

Chapter

Biofilm Biology of *Staphylococcus aureus* and *Staphylococcus epidermidis*

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Abstract

Staphylococcus aureus (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) are the primary agents of chronic and device-associated infections, mainly due to their ability to form biofilms. These biofilms shield the infectious agents from immunological responses and antibacterials, serving primarily as a survival strategy. The accessory gene regulator (*agr*) quorum-sensing system, staphylococcal accessory regulator A (*SarA*), sigma factor B (*sigB*), nitric oxide synthase (*NOS*), vancomycin-resistance-associated sensor/regulator (*VraSR*), intracellular adhesin (*ica*)-dependent and *ica*-independent pathways regulate the early surface attachment, accumulation, maturation, and dispersal stages of biofilm growth. Biofilm formation in *Staphylococcus* is greatly influenced by host matrix proteins, iron availability, plasma proteins, immunological responses, and environmental factors. *S. epidermidis* biofilms are composed primarily of polysaccharide intercellular adhesin/poly-N-acetylglucosamine (*PIA/PNAG*), whereas *S. aureus* biofilms are more dependent on extracellular DNA (*eDNA*) and proteins. Biofilm is a key virulence factor in both *S. aureus* and *S. epidermidis*, but it is the defining mechanism of pathogenicity in *S. epidermidis*, leading to chronic conditions, whereas *S. aureus* relies on a broader array of virulence factors, including surface adhesins, toxins (e.g., hemolysins, leukotoxins), biofilms, and immune evasion proteins, and leads to acute infections.

Keywords: *Staphylococcus aureus*, *Staphylococcus epidermidis*, biofilm, antibiotic resistance, biofilm-associated infections

1. Introduction

1.1 Staphylococcal biofilms

Staphylococci are gram-positive cocci that form part of the normal microbiota of human skin and mucous membranes, yet they are also among the most important opportunistic pathogens in modern medicine. In particular, *Staphylococcus aureus* (*S. aureus*) and *Staphylococcus epidermidis* (*S. epidermidis*) dominate as causes of chronic and device-associated infections, largely because of their remarkable capacity to grow as biofilms on biotic tissues and indwelling medical devices [1–3].

In this sessile lifestyle, bacterial cells adhere to a surface, proliferate, and become embedded in a self-produced extracellular matrix composed of polysaccharides, proteins, and extracellular DNA (eDNA), forming a structured microbial community that is phenotypically distinct from free-living planktonic cells [2, 4, 5].

1.2 Clinical relevance and burden

The expansion of implant-based and invasive medical procedures has greatly amplified the impact of staphylococcal biofilms. *S. aureus* and *S. epidermidis* are now leading etiological agents of catheter-related bloodstream infections, prosthetic joint and orthopedic device infections, cardiac valve and vascular graft infections, and infections of breast and other soft-tissue implants [1, 3, 6]. According to Centers for Disease Control and Prevention (CDC) estimates, methicillin-resistant *S. aureus* (MRSA) causes over 70,000 serious infections and 9,000 fatalities annually [7]. In orthopedics, *S. aureus* is a major cause of surgical site and implant-related infections, whereas *S. epidermidis* accounts for roughly one-fifth of orthopedic device-related infections overall and an even higher proportion of late-onset cases [6, 8]. These infections are typically indolent in onset but persist for months or years, often necessitating repeated surgical debridement, device removal, prolonged antimicrobial therapy, and, in some cases, staged re-implantation [8, 9]. The resulting morbidity, healthcare costs, and contribution to antimicrobial resistance place staphylococcal biofilms among the foremost challenges in hospital infection control [5, 10].

1.3 Biofilm as a survival and virulence strategy

Biofilm growth represents a central survival strategy for *staphylococci* in the host. Once attached to a surface, staphylococcal cells multiply to form multilayered microcolonies and elaborate an extracellular polymeric matrix that confers mechanical stability and creates steep gradients of nutrients, oxygen, and pH [2, 5]. This microenvironment drives heterogeneity in metabolic activity, with dormant or slow-growing subpopulations that are inherently less susceptible to antibiotics targeting active cellular mechanisms [4, 11]. The matrix also slows or prevents penetration of many antimicrobials, while binding or inactivating others, so that conventional susceptibility testing with planktonic cultures often underestimates the drug concentrations required to impact biofilm-embedded bacteria [4, 10, 12]. Equally important, staphylococcal biofilms profoundly modulate host immune responses. Biofilm communities limit exposure of bacteria to phagocytes and antibodies and can alter expression of toxins and other virulence factors in ways that blunt effective clearance [5, 13, 14]. The *S. aureus* and *S. epidermidis* biofilms are therefore poorly cleared even in immunocompetent hosts, promoting chronic, relapsing infection that frequently persists despite appropriate systemic therapy [6, 13, 14]. In *S. epidermidis*, a tendency to induce low-grade inflammation with elevated anti-inflammatory cytokines, such as interleukin-10 (IL-10), may further favor long-term persistence on implants [6].

