Chapter

Genetic Diversity in *Staphylococcus aureus* and Its Relation to Biofilm Production

Furqan Awan, Muhammad Muddassir Ali, Muhammad Hassan Mushtaq and Muhammad Ijaz

Abstract

Staphylococcus aureus (*S. aureus*) has been a substantial economic problem due to its antibiotic resistance, persistence inside host and recurrence of disease. It escapes from immunity because of its intra-cellular growth. Moreover, it forms biofilm on both living and in-animate surfaces that leads to recurrent infections and growth in food industry, respectively. Further, *S. aureus* undergoes the vertical and horizontal evolution that has genetically diversified the bacterial population. All the factors such as point mutations, plasmids, phages etc. have played their roles in diversifying this bacterium. Many bacterial physiological characteristics have been affected by genetic diversity. Biofilm forming ability is also considered as a variable characteristic of *S. aureus* that can help the bacteria to survive in different environments with different levels of biofilm production. In adapting the environment, *S. aureus* also forms different types of biofilm for its better survival. How genetic diversity is playing its role in this division of *S. aureus* is yet to be revealed. This chapter focuses on the factors related to genetic diversity and biofilm formation of *S. aureus*.

Keywords: Genetic diversity, Non-synonymous mutations, ica-operon, biofilm production, agr-operon

1. Introduction

Staphylococcus aureus is a mammalian commensal [1] that colonizes mucosal membranes of its hosts around the world. The virulent *S. aureus* strains promote infections by producing potent protein toxins, colonizing factors and cell surface proteins that inactivate antibodies [2]. Contrastingly, genetic diversity in the *S. aureus* causes the variation in disease severity of the clinical strains [3]. This genetic diversity among *S. aureus* population around the world suggests the variation in spatial distribution. The development of different techniques such as multi-locus sequence typing (MLST) [4], Pulsed-field gel electrophoresis (PFGE) [5], and core genome phylogenetic reconstruction [6] have facilitated analysis of the genetic diversity in *S. aureus* population. MLST is commonly used to understand the *S. aureus* lineages [7]. It relies on the allelic profiles of housekeeping genes present throughout the core genome [8]. Previous studies showed that *S. aureus* population structure is composed of limited clonal complexes (CCs) that further comprises of new sequence types (STs) [9]. ST precisely defines a strain with a unique allelic

profile that have descended from the same recent common ancestor. Such ST types indicate the evolution based on point mutations. Additionally, recombination also appears to have played a relatively minor role in shaping *S. aureus* population [10]. Such techniques and studies are well suited to undermine the global epidemiology and genetic diversity of *S. aureus* population [11].

S. aureus infections can be recurred and costs for a long-term treatment along with productivity losses [12]. This recurrence is the result of biofilm formation and persistence inside body. Similarly, *S. aureus* biofilms also poses a major problem in the device-related infections (DRIs) [13]. Biofilms provides a shelter to *S. aureus* that resist antibiotics and other cellular immunity defenses [14]. *S. aureus* biofilms are more potent as it can be formed on the fomites, pipelines in the food industry and on the skin [15]. In this way, biofilms can also act as source of spread for long term without being observed. Recent findings have shown that staphylococcal biofilm mechanisms are adaptable to the environmental changes and help the pathogen in adherence to the surfaces at any cost [12]. Genetic diversity could be one of the influencers among the biofilm production ability of this pathogen [16]. A deep understanding of mechanisms for such variation in biofilm production is yet to be discovered.

Here we will review how diversity has affected the *Staphylococcus aureus* population structure and its biofilm mechanisms.

2. Mechanism of genetic diversity in *S. aureus*

The architecture of *S. aureus* population is mainly based on its genetic markers. Its genome consists of a single chromosome of 2.7–3.1 Mbp [17]; mainly represents the core genome that undergoes the vertical evolution. While accessory genome is dominated by mobile genetic elements (MGE) that include plasmids, transposons, phages and insertion sequences (IS) [18]. Horizontal evolution in MGE is driving the genetic diversity in this fraction of genome. Therefore, diversity in *S. aureus* population include a highly varying accessory/disposable genome with variable distribution of antimicrobial resistance (AMR) genes, virulence factors (VF), sequence types (ST), and clonal complex (CC)-specific pathogenic potentials [19]. The causes of genetic diversity among *S. aureus* strains are: vertical evolution (mutation) [20] and horizontal evolution (transformation, conjugation, transduction, and transposition) [18].

