Chapter

Staphylococcus aureus and Virulence-Related Small RNA

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Abstract

Staphylococcus aureus causes a wide range of diseases, including both community-associated and hospital-acquired infections such as abscesses, wound infections, osteomyelitis, endocarditis and septicemia. Regulation of the expression of various virulence factors is initiated through complex coordination between two-component systems, transcriptional regulatory proteins and regulatory small RNAs (sRNAs). *S.aureus* uses many sRNA and RNA–RNA interactions mediated the regulation of the expression of genes post-transcriptionally, but it uses few sigma factors to initiate the transcription function. sRNA transcripts are encoded within intergenic regions or in antisense orientation to mRNA transcripts, and sRNA regulation plays a central role in the response to stress stimuli encountered by pathogens during infection. One of the most intriguing examples of sRNA-mediated post-transcriptional regulation is RNAIII from *S.aureus*, which interacts with and regulates various RNA targets involved in virulence. Several genes known to be regulated by RNAIII have been demonstrated to be regulated by the *sarA* locus, independent of its effect on the expression of RNAIII. We discuss the potential role of small RNA (sRNA) in the pathogenesis and virulence factors production of *Staphylococcus aureus*.

Keywords: *Staphylococcus aureus*, regulatory small RNA (sRNA), virulence factors, RNA–RNA interactions

1. Introduction

Staphylococcus aureus is a human symbiotic microorganism that commonly colonizes in the anterior nasal regions and on the skin surface for 20–25% of the world population [1–3]. The distribution of multi-drug resistant strains among asymptomatic individuals is responsible for spreading the infections among the population very quickly [4]. Among the human populations, the carriage percentages of *Staphylococcus aureus* vary based on different factors. Broadly human carriers are classified into three categories: 20% non-carriers, 25% persistent carriers, and 60% population are intermediate carriers [5]. Usually, *Staphylococcus aureus* forms colonization in the nasal passage and axillae in humans and found its occurrence as flora in vaginal tracts and digestive tracts [6].

Among the various factors responsible for the regulation of virulence, small RNA (sRNA) has a major role in determining the virulence of the bacteria. sRNA are short 50–250 nucleotide long transcripts involving bacterial gene expression

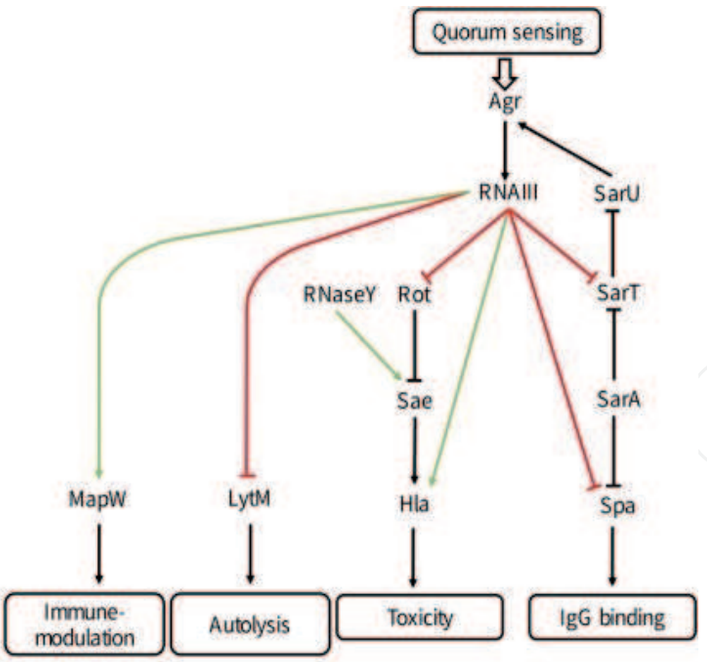

Figure 1.
Virulence factors regulation by RNAIII. RNAIII is regulated positively by the quorum-sensing agr operon. Post-transcriptional regulation is marked with colored lines, whereas up-regulation represented by green arrows and down-regulation by red cross bars. RNAIII in turn, positively regulates MapW and hla at a post-transcriptional level. MapW and hla prevents leucocyte attachment and promote dissemination by lysing host cells. RNAIII also negatively regulates LytM, rot, Spa and SarT, which will promote autolysis via LytM, blood cell toxicity by rot and expression of an IgG binding protein via SarT.

for rapid adaption to stress conditions. Small RNAs play a major role by pairing with bases of target mRNA or by interacting with the modulating proteins for both the positive and negative mechanism of biofilm formation. *Staphylococcus aureus* becomes more adherent resulting in increased biofilm formation when *agr* mediated mechanism is inhibited. Regulation of gene expression mediated by sRNAs is more beneficial when compared to proteins during a rapid response because it takes a short time for sRNAs to either synthesize or degrade. Various regulatory mechanism of sRNAs is similar to the regulation of quorum sensing in the bacteria. The quorum-sensing mechanism regulates the expression of virulence-related genes. Since the quorum sensing mechanism controls bacteria's virulence factor, it is considered a major target for finding out the new therapeutic methods [7]. We discuss the potential role of small RNA (sRNA) in the pathogenesis and virulence factors production of *Staphylococcus aureus* (**Figure 1**).