1.4 Transition from commensalism to pathogenicity

Both *S. aureus* and *S. epidermidis* illustrate the dual nature of *staphylococci* as commensals and pathogens. *S. epidermidis* is a permanent resident of healthy skin,

where, under normal conditions, it may help exclude more virulent organisms [6]. *S. aureus* colonizes the anterior nares and skin of a substantial fraction of the population, serving as a reservoir for subsequent invasive disease [3, 15]. The introduction of a foreign body, tissue damage, or host immunosuppression creates opportunities for these otherwise benign colonizers to adhere, form biofilms, and establish infection [1, 6, 16]. The rapid conditioning of implant surfaces by host proteins, along with an array of staphylococcal adhesins and redundant attachment mechanisms, ensures that the bacteria can adhere to surfaces at all costs and initiate biofilm formation in a wide variety of anatomical sites [3, 17].

1.5 Therapeutic implications

The combination of immune evasion, a protected microenvironment, and antibiotic tolerance makes staphylococcal biofilm infections intrinsically difficult to eliminate. Standard treatment often relies on a combined surgical–medical approach, including debridement or removal of colonized material, combined with prolonged, high-dose antimicrobial regimens [8, 9]. Recognition of the central role of biofilms has spurred intensive efforts to understand the molecular basis and regulation of biofilm development and to design novel therapies and surface modifications that prevent colonization, damage established biofilms, or sensitize biofilm cells to existing drugs [2, 10, 13, 18, 19]. A detailed exploration of these mechanisms and strategies is essential to improve outcomes in patients suffering from chronic and device-associated staphylococcal infections.

2. Biofilm in *S. aureus*

2.1 Formation

Biofilm development in *S. aureus* is a staged, highly coordinated process that transforms free-living planktonic cells into a structured multicellular community. It can be divided into initial attachment, proliferation and accumulation, maturation, and dispersal, as shown in **Figure 1**. In the attachment phase, planktonic cells encounter biotic or abiotic surfaces that are often pre-coated with host extracellular matrix proteins such as fibrinogen, fibronectin, collagen, and laminin. A combination of cell wall-anchored adhesins, mainly microbial surface components recognizing adhesive matrix molecules (MSCRAMMs), mediates this initial stable contact. Key adhesins include fibronectin-binding proteins (FnBPA and FnBPB), serine–aspartate repeat proteins (SdrC, SdrD, SdrE), clumping factors (ClfA and ClfB), fibrinogen-binding protein, and *S. aureus* surface proteins (Sas) such as SasC and SasG, which in combination support the adhesion to host tissues and conditioned biomaterials [4, 20–22].

Following attachment, *S. aureus* cells proliferate on the surface and transition to an aggregation phase characterized by intercellular adhesion and microcolony formation. This step may be driven by different matrix strategies. In many strains, the polysaccharide intercellular adhesin/poly-N-acetylglucosamine (PIA/PNAG), synthesized by the intracellular adhesin ADABC (*icaADABC*) locus, plays a central role in promoting cell–cell cohesion, accumulation, and protection from host defenses [11, 23, 24]. Other strains rely mainly on proteinaceous matrices, in which

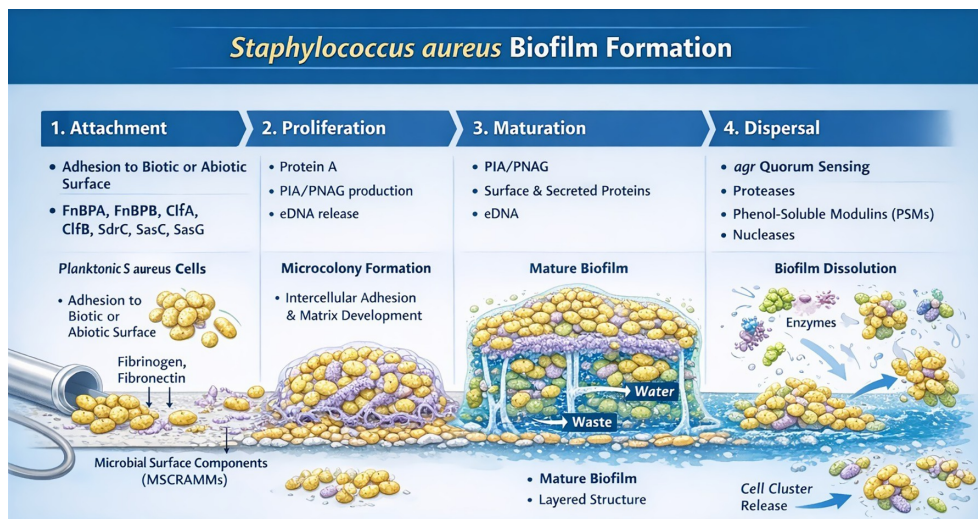


Figure 1.