2.1 Vertical evolution and genetic diversity

The majority of *Staphylococcus aureus* population has highly conserved core genome [21, 22] that has evolved mainly through mutations. This conserved core genome can further undergo to single nucleotide polymorphisms and SCV formation. Such mutations are detected by MLST of selected housekeeping genes (*arc, aroE, glpF, gmk, pta, tpi, yqiL*) or through whole-genome sequencing that helps the researchers to identify the phylogenetic relations in *S. aureus* populations. According to the pubmlst database, there are 632,297 allele sequences in the 35,804 isolates. Furthermore, there are total 6569 MLST types divided into 10 clonal complexes including the untypeable clonal complex [4]. However, mutations in core genome points at the continuous evolution of this bacterial pathogen.

Previous studies have mentioned the synonymous and non-synonymous mutations in bacterial genomes as two main types of mutations [23]. Synonymous mutations are mostly less diversifying and cause least impact due to presence of amino acids against different pairs of codons and introduction of amino acid from same groups [24]. Thus, these mutations are considered as mostly harmless for

bacterial physiology. The other mutation type is non-synonymous mutation that causes gene rupturing by introducing a stop codon that further leads to significant changes in bacterial physiology [25]. Among *S. aureus* population, nonsynonymous mutations generate the irreversible small colony variants (SCVs) that play main role in this genetic diversity [26]. Irreversible mutations introduced in such variants are mainly shaped by parallel evolution and are generated due to environmental stress factors such as cationic peptides, oxidative stress, low pH, bacterial competition [27]. These SCVs have attributes of high biofilm formation, antibiotic resistance and low metabolism that reduce the cure rates [16, 28, 29]. Human joint infections, cystic fibrosis in lungs, and bovine chronic mastitis are some common examples [30]. Studying the underlying mechanisms is important to assess the physiological processes and genetic diversity.

Some recent reports have determined that *S. aureus* also undergo genome reduction similar to mycobacterium spp. [31]. Genome reduction is mainly caused by removal of the ruptured genes and pseudo genes that eventually shortens the genome size [31]. A recent example of such genome reduction in *S. aureus* is isolates from ST-228 that are believe to lose 522 genes in their history of evolution [21]. It can be estimated that persistence of *S. aureus* in an environment for very long time causes genome reduction. The possible reason behind this is the least utilization of genes that are not required in that particular environment [31]. In other words, these proteins could have evolved to fulfill specific nonessential innovations and hence could easily be lost in reductive evolutions. Such complex genetic diversity also points at the continuous evolution of this *S. aureus*. A deep understanding of the mechanisms behind this evolution and genetic diversity is required.

2.2 Horizontal evolution and genetic diversity

The accessory genome is highly diverse among *S. aureus* populations [9]. It mainly encodes proteins necessary for bacteria's adaptation to various environmental conditions via resistance genes or virulence factor [14]. Such exogenous genes are often shared by other bacteria/environment therefore containing different rate of G-C in as compared to the core genome [32]. Generally, these exogenous genes can be obtained through one or multiple ways of horizontal evolution such as transformation, conjugation, transduction, and transposition. The mobile genetic elements (MGEs) are responsible for such kind of genes transfer. MGEs prevalent in *S. aureus* population include plasmids, transposable elements, bacteriophages, and pathogenic islands [18]. A deep knowledge of these MGEs and their mobility methods are of great concern for understanding the horizontal evolution.