2. Bacterial small RNA

sRNAs mediate the regulation of mRNAs through direct binding interactions between the sRNA and the target. The sRNA usually binds to the 5′ end of the mRNA and blocks ribosomes binding. Although sRNAs often stimulate degradation of the target as well [8]. The interaction is initiated by a short sequence of perfect complementarity between the sRNA and target termed the seed region. Seed regions are generally 6–8 nt long, and a single sRNA can have one seed region that regulates all of its targets or multiple seed regions that each regulate a subset of targets. Additionally, seed regions are highly conserved, and mutations to the seed region lead to complete abrogation of target regulation. In order to facilitate intermolecular interactions with target mRNAs, seed regions are usually single-stranded in the folded sRNA and disruption of the sRNA secondary structure can drastically

reduce sRNA function. However, seed regions alone are generally not sufficient to mediate target binding, most sRNA characterized to date rely on the assistance of an sRNA chaperone protein [9, 10].

The regulation mechanism for the SpoVG and SprX and their targets with interactions have been discussed below, which involves regulating virulence factors. Production of capsule, virulence factors, and the cell wall's metabolism is regulated by a transcription factor *SpoVG* also called a master regulator. It is also responsible for resistance against methicillin and glycopeptide antibiotics [11]. Synthesis of pentaglycine crosslinks between peptidoglycan strands carried out by *lytSR* operon and glycine glycyl transferases is positively regulated by SpoVG, whereas murein hydrolysate lytN regulated negatively. Base pairing of *sprX* (Highly conserved RNA) with the *SpoVG* mRNA during the translation process prevents loading of the ribosome. Small RNA *sprX* negatively regulated *SpoVG*, in four phases of exponential growth (lag, log, linear and late phase), but it decreases during the stationary growth phase. The SpoVG dependent process increases glycopeptides susceptibility and disrupts the cell membrane metabolism and other independent *SpoVG* mechanisms [12].

SpoVG and *SprX* both seem to contain extra regulatory targets, and antibiotic susceptibility through the *SprX*-dependant mechanism can be bound to susceptibility tied to extra phenotypic advantages that continue to be studied [13]. Various strains of *S.aureus* additionally show an antibiotic determination-related phenotypic variation called as small colony variants (SCVs), which stimulates the biofilm formation and reduced sensitivity to aminoglycosides [14]. The different variation form of small colony variants (SCV) of *S. aureus* has disturbed the expression of 18 sRNAs and increased regulation of the *RsaA*, which is a *sigB* dependent small RNA [15]. However, the network link among small colony variants, antibiotic resistance, and expression profiling of sRNAs has now no longer, but been delineated, those outcomes infer that regulatory sRNAs make contributions to antibiotic resistance through phenotypic maintenance of small colony variants [16].

2.1 RNAIII

RNAIII regulates the expression of genes encoding exoproteins and cell wall associated in *Staphylococcus aureus*. In the quorum sensing mechanism, RNAIII transcribed from the P3 operon, acts as an initiator for agr system in *Staphylococcus aureus*. It also contains the hld gene (delta haemolysin), which is 26 amino acid long sequences. By blocking the translation of transcription factor rot, it regulates its expression. It has been reported that binding of RNAIII with mRNA of transcriptional factor rot in an antisense manner, thus blocking the Shine-Dalgarno sequence [17, 18].

2.2 Teg49

It is a small RNA found in the extended promoter region of sarA, which is an accessory regulator of Staphylococcus bacteria. Confirmation and identification were performed by Northern blotting and RNA-sequencing method. Modulation of the expression of SarA it regulates the virulence factor of *Staphylococcus aureus* [19].

2.3 SprF1-SprG1

There are two types of Toxin-Antitoxin system, whereas Type I has two sub-type types, and Type II has three sub-types but remains uncharacterized. The first type I TA system was SprA1-SprAAS in, which the former denotes the toxin and the latter denotes the antitoxin in *Staphylococcus aureus.* Inhibition of translation and cell wall

damage is done by toxin SprA1, a short peptide. txpA-ratA family, also referred to as SprF1-SprG1, is the second type I TA system, whereas SprG1 belongs to pore-forming toxin and antitoxin are SprF1. SprG1 consists of 44 amino acid sequence long peptide and 31 amino acid sequence of short peptide and they have cytotoxic properties [20].