Biofilm formation in S. aureus. (During the preparation of this work, the authors used Chat-GPT to create the figure for conceptual visualization).

fibronectin-binding proteins, SasG, SasC, serine–aspartate repeat proteins, and the biofilm-associated protein (Bap), when present mediate tight bacterial aggregation and three-dimensional architecture formation even in the relative absence of visible [22, 25]. Extracellular DNA, released in part through controlled autolysis pathways such as the cidABC–lytSR regulatory axis, is an integral scaffold that stabilizes early biofilm structures and promotes irreversible attachment [4, 24, 26].

As the biofilm matures, the community acquires a complex three-dimensional organization with channels that facilitate nutrient diffusion and waste removal. The extracellular matrix becomes richer and more heterogeneous, containing varying proportions of PIA/PNAG, surface and secreted proteins, including the extracellular adherence protein (Eap), which reduces matrix porosity and enhances structural integrity, amyloid fibrils, and abundant eDNA [11, 22, 24, 27]. These components can act synergistically; for instance, positively charged PIA can associate with eDNA and lipoproteins to anchor the matrix to the cell surface, reinforcing cell aggregation and increasing tolerance to environmental stresses [26]. Mature *S. aureus* biofilms are dynamic, harboring metabolically diverse subpopulations, including dormant or slow-growing cells that contribute to antibiotic tolerance and persistence [4, 28].

Dispersal represents the final developmental stage and is critical for dissemination to new sites. In *S. aureus*, detachment is closely linked to the activation of the accessory gene regulator (*agr*) quorum-sensing system once a critical cell density and environmental threshold are reached. Agr upregulates the production of extracellular proteases such as Aureolysin (Aur) metalloprotease, serine protease-like (Spl) proteins, and nucleases, which degrade surface adhesins and eDNA and weaken the matrix, leading to the release of planktonic cells or small aggregates [20, 23, 29]. This controlled dismantling of the biofilm restores motile, more antibiotic-susceptible populations, but also seeds new foci of infection, allowing *S. aureus* to alternate between persistent and invasive phases [28–30].

2.2 Regulation

Biofilm formation in *S. aureus* is not a fixed trait but the outcome of a multilayered regulatory network that integrates cell density, nutrient status, stress signals, and host-derived cues. Central to this network is the agr quorum-sensing system, which senses an autoinducing peptide (AIP) and coordinates a global transcriptional switch. At low agr activity, expression of many cell wall-anchored adhesins is favored, supporting surface attachment and biofilm accumulation. When AIP reaches a threshold concentration, agr signaling is activated via the AgrC–AgrA two-component pair, inducing RNAIII and downstream targets that repress surface proteins and strongly promote secretion of proteases, phenol-soluble modulins (PSMs), and other factors that drive matrix degradation and dispersal [20, 23, 29, 31]. Thus, agr functions as a key determinant of this transition from a sessile to a motile lifestyle. Agr-defective mutants frequently show hyper-biofilm phenotypes.

The SarA is another major hub that generally promotes biofilm formation. SarA enhances the expression of icaADBC and various adhesins, suppresses extracellular proteases and nucleases, and stabilizes the matrix, thereby favoring accumulation and persistence [4, 11, 23]. The sigB also modulates stress responses and indirectly influences biofilms by upregulating adhesin genes and repressing the agr system, especially under adverse environmental conditions such as oxidative stress or nutrient limitation [4, 31]. Other regulatory loci, including saeRS, arlRS, CodY, and icaR, control the balance between matrix production, autolysis, and dispersal in response to specific stimuli such as nutrient availability, osmolarity, pH, and antibiotics [4, 24, 26, 32].

This complex and often overlapping circuitry allows *S. aureus* to flexibly shift between PIA-dominated and protein/eDNA-dominated matrices, to alter the relative contribution of adhesins and degradative enzymes, and to adapt its biofilm phenotype to distinct host niches. Importantly, regulatory programs *in vivo* may differ substantially from those observed in laboratory media, and interactions between global regulators remain incompletely understood, highlighting significant gaps in current knowledge [4, 23, 28, 31].