2.2.1 Plasmids and their role in genetic diversity

Plasmids are small self-replicating DNA molecules (ranging 1–60 kbp) that can be transferred from one bacterium to other [18]. *S. aureus* has three classes of plasmids based on their sizes and other properties. Class I plasmids include small sized (<4.6 kbp) but multicopy plasmids often with a single resistance determinant [33]. Such type of plasmids is never reported to bear transposons or prophages [34]. Class II plasmids are of intermediate size (15–46 kbp) with lesser number of copies as compared to class I [33]. But some of the plasmids included in this class are antibiotic resistance plasmids such as pencillinase and aminoglycoside resistance plasmids [35]. In addition, there are different resistances genes do present on this kind of plasmids. Class III consisted of large and complex plasmids with determinant of transfer (*tra*) by conjugation along with different combinations of resistant markers [36]. Such plasmids also possess few transposons and insertion sequences.

Staphylococcus aureus strains commonly resist against Penicillin and glycopeptides such as vancomycin [15]. The resistance to methicillin is commanded by the *mecA* gene, responsible of the 76 kDa penicillin binding protein (PBP) synthesis. This protein with a low affinity to β-lactams is called PBP 2′ or PBP 2a [37]. The *blaZ* gene encodes for β-lactamase in *S. aureus* strains and both the two adjacent regulatory genes *blaI* (repressor) and *blaR1* (antirepressor) control this gene [38]. There are five different phenotypes of resistance genes (*vanA*, *vanB*, *vanC*, *vanD* and *vanE*) to vancomycin in enterococci [39]. *vanA* and *vanB* resistance operons in the plasmids possess the Tn1546-like and Tn1547 transposon elements [40].

2.2.2 Transposable elements (Tn) and insertion sequences (IS)

The genome of *S. aureus* carries heterogeneous MGE. The mobile genetic elements contain insertion sequences (IS), transposons (Tn), and transposon-like elements [40]. These mobile genetic elements are involved in evolution of bacteria and these can be found on chromosomes as single or multiple copies. MGE can also be found in association of other genetic elements.

IS sequences are the segments of DNA which can be transposed from one site of genome to another [18]. The genetic information required for their transposition is carried by these transposable elements. They are responsible for the recombination and stabilization of some genes which are responsible for resistance, though they do not code for resistance. These IS sequences are responsible for inducing changes in the expression levels of chromosomal genes and thus are very important in the process of evolution of the bacterial genome [41]. IS sequences can affect the transcription of other genes which are nearby, either by direct insertion or by polar effect, in order to inactivate them. IS sequences which also contain some other genes are called as composite transposons i.e. Tn4001 and Tn4003 which are composite transposons are known to contain IS256 and IS257 respectively which mediate resistance to gentamycin (Gmr), kanamycin (Kmr), and tobramycin (Tmr) [18]. IS256 and IS257 on styphylococcal chromosome have been observed in both contiguous and independent form. It suggested that these genetic elements in the genome may have a role in molecular rearrangements. The circular chromosome of *S. aureus* contains two copies of IS257. The recombination of either of IS257s of the plasmid (pJ3356) mediating ertgromycin resistance, in the pOX7 has been observed.

Transoposons present in staphylococcal genome are relatively small and they carry genes for resistance. Tn552 carries 'bla' gene for pencillinase and Tn554 carries gene for resiatance against spectinomycin, erythromycin and mactolide-lincosamide-streptogramin B [18, 40]. These elements are present in staphylococal cassette chromosome, plasmids or on the chromosome in multiple copies. Two copies of transposon 554 (Tn544) are commonly observed in N315, Mu50 and MRSA252 genome, while three addition copies were reported in N315 genome [33]. A unique conjugative transposon i.e., Tn5801 that carries '*tetM*' gene mediating resistance to tetracycline and minocycline was found in Mu50 genome. The single copies of transposons which are larger than 18 kbp are rare to find relatively. They encode genes mediating resistance to tetracycline, trimethoprim, aminoglycosides, or vancomycin. A specific transposon is present on the penicillinase plasmid (pl524) which carries methicillin resistance gene.