It has been reported that a small RNA, which expresses from pathogenicity islands of SprD upon binding with antisense base pairing of sbi mRNA (encoding an immunoglobulin binding protein) will lead to an impaired host immune response [21]. Besides direct base-pairing with target mRNA, several other mechanisms, including dual-function sRNA that acts as an antisense molecule and codes for a small peptide (e.g., Hld in RNAIII), have been proposed to act on the same or other pathway genes, and also riboswitches that exhibit a structured receptor domain specifically recognized by a small molecule or metabolite [22].

3. Multifunctional small RNA couples QS to virulence

Regulation of virulence factors through quorum sensing mechanism involves the agr mediated pathway and the two-component system. RNAIII plays a major role in regulating the agr dependent transcriptional regulation in MRSA (Methicillin-Resistant *Staphylococcus aureus*). The significance of agr mediated regulations of *S. aureus* pathogenesis is the situation of an obvious paradox. By comparing with different *S. aureus* sRNAs, that has been discovered through bioinformatic strategies or RNA sequencing, RNAIII was the first predicted sRNA in transposon mutagenesis, which defines the epistasis outcomes for a point insertion [23]. The primary factor for virulence is agr and RNAIII, its effector molecule involved in producing virulence factors. Clinically isolated strains from acute infections of *S.aureus* have both virulence factor regulation through agr mediated along with RNAIII involvement. However, mutated strains of agr mediated pathway, which arose at some point of infection, also have been isolated from patients [24].

RNAIII as an effector regulates the expression of important virulence genes, including proteins associated with cell wall metabolism and exotoxins. Also involved in the expression of two-component systems, different global regulators such as *arl*, *sae*, *srr*, *rot* and other mechanisms in the formation of biofilm, synthesis of amino acid and peptidoglycan [25]. These factors vary quantitatively but not qualitatively in different *staphylococcal* strains.

Compared to UAMS-I (Virulent oxacillin susceptible clinical isolates) strain, the agr inactivation effect was observed more in the transcriptome of the *NCTC 8325* strain [26], but whether it exerts direct or indirect effect was studied only in certain genes from the structural prediction of RNAIII. Structurally *RNAIII* comprises 14 loop and two long helices aligned through the long-range base pairing, which blocked off self-reliant structural domains [27]. Some particular site-defined *RNAIII* domains are responsible for the regulation of various targets. The secondary structure of intramolecular RNA removes the hla ribosomal binding sites upon directly competing with the 59 ends of *RNAIII,* which positively induces translation of hla and alpha-hemolysin (**Figure 2**) [29, 30].

Production of various virulence determinants such as coagulase, protein A, and the rot (repressor of toxins) are repressed with minor variations by conserved regions or domains at the post-transcriptional level. These are repressed either individually or in combination by the H13 *RNAIII* hairpin and H14 terminator of the 39 domain, and central domain hairpin H7. The mechanism behind the repression of these virulence factors by the *RNAIII* is mediated through repression of the initiation process in translation mechanisms wherein the degradation of mRNA is initiated by *RNAIII* [31].