2.3 Host–Bacterial factors

Biofilm formation by *S. aureus* is profoundly shaped by the interplay between bacterial determinants and host-derived factors. Upon contact with blood or tissue fluids, foreign materials and damaged surfaces are rapidly coated with a layer of human extracellular matrix (hECM) proteins, including fibrinogen, fibronectin, collagen, elastin, laminin, vitronectin, and proteoglycans. *S. aureus* expresses more than 30 adhesins capable of recognizing these substrates, enabling colonization of virtually any tissue niche. The FnBPs mediate binding to fibronectin and, via integrin $\alpha 5 \beta 1$, can also drive internalization into non-professional phagocytic cells, providing a protected intracellular reservoir that complements surface biofilms [20, 21, 33]. The ClfA and ClfB interact with fibrinogen and cytokeratin, supporting colonization of vascular devices and nasal epithelium, while Sdr family proteins contribute to adhesion, immune evasion, and abscess formation [4, 20–22].

Host immune responses further influence biofilm architecture and persistence. Neutrophil attack and oxidative burst can trigger bacterial autolysis and the release of eDNA, which becomes incorporated into the matrix and enhances structural stability [11, 24, 34]. Inflammatory exudates supply additional host DNA, actin, and other

polymers that interweave with bacterial components. Plasma and serum factors can promote the rapid formation of suspended aggregates that are poorly accessible to phagocytes, reducing the necessity for certain self-produced polysaccharides observed *in vitro* [35]. At the same time, biofilm-embedded *S. aureus* modulates the expression of toxins, leukocidins, and immunomodulatory proteins, such as Eap and protein A, to impair phagocytosis, complement activation, and cytokine signaling, thereby skewing local immunity toward a state that favors chronic infection [11, 24, 27].

Environmental conditions at the infection site, such as pH, osmolarity, oxygen tension, iron availability, and nutrient composition, also have decisive effects on matrix composition and regulatory circuits. For example, high glucose often enhances PIA production and biofilm biomass, whereas elevated NaCl or osmotic stress can promote icaADBC-dependent biofilms in certain strains but not others [23, 34, 36]. Iron limitation can induce the expression of specific surface proteins that enhance proteinaceous biofilm formation, while hypoxic or acidic microenvironments enrich metabolically adapted subpopulations that contribute to antibiotic tolerance [24, 28]. Together, these host–bacterial interactions generate a spectrum of *S. aureus* biofilm phenotypes that differ across anatomical sites and clinical settings, complicating eradication and underscoring the need for context-aware therapeutic strategies.

3. Biofilm in *S. epidermidis*

3.1 Formation

Staphylococcus epidermidis is a skin commensal that becomes a highly efficient biofilm former when given access to abiotic surfaces such as catheters, prosthetic joints, and other indwelling devices. Its biofilm lifecycle is typically divided into adherence, accumulation, maturation, and dissemination, as shown in **Figure 2**, but

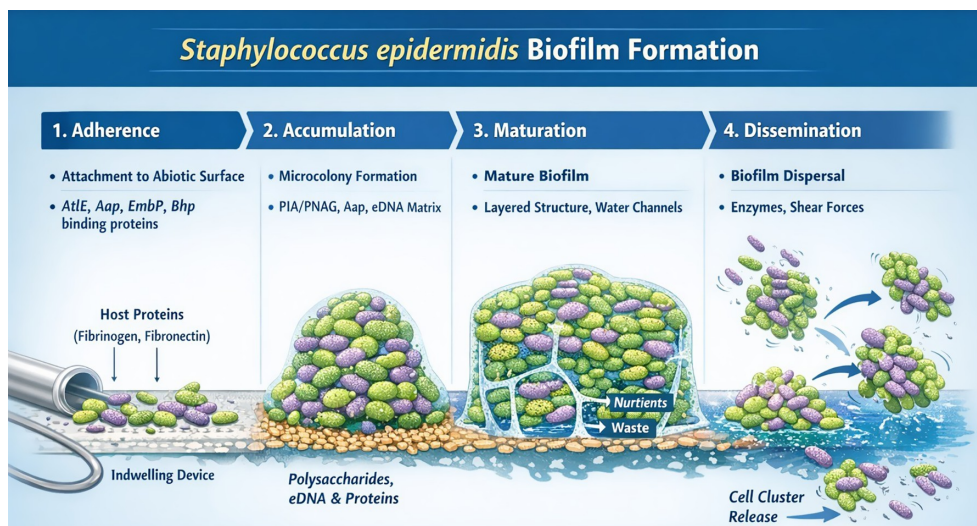


Figure 2. Biofilm formation in *S. epidermidis*. (During the preparation of this work, the authors used Chat-GPT to create the figure for conceptual visualization).

the molecular strategies underlying each stage are distinct from those of *S. aureus* and are adapted to a less aggressive, more persistent pathogenic lifestyle [3].