2.2.3 Bacteriophages and S. aureus *diversity*

The presence of mobile genetic elements, especially prophages, help to determine the diversity of *S. aureus* species [34]. Both the horizontal and vertical

evolutions are closely linked to phages. In horizontal evolution, the phages being a mobile genetic element can be transferred to the recipient bacterial cell present in the environment. The prophages carry the many accessory genes in their genomes that are responsible for staphylococcal virulence factors and help in the survival of certain *S. aureus* strains [34, 42, 43]. The phages aid in the genomic island induction and its transfer. Additionally, phage transduction also transfers plasmids and chromosomal markers. Phages, in this way, diversify the *S. aureus* population and directs the horizontal evolution.

Currently, *S. aureus* strains isolated from non-human mammals are being sequenced and studied. Such strains have been shown adaptation to different host species through mutations in the core genome and through potential phage-encoded virulence genes [34]. Recent examples are the cattle-associated strains that were shown to originate in humans [43]. Furthermore, isolates from birds were shown to possess Sa3int phages with unique genes [44]. Therefore, phages are believed to be the one of the tools for host-diversification.

The phages are often regarded as selfish elements even though bacteria are utilizing them for their own survival. In this context, lysogeny could only serve as a short-term strategy of evolution. There are many reports indicating that phages provide *S. aureus* with additional genes that allow them to survive and persist. Several genes are the examples of introduction such as Panton-Valentine leukocidin (*lukSF*), exfoliative toxin A (*eta*), cell wall anchored *SasX* protein and the immune evasion group (IEC) composed of enterotoxin S (*mar*), staphylokinase (*sak*), the chemotaxis inhibitor protein (*chp*) and the inhibitor of the staphylococcal complement (*scn*) [45]. Such gene transfers between species and between different strains is limited due to receptor modifications in restriction barrier and phage exclusion. These effects most likely play an important role in species diversification of staphylococci. Hence, deeper insights into phage biology will be beneficial in understanding bacterial evolution.

3. Biofilm formation in *S. aureus* population

Biofilm production in *S. aureus* is comprised of three-steps. In each step, there is distinct bacterial physiology with expression of different sets of genes [46]. These steps can be described as follows: (i) initial attachment; (ii) colonization; and (iii) dispersion [47, 48]. In the initial attachment step, bacterial cells attach to the surfaces (6 h–11 h). This step is characterized by active metabolism of the bacterial cells and higher production of adhesion factors. In maturation step, the biofilm production is increased due to bacterial multiplication (18 h). During this step, metabolically active cells and slow metabolism cells both are present and subject to QS signals gene expression changes. At this step, persister cells can be found here. In the third and last step, upon finding the favorable conditions, the metabolically active cells separate from the colonies and begin to function as free cells [49]. Gene expression changes also force the bacteria to decrease the biofilm production [50]. Biofilm production in *S. aureus* is a complex phenomenon that secures this pathogen from environmental stress factors.

3.1 Role of outer surface proteins

Outer surface proteins play a very important role in initial adhesion and helps bacteria to adhere any surface; playing an important part in beginning of biofilm formation. Previous studies have focused on human isolated bacteria and whole

proteome comparison of biofilm and planktonic states of *S. aureus* [47, 51]. But recent studies have shown that MRSA can also form a varying form of protein based biofilm that is not present in other *S. aureus* bacteria [52]. This difference includes the biofilm components, outer surface protein expression and encoding operons. For example, *S. aureus* can produce two types of biofilms (i) ica-operon dependant/ Polysaccharide intercellular adhesion-based biofilm (ii) ica-operon independent biofilm [13, 53]. Ica-operon independent biofilm is important for persistence in Hospital Associated infections (HA-MRSA) that is structurally different to the former type of biofilm. Hence drugs designed for the former type of biofilm might be not suitable for this type. This implicates that the drugs designed for other *S. aureus* biofilms will not be effective for native or highly antibiotic resistant strains.

Surface proteins are mainly classified into structural based classified groups (i) microbial surface component recognizing adhesive matrix molecule (MSCRAMM) (ii) Near iron transporter (NEAT) motif proteins. (iii) Three-helical bundle proteins (iv) G5–E repeat family (v) Structurally uncharacterized proteins [54]. Among these the last group is least studied and has potentially important proteins such as biofilm associated protein (bap). In this group, SasL and SasD proteins are also included that are expected to have important role in pathogenicity and biofilm formation [55]. But there are still no studies regarding gene mutation and characterization. Furthermore, all the proteins in this fraction are never studied for their role in ica-independent biofilm formation.

