease because of the signals secretion that blocks an appropriate host immune response. Whereas, each species of biofilm is diverse in specific properties, make-up, and antibiotics response. In general, treatment of biofilms are extremely difficult by antibiotics because the EPS block the diffusion of the antibiotic and create a microenvironment that allows gene transfer between the cells inside the biofilm, thus the cells become more resistant, slowing metabolically, and finally dispersion or escape to establish a new biofilm at different places inside the body.

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