In the adherence phase, *S. epidermidis* encounters host-conditioned biomaterials rapidly coated with plasma and extracellular matrix proteins (e.g., fibrinogen, fibronectin, vitronectin). Initial, reversible attachment is mediated by physicochemical interactions and by surface proteins such as the autolysin (AtlE) and the accumulation-associated protein (Aap), which can bind abiotic surfaces and host components [3, 35]. This early attachment is reinforced by specific adhesins, including the extracellular matrix-binding protein (Embp) and the biofilm-associated homologue (Bhp), which recognize a broad spectrum of host matrix proteins and stabilize colonization on plastic and metal implants [35, 37].

The accumulation stage involves intercellular adhesion and microcolony formation through both ica-dependent and ica-independent routes. In the canonical pathway, the icaADBC operon directs the synthesis of PIA/PNAG, a cationic polymer that bridges neighboring cells, builds dense three-dimensional clusters, and confers resistance to phagocytosis and antibiotics [3, 37]. In parallel, many clinical isolates rely predominantly on proteinaceous and eDNA-rich matrices. The Aap protein, once proteolytically processed, self-associates via its B-repeats to mediate tight cell-cell aggregation; Embp and Bhp likewise contribute to accumulation by forming extended, fibrillar connections within the bacterial community [37]. eDNA, released through autolysis of a subpopulation of cells, serves as a negatively charged scaffold that entangles with proteins and polysaccharides, further stabilizing the growing biofilm [3].

As biofilms mature, *S. epidermidis* develops a heterogeneous, stratified architecture with water channels that permit nutrient and waste exchange. The relative contribution of PIA, proteins, and eDNA to the matrix varies markedly among strains and environments. In nutrient-rich laboratory media lacking host factors, many isolates require PIA to form compact, firmly attached biofilms [35]. By contrast, in “humanized” media containing plasma or serum, *S. epidermidis* can form abundant, loosely attached biofilms and suspended aggregates even when PIA and Embp are absent, indicating that incorporated host proteins can replace self-produced polysaccharides and adhesins as structural scaffolds [35]. These suspended clusters rapidly reach sizes that hinder phagocytosis and exhibit antibiotic tolerance comparable to classical surface-attached biofilms [35].

The dissemination phase involves detachment of single cells or clusters that seed new sites. Protease-mediated processing of Aap and other matrix proteins, modulation of PIA production, and degradation of eDNA by nucleases all contribute to localized weakening of the matrix and release of biofilm fragments [10, 37]. *In vivo*, shear stress from blood flow, device micromovement, and fluctuating host factors (e.g., complement, antibodies) can further promote embolization of aggregates, leading to catheter-related bloodstream infection or spread to secondary foci. Overall, *S. epidermidis* biofilm formation is best viewed as a flexible toolkit that shifts between polysaccharide-dominated, protein/eDNA-dominated, and host-matrix-dominated modes depending on the surrounding conditions [3, 35, 37].

3.2 Regulation

Biofilm formation in *S. epidermidis* is governed by an intricate regulatory network that integrates environmental signals, metabolic status, and host-derived cues.

Although less extensively characterized than in *S. aureus*, several global regulators play pivotal roles, notably SarA, SigB, the agr quorum-sensing system, NOS, and the VraSR two-component system [10, 27, 31].

The SarA in *S. epidermidis* exhibits strain- and matrix-dependent effects. In some clinical isolates, SarA functions as a positive regulator of PIA-mediated biofilms by upregulating the ica operon and directly binding the icaA promoter to enhance transcription [3]. In others, particularly ica-negative or Aap-negative strains, SarA acts as a repressor of protein/eDNA-rich biofilms. Loss of sarA can markedly increase biofilm formation through two mechanisms: elevated expression of Embp, which strengthens protein-dependent accumulation, and increased production of the metalloprotease SepA, which processes the autolysin AtlE, thereby enhancing autolysis and eDNA release [3]. This dual behavior highlights that SarA coordinates the balance between polysaccharide- and protein/eDNA-based strategies in a background-specific manner.

The alternative sigma factor SigB is a major stress-response regulator that generally promotes a biofilm-prone phenotype. SigB upregulates adhesins and icaADBC while repressing the agr system, fostering conditions that favor stable attachment and accumulation, especially under host-like stresses such as osmotic shock, oxidative damage, or nutrient limitation [10, 27].

The agr quorum-sensing system in *S. epidermidis* shares core components with *S. aureus* and similarly links cell density to the expression of surface proteins, exoenzymes, and PSMs. Low agr activity is associated with robust, persistent biofilms, whereas agr activation induces the production of surfactant-like PSMs and proteases that dismantle the matrix and trigger dispersal [3, 10]. Accordingly, agr mutants typically form thick, highly adherent biofilms *in vitro*. However, *in vivo*, agr expression is modulated by host factors, antibiotics, pH, and oxygen, so the timing and extent of dispersal can vary widely across niches [31]. *S. epidermidis* also expresses a bacterial nitric oxide synthase (bNOS), which produces low levels of NO that function as a signaling molecule rather than a simple toxin. Endogenous NO modulates redox homeostasis, influences autolysis and eDNA release, and can promote or inhibit biofilm formation depending on concentration and environmental context [27].