3.2 Quorum-sensing regulation system and biofilm formation

In Staphylococci spp., accessory gene regulator (agr) system acts as a main quorum sensing (QS) regulating system. Another QS regulation system i.e., luxS regulating system is also present but its role is less significant in the physiology of this bacteria [56]. Autoinducing peptide (AIPs) forms basis of agr-mediated QS system and acts as main signal peptides that regulates the biofilm formation and virulence. The main functions of agr mediated QS system are to sense the bacterial cell density in the surrounding environment and to respond with genetic adaptations.

S. aureus possess four main genes in the agr-operon such as agrA, agrB, agrC, agrD and divergent transcriptional units, such as RNAII and RNAIII, with promoter-2 (P2) and promoter-3 (P3), respectively. agrD gene in this operon produces a small oligopeptide that further undergoes maturation and transported in extracellular environment via agrB [57, 58]. These mature oligopeptides act as AIs in the extracellular environments. After reaching a certain threshold value, these AIs interacts with extracellular segment of histidine kinase, agrC. This agrC acts as a transmembrane receptor which activates the kinase leading to phosphorylation of agrA response regulator; resulting in the expression of biofilm related genes [59]. This activated agrA regulates the promoters P2 and P3 that further activates or deactivates transcriptional units.

It has been determined to maintain a balance between production of virulence factors and biofilm formation. The *agr* based QS system plays a major role in the dispersion step of biofilm formation [60]. Because agr system activation supports the free-living and more mobile lifestyle. On the other hand, its deactivation supports the colonization and sessile lifestyle. Therefore, *agr* mutants are shown to form a higher biofilm production as compared to the wild type. As mentioned, this increased biofilm production and thickness is associated with the inability of cells in dispersion from the mature biofilm. Thus production of factors that stops the bacteria to enter into mobile phase and not due to cell growth or death [61]. However, this agr-QS system needs a deep understanding of pathways and mechanisms.

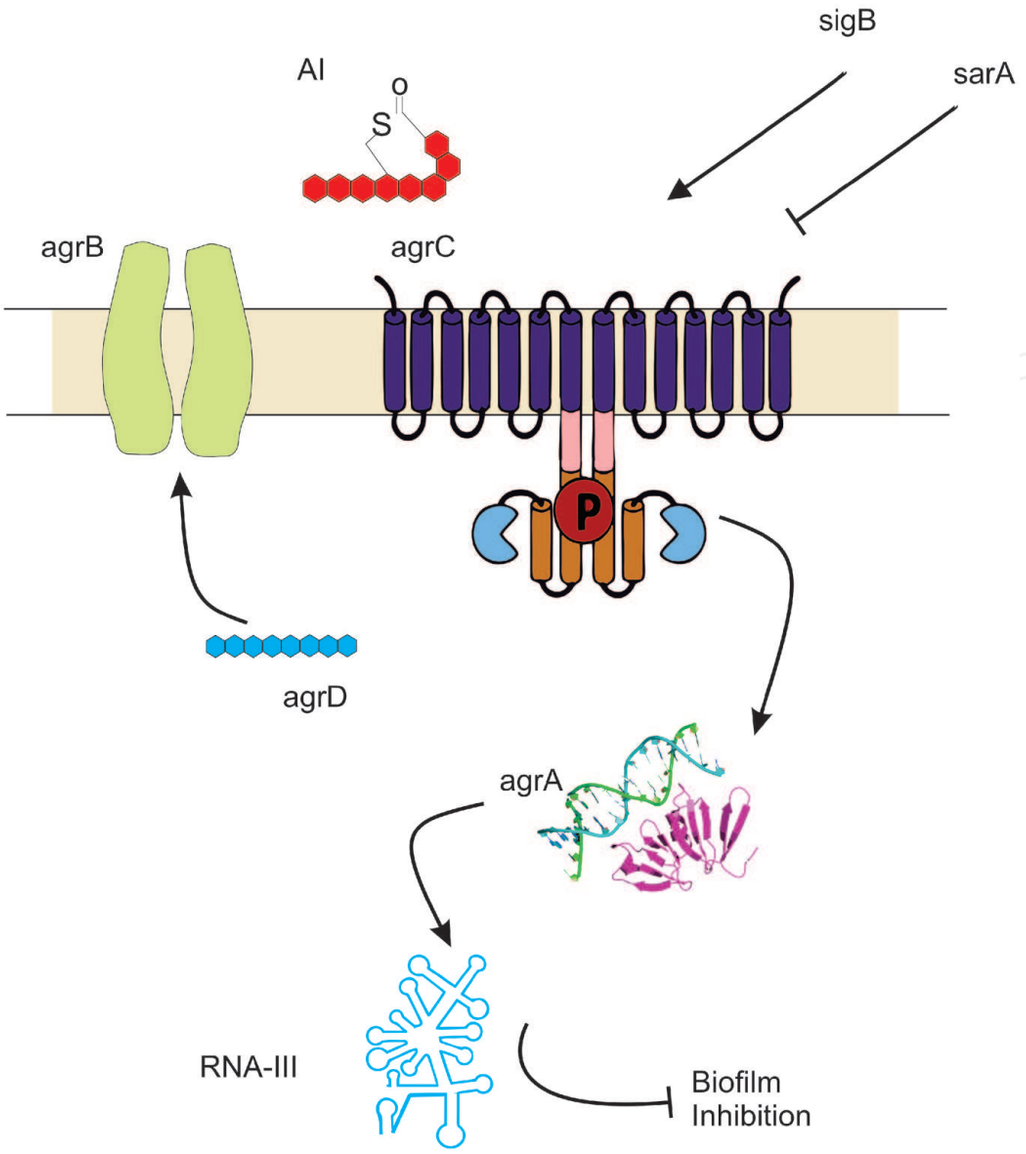

Figure 1.
Regulatory networks in biofilm formation. Sigma factor B (SigB) inhibits agr expression, while SarA has been shown to directly enhance it [62].

3.2.1 Role of alternative sigma B (sigB) operon and agr operon

Alternative sigma B (sigB) factor-regulated genes include those involved in general stress response, virulence, capsule formation, and biofilm formation (**Figure 1**) [62]. sigB operon is composed of *rsbU, rsbV, rsbW,* and *sigB* genes. It represses the agr operon that is important in depressing the biofilm production. Disruption of any gene from this operon could result in mal-function and enhance the biofilm production. Recently, this operon found to be playing an important role in counterfeiting the oxidation stress in *S. aureus* that are very important risk factors for mastitis infections.

4. How genetic diversity affects the biofilm production

Biofilm forming ability is a variable characteristic of Staph aureus that can categorize the bacteria into different categories such as level of biofilm formation, certain STs with high biofilm formation and types of biofilm formation (discussed earlier).

4.1 Relation of MLST with biofilm production

There is a proposal that genetic diversity could affect the biofilm production. A recent study has demonstrated that MLST types such as ST59 and ST188 isolated from human and canine sources were found to be associated with strong biofilm production [15]. This shows that the biofilm production capacity is strongly affected by evolutionary process that changed the biofilm production among different strain types. Parallel evolution could vary the biofilm production by introducing new mutations. But the genes and pathways in specific sequence types related to biofilm production affected by parallel evolution are not well understood. Further studies are underway to reveal this relation. As discussed previously, parallel evolution could help in emergence of new strains but its relation to biofilm production is still unknown.