The VraSR two-component system senses cell wall stress (often induced by antibiotics such as glycopeptides or β -lactams) and induces a protective response that includes remodeling of the peptidoglycan and alterations in biofilm behavior. Activation of VraSR can enhance survival in the presence of cell wall-active antibiotics and has been implicated in promoting biofilm resilience, although its precise effects on matrix composition and dispersal remain under investigation [31].

Collectively, these regulators create a layered network in which stress, nutrient limitation, antibiotic exposure, and host conditions can flip *S. epidermidis* between low-matrix, more invasive states, and high-matrix, persistent biofilms. Importantly, findings from reference strains in standard media do not always mirror regulatory wiring in clinical isolates growing in plasma or serum, emphasizing the importance of studying regulation under host-like conditions [3, 35].

3.3 Host–bacterial factors

Host–bacterial interactions profoundly shape *Staphylococcus epidermidis* biofilm initiation, architecture, and antibiotic response. Because this species is a dominant

resident of human skin, it is frequently introduced into the bloodstream or deep tissues during device implantation, where it exploits host components to establish a chronic, low-grade infection. Host immune responses exert a dual influence. On one hand, neutrophils, macrophages, complement, and antimicrobial peptides attempt to clear colonizing bacteria. These attacks can stimulate bacterial autolysis and the release of eDNA, which then becomes incorporated into the biofilm matrix and strengthens structural integrity [3]. Inflammatory exudates supply additional host DNA, actin, and other polymers that interlace with PIA and bacterial proteins. On the other hand, *S. epidermidis* biofilms tend to elicit a muted, anti-inflammatory profile characterized by increased IL-10 and reduced pro-inflammatory cytokines, which dampens effective clearance and promotes long-term persistence on implants [3].

Iron availability is another critical determinant. The host sequesters iron using transferrin, lactoferrin, and other binding proteins as a nutritional immunity strategy. Iron restriction limits *S. epidermidis* growth and can suppress biofilm formation and survival, particularly in strains that rely heavily on iron-dependent metabolic pathways [34, 35]. To counter this, *S. epidermidis* upregulates high-affinity iron acquisition systems and may adjust its matrix composition toward proteinaceous or eDNA-dominated structures that are less metabolically demanding than thick PIA layers. Interactions between iron-responsive regulators and global systems such as *agr* and *SigB* likely fine-tune this adaptation, although these connections are only partly mapped.

Plasma and serum proteins strongly influence both the quantity and quality of *S. epidermidis* biofilms. In the presence of human plasma, bacteria rapidly form large, loosely attached suspended aggregates rather than solely surface-attached films, and these aggregates arise even in mutants lacking PIA or *Embp* [35]. Adsorbed host proteins (including fibrinogen, fibronectin, immunoglobulins, and complement components) become integral parts of the matrix, providing binding sites for surface adhesins and contributing to mechanical cohesion. Remarkably, under these host-like conditions, traditional major adhesins such as PIA and *Embp* become dispensable for aggregate formation, indicating that host factors can essentially replace self-produced matrix components [35]. Despite this shift, aggregates display antibiotic penetration and vancomycin susceptibility profiles similar to PIA-rich biofilms, underscoring that host-augmented matrices can confer comparable protection [35].

Soluble immune effectors, including antibodies and complement, can also reshape biofilm architecture and drug responses. Binding of opsonins may promote localized phagocytosis at the biofilm periphery, leading to the release of additional host DNA and cytoskeletal proteins that integrate into the outer matrix layers. Conversely, sub-inhibitory antibiotic concentrations and inflammatory mediators can activate *sigB*, *VraSR*, or other stress regulators, driving compensatory increases in matrix production and thickening of the biofilm in response to chemotherapy [10, 31, 32].

Finally, environmental factors created by the host, such as pH, osmolarity, oxygen gradients, and nutrient composition, define microenvironments within *S. epidermidis* biofilms. Low oxygen tension and nutrient limitation in deeper layers select for slow-growing or dormant cells with high antibiotic tolerance, while surface-exposed regions experience fluctuating immune attacks and shear forces that favor constant remodeling of adhesins and matrix components [3, 35]. The combined

effect of these host–bacterial interactions is the emergence of *S. epidermidis* biofilms that are exquisitely tailored to device-associated and tissue niches, inherently tolerant to antibiotics, and capable of persisting for months or years with minimal overt inflammation.