4.2 Level of biofilm formation

Level of biofilm formation is another complex mechanism that shows the diversity among the strains and within the member of strains [46]. There are multiple estimations that can explain these variations [46, 50]. But most importantly these variations in expression of genes are associated with the environmental signals [63]. For instance, some bacterial cells in same colony can produce PNAG to capture water [13]. On the other hand, some pathways like c-di-AMP respond to external environmental chemicals such as glucose and drop in biofilm formation is measured [64]. Biofilm formation and eDNA release from bacterial cells are triggered by significant reduction in c-di-AMP levels and this reduction is related to low *agr* operon expression [65]. Importantly, *gdpP, xdrA* and *apt* genes also play important role in biofilm formation [66]. Although this pathway shifting is notified but environmental factors that drive this reduction in agr operon expression are still under study.

4.3 Types of biofilm

S. aureus biofilms can be classified as ica-dependent and ica-independent based on their matrix composition. Biofilm matrix composition in ica-dependent biofilms is synthesized by the icaADBC operon that is composed of polysaccharide intercellular adhesion (PIA) or polymeric N-acetylglucosamine (PNAG). On the other hand, ica-independent biofilms are further consisting of three types of biofilms based on their biofilm matrix. Protein/e-DNA biofilm, Fibrin biofilm, and Amyloid biofilm are included in this ica-independent classification (**Table 1**). There is an interesting comparison of biofilm types among MSSA and MRSA isolates also exists. It was reported that ica-dependant biofilm was more common in MSSA while ica-independent biofilms were more frequently observed among MRSA isolates [67]. It is possible that multiple types are present at same place [47]. *S. aureus* biofilms can be found everywhere in body after inoculation. These biofilms could of different types with different EPS, places of origin, and genes/operon controlling them.

5. Role of biofilm environment itself in SCV generation

Biofilm acts like a micro-environment with its own conditions and stressors. There are many studies demonstrated that chronic cases with biofilm formation for a certain period of time also cause the mutation in genomes via natural selection or parallel evolution [68–71]. This reshaping of genome could result in non-synonymous mutations or shortening of genome. In chronic mastitis, *S. aureus*

Characteristics	Polysaccharide-type biofilm	Protein/e-DNA biofilm	Fibrin biofilm	Amyloid biofilm	References
Extracellular Polymeric Substance (EPS)	Poly-N-acetylglucosamine (PNAG)/ Polysaccharide intercellular adhesin (PIA)	Autolysin-mediated release of cytoplasmic proteins and extracellular DNA	Coagulase-mediated fibrin production	Phenol-soluble modules and amyloid accumulation	[10, 64]
Gene/operon	Intracellular adhesion (icaADBC) operon	Surface proteins i.e. Bap, FnBPs, Aap/SasG etc.	Coagulase gene (*coa*) and von Willebrand factor binding protein (*vWbp*)	*psmα1-4*, *psmβ1-2*, *pmt* operon, SaeRS-two component system	[43, 48]
Location	Skin with higher NaCl concentrations and lower water availability	Low pH regions (e.g., urinary tract, vagina, mouth, and skin)	Inside blood or regions with fibrinogen	Iron- and nutrient-limiting conditions in blood.	[10, 65]

Table 1.
Comparison of different types of S. aureus *biofilms. Polysaccharide type biofilm is only considered as ica-dependent biofilm while all the remaining are considered as ica-independent biofilms.*

also form biofilm and remains sub-clinical for very long time that could be helpful in causing non-synonymous mutations. Non-synonymous mutations often also involve the introduction of stop codons that disrupt the gene leading to non-functional or pseudogene formation. Loss in gene function irreversibly changes the phenotype of bacterium. This newly formed phenotype could be more antibiotic resistant, highly biofilm forming or reduced metabolic form of persisters [72–74]. This phenotypic variation should be considered during therapeutic developments and treatment regimes. Hence, it is necessary to study and mimic those conditions to understand which genes undergo mutation formation.

6. Role of SCVs in persistence

Biofilm formation helps the *S. aureus* to persist and multiply sub-clinically in inhospitable environment. As mentioned earlier, the Small Colony Variants (SCV) phenotype are found potentially responsible for the sub-clinical and chronic infections. Such SCVs phenotypes share some common features of slow-growth and quasi-dormancy with low virulence potential [75]. SCVs further express some distinctive features such as small colony formation, a dormant metabolism, less enzymatic activities, and elevated antibiotic resistance [76]. Such SCVs are mostly point mutations in the important genes. Therefore, during proof-reading mechanisms, SCVs can be return to a wild-type (WT) or converted to a different phenotype. Later, clinically observed phenotypes are stable and permanent genetic changes showing irreversible SCVs. Such irreversible SCVs are examples of parallel evolution or evolution with-in population [77]. External environmental stress factors can also trigger the emergence of SCVs such as reactive oxygen species, low pH, cationic peptides, limited nutrition and bacterial biofilm competition [78, 79].

SCVs can be generated spontaneously under any sub-clinical and chronic disease condition. Considering bovine mastitis as an example, SCVs will be discussed now. The detection of mastitis origin SCVs, especially permanent genetic changes within population, in routine laboratories and their accurate studies in research laboratories are challenges not overcome yet. Among these mastitis studies, such isolates were also found positive for biofilm producing genes i.e., ica operon, adhesive proteins, bap operon [80]. According to a study based on different food samples, approximately 72% of the isolates produced biofilms. As discussed above, biofilm producing *S. aureus* are really important in chronic and sub-clinical mastitis infection. Moreover, a few studies have also studied the SCVs formed and found that SCVs formed can cause different level of mastitis based on their severity. Another study has also pointed out the isolation of *S. aureus* irreversible mutation variant from dairy cows in Yunnan province that was responsible for chronic mastitis [26]. This mutation was found in thymine related pathway that promotes the resistance against Sulfamethoxazole (SXT) and helps the bacterium to develop in fibrotic conditions. Similarly, a Beijing based study described the slow growth, antibiotic resistance and chronic mastitis as features of isolated irreversible thymidine SCV [81]. Most of the studies have focused on the antibiotic resistance profiles of *S. aureus* isolated from mastitis infection. On the other hand, there are very few studies that determined the SCVs and relation of chronic sub-clinical mastitis. Additionally, in Austria, a study related to chronic mastitis also revealed that irreversible mutations in rsbU, one of sigB genes, generated from SCVs caused the bacterium to persist and resist the therapy [16]. Further, SCVs related to regulatory circuits have also been revealed such as agr genes, hemin (hemB), menadione (menD), α-Toxin (hla), γ-Hemolysin (hld), Coagulase (coa), L-lactose dehydrogenase (ldh), Alcohol dehydrogenase (adh), Arginine deiminase (arcA), Capsular biosynthesis (capA) and Alkaline shock Proteins (Asp) [82]. Some experimental studies have reported the induction of SCVs by growing *S. aureus* with antimicrobial peptides, and magnesium ions (Mg^{+2}). Further these studies have also mentioned the need of in vivo experiments for complete understanding. This indicates that there is lack of animal experiments based comprehensive studies explaining the factors of this within population and parallel evolution.

7. Conclusions

Genetic diversity can generate new strains and ST types that behave like a different bacterium. Both horizontal and vertical evolutions are the ways to genetic diversity that can help the *S. aureus* to survive under various environments. Biofilm formation ability is also affected by the genetic diversity and can help our pathogen in not only surviving but also in pathogenesis. Plasmids, bacteriophages, Tn and IS elements are much more faster ways of evolution as compared to SCVs and point mutations. SCVs generation could be a slow phenomenon but once these are generated their characteristics can change the behavior of *S. aureus*. Understanding these mechanisms underlying these evolutions could help us in designing suitable strategies and anti-biofilm therapies against *S. aureus*.

Conflict of interest

The authors declare no conflict of interest.

Author details

Furqan Awan[1*], Muhammad Muddassir Ali[2], Muhammad Hassan Mushtaq[1] and Muhammad Ijaz[3]

1 Department of Epidemiology and Public Health, University of Veterinary and Animal Sciences, Lahore, Pakistan

2 Institute of Biochemistry and Biotechnology, University of Veterinary and Animal Sciences, Lahore, Pakistan

3 Department of Clinical Medicine and Surgery, University of Veterinary and Animal Sciences, Lahore, Pakistan

*Address all correspondence to: furqanvet@gmail.com

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