4. Comparative analysis of biofilm formation in *S. aureus* and *S. epidermidis*

4.1 Matrix composition and structural organization

A central difference between *S. aureus* and *S. epidermidis* lies in the dominant matrix polymers that stabilize their biofilms. In contrast, *S. aureus* biofilms show a broader and more protein/eDNA-centered architecture. Large surveys of clinical isolates demonstrate that eDNA is present in virtually all *S. aureus* biofilms and that DNase I is the most effective enzyme for both preventing and dispersing massive biofilms, far out-performing PIA-targeting Dispersin B [34]. Proteinase K also markedly inhibits and disrupts the majority of *S. aureus* biofilms, indicating that surface and secreted proteins constitute key load-bearing elements of the matrix [34]. PIA/PNAG contributes substantially in only a minority of *S. aureus* strains, where its importance correlates poorly with overall biofilm robustness [34, 38, 39]. The eDNA not only serves as a ubiquitous electrostatic mesh but also promotes functional amyloid fiber formation from PSMs, further strengthening *S. aureus* biofilm structure and resilience [40, 41].

In *S. epidermidis*, the biofilm matrix is classically polysaccharide-rich, with PIA/PNAG frequently serving as the major structural scaffold. Enzymatic disruption with Dispersin B or sodium metaperiodate selectively collapses these polysaccharide-dominated communities, underscoring the key mechanical role of PIA/PNAG in many clinical isolates [38, 39, 42]. Strong biofilm-producing *S. epidermidis* strains from periprosthetic joint infections are significantly more likely to have polysaccharidic extracellular polymeric substances (EPS), either alone or combined with proteins, and this phenotype is associated with treatment failure [39]. Even when *S. epidermidis* employs alternative, ica-independent strategies, such as proteinaceous or eDNA-rich matrices based on Aap, Bhp, or other proteins, PIA/PNAG remains a hallmark of many high-biofilm, device-associated lineages [38, 42, 43].

Direct enzymatic comparisons highlight these structural contrasts: PNAG-degrading enzymes detach and sensitize *S. epidermidis* biofilms but have little effect on preformed *S. aureus* biofilms, whereas DNase I efficiently detaches *S. aureus* biofilms but not *S. epidermidis*, confirming that PNAG and eDNA occupy fundamentally different structural roles in the two species [38]. Thus, *S. epidermidis* tends toward polysaccharide-dominated, cohesive matrices, while *S. aureus* relies more on eDNA–protein networks and amyloids, with polysaccharides playing a subsidiary or strain-restricted role [34, 38, 40].

4.2 Regulatory networks and phenotypic plasticity

Both *staphylococci* use complex global regulatory systems to control biofilm formation, but the functional emphasis of these networks differs. In *S. aureus*,

quorum-sensing via the agr system orchestrates a switch between adhesive, low-secretory states and dispersal phases characterized by high expression of proteases, PSMs, and nucleases that dismantle protein and eDNA scaffolds [41, 44]. SarA and other regulators favor matrix preservation by sustaining adhesin and ica expression while repressing degradative enzymes, creating a dynamic tension between growth and dispersal that underpins acute–chronic transitions [45, 46].

Staphylococcus epidermidis shares homologous regulators (agr, SarA, SigB, VraSR); yet, these are tuned toward stable, device-associated persistence. SarA not only enhances ica-dependent, PIA-mediated biofilms but also modulates proteinaceous and eDNA-rich matrices by controlling Embp and autolysis, whereas SigB promotes stress-resilient, high-matrix states [6, 42]. The Agr activity in *S. epidermidis* is frequently attenuated or variable in chronic infections, with certain agr specificity groups enriched among device-related isolates, suggesting selection for low-dispersal, high-biofilm phenotypes rather than aggressive toxin production [6, 47]. Small RNAs, such as RsaE, further increase phenotypic heterogeneity by coordinating eDNA release and switching between PIA- and protein-based matrices within the same strain, leading to spatially diversified communities [48].

Overall, *S. aureus* regulatory logic is closely tied to the transition between invasive and sessile lifestyles, whereas in *S. epidermidis*, the same or analogous regulators are biased toward long-term surface colonization, matrix diversification, and stress survival rather than overt virulence [6, 39, 48].

4.3 Host interaction, immune evasion, and clinical manifestations

Differences in matrix composition and regulation translate into distinct host–pathogen interactions and disease patterns. *S. aureus* possesses a rich arsenal of secreted immune-evasion proteins and toxins that enable rapid tissue invasion, intracellular survival, and destruction of neutrophils, making it a leading cause of acute bacteremia, endocarditis, osteoarticular infections, and severe skin and soft tissue diseases [44, 45]. In acute planktonic-dominated infections, *S. aureus* triggers strong pro-inflammatory cytokine and chemokine responses; when it adopts a biofilm lifestyle on implants or tissue, the eDNA–protein–amyloid matrix supports immune evasion and antibiotic tolerance, contributing to chronic, relapsing courses [6, 41, 46].

Staphylococcus epidermidis, by contrast, is a low-toxicity opportunist whose principal virulence factor is its biofilm itself. On orthopedic and other implants, *S. epidermidis* frequently accounts for 20%–30% of device-related infections and up to 50% of late-onset cases [1, 6]. These infections are typically sub-acute, with subtle clinical signs, reflecting limited classic virulence factors but potent biofilm-mediated shielding. PIA/PNAG and related polysaccharides hinder complement deposition and phagocytosis and promote survival in the face of neutrophil attack [6, 49, 50]. Comparative studies even suggest that *S. epidermidis* biofilms are less readily infiltrated and engulfed than *S. aureus* biofilms, despite *S. aureus* possessing more mechanisms to survive inside phagocytes, illustrating different immune-evasion choices. *S. epidermidis* favors exclusion and dampened inflammation, *S. aureus* tolerates contact but subverts killing [6].

Clinically, these strategies underpin a division of labor in pathogenesis. *S. aureus* is the prototypical agent of acute, invasive infections (rapidly progressive osteomyelitis, bacteremia, and destructive endocarditis), while *S. epidermidis*

predominates in chronic, device-related infections such as prosthetic joint infections, catheter sepsis, and late breast implant infections that may smolder for months or years [1, 6, 44]. Epidemiological analyses of osteoarticular infections confirm this dichotomy: *S. aureus* is more frequent overall and associated with community-onset and aggressive courses, whereas coagulase-negative *staphylococci*, particularly *S. epidermidis*, cluster in older patients with prosthetic devices and prior periprosthetic infection [43].

4.4 Therapeutic and diagnostic implications

The contrasting biofilm architectures and clinical profiles of *S. aureus* and *S. epidermidis* have direct implications for prevention and treatment. Because eDNA is the most ubiquitous and functionally central matrix component in *S. aureus*, DNase-based strategies and agents targeting eDNA-stabilizing proteins (such as DNABII-binding antibodies) are promising adjuncts to antibiotics for dispersing *S. aureus* biofilms [34, 41]. Protease-based or anti-amyloid interventions may further destabilize protein-rich and PSM-dependent matrices [34, 40]. For *S. epidermidis*, therapies aimed at disrupting PNAG (e.g., Dispersin B or anti-PNAG antibodies) and interfering with *ica* regulation, as well as blocking Aap/Bhp-mediated aggregation or host protein incorporation, are more rational targets [35, 38, 42].

Genomic and phenotypic profiling shows that strong PIA-rich biofilms in *S. epidermidis* and robust biofilm phenotypes in *S. aureus* correlate with treatment failure in periprosthetic joint infections, while multidrug resistance is particularly problematic in *S. epidermidis* lineages [39]. Consequently, device-related infections by *S. epidermidis* often require both aggressive surgical debridement or implant exchange and tailored antibiotic regimens guided by resistance testing, whereas acute *S. aureus* infections demand early, potent systemic therapy to prevent dissemination [1, 39, 44].

Taken together, *S. aureus* and *S. epidermidis* share a core capacity to form biofilms and colonize implants, yet their matrix composition, regulatory emphasis, immune engagement, and clinical behavior diverge significantly. Recognizing these species-specific patterns is crucial for designing diagnostics, anti-biofilm strategies, and infection-control policies that are appropriately targeted rather than one-size-fits-all.

Abbreviations

<i>S. aureus</i>	<i>Staphylococcus aureus</i>
<i>S. epidermidis</i>	<i>Staphylococcus epidermidis</i>
agr	Accessory gene regulator
SarA	Staphylococcal accessory regulator A
sigB	Sigma factor B
NOS	Nitric oxide synthase
VraSR	Vancomycin-resistance associated sensor/regulator
ica-dependent	Intracellular adhesin-dependent
PIA/PNAG	Polysaccharide intercellular adhesin/ poly-N-acetylglucosamine
eDNA	Extracellular DNA

CDC	Centers for Disease Control and Prevention
MRSA	Methicillin-resistant <i>S. aureus</i>
IL-10	Interleukin-10
MSCRAMMs	Microbial surface components recognizing adhesive matrix molecules
FnBP	Fibronectin-binding proteins
Clf	Clumping factors
Eap	Extracellular adherence protein
PSMs	Phenol-soluble modulins
hECM	Human extracellular matrix
Aap	Accumulation-associated protein
Embp	Extracellular matrix-binding protein
Bhp	Biofilm-associated homologue
EPS	Extracellular polymeric substances

Conflict of Interest

The authors declare no conflict of interest.

AI usage disclosure

During the preparation of this work, the authors used Chat-GPT to improve the readability and language of the chapter. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the published chapter.

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