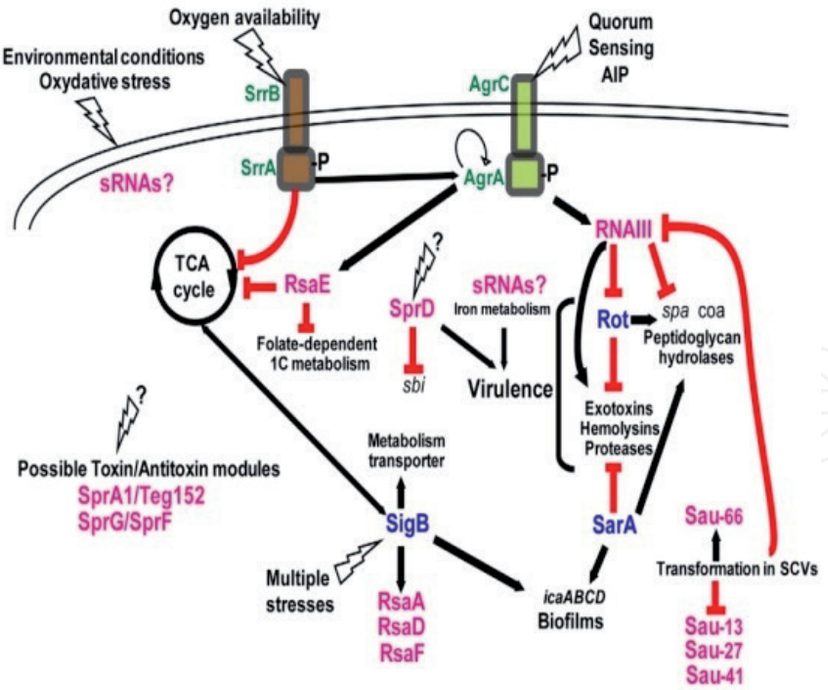

Figure 2.
Integration of sRNAs into gene cascades regulation. The "agr-RNAIII" auto activation circuits is indicated with two feed-forward loops involving RNAIII. The autoinducing peptide (AIP) activates the agr autocatalytic circuit, leading to RNAIII transcription on attaining optimal cell density. RNAIII represses the expression of rot, which activates spa transcription and represses that of hla. RNAIII also activates hla mRNA translation and represses spa mRNA translation. The white and broken lines indicate the direct or indirect gene activations. The red lines represent the down regulations through different RNAs. The black question marks above the see-sawing triangles point to the unknown triggering factors. The transcriptional regulatory proteins are in blue [28].

Complex structure was dependent on their target mRNA and included two factors (i) presence of an extended duplex between the mRNA of Ribosomal Binding Site (RBS) and *RNAIII* and (ii) an imperfect duplex which removes the finished RBS by the interaction between the loops in the coding region [32]. In the above two factors, an individual interaction between the loops is not enough for complete repression, accordingly proscribing the capability of *RNAIII* to behave as a repressor to the mRNA targets. Hence it will not have Shine Dalgarno (SD) series complementary to H7, H13, or H14 of RNAIII, however, it still show a further vicinity of communication or the potential to produce prolonged duplication. *Hfq* is an RNA binding protein and an important chaperone present in different staphylococcus species, but it does not play a role in the *RNAIII* dependent regulatory mechanisms. Whereas in the in-vitro assay, it binds to the *RNAIII* [33, 34].

The repression of all the target mRNA is carried out by the direct effects of *RNAIII* except the translational initiation of hla protein. The repression of Rot (a transcriptional regulatory protein) by RNAIII leads to indirectly regulating transcription for several genes, particularly the protein A repression and the alpha-toxin activation [35].

4. sRNA dependent mechanism of antibiotic resistance

Small RNAs play a major role in altering bacterial cell wall and hence would contribute to the antimicrobial-resistance mechanism. Small RNAs are present prominently on mobile genetic elements on which the resistance pattern for the AMR pathogens is found. SmallRNAs do not exert direct regulation on the resistance gene expression [36]. For example, Fudoh, a regulatory RNA present in

Staphylococcal species is encoded by the SCC mec family of methicillin resistance cassettes. SCCmec is a mobile element that is responsible for the antimicrobial resistivity of methicillin-resistant *Staphylococcus aureus* (MRSA). It also involves regulating the cell distribution process and the expression of alpha phenol soluble modulins, a catalytic peptide [28].

However, the resistance pattern of methicillin through fudoh is still not known. Regulatory small RNA is responsible for the expression of intrinsic antibiotic resistance and tolerance in different bacterial species. Since only some of the small RNA related research has been performed on the clinical strains, whereas most of the studies for RNA-dependent intrinsic antibiotic resistance were performed on the AMR-related pathogens [37].

5. sRNA and stress responses

Specific mechanisms and certain sRNAs involvement regulate the expression pattern of virulence factors under different stress conditions. Small RNA regulation can produce an immediate action to regulatory networks adapted to the acute stress induced by antibiotics. Emergency responders are referred to as Class I small RNAs because they enhance rapid stress responses and aids co-operative degradation of different mRNA targets. Class I sRNAs act in direct mode on the pre-existing mRNA clusters to alter the translation process or deterioration for the acute stress response. Mostly they are involved in disassociating the regulation of transcriptional responses and half-life kinetics of mRNA [38].

It has been reported that during the host infection, variations of temperature and pH, oxidative stress, quorum sensing, biofilm formation and nutrient starvation were related to the functional regulation of small RNAs in *Staphylococcus aureus* [39]. Such responses were controlled by alternative sB (sigma B factor). sB factor regulates several genes that regulate stress-mediated responses, biofilm formation, virulence factor expression, antibiotic resistance, and membrane transport mechanism [40].

Sigma B factor also represses several genes expression by an indirect pathway with the involvement of small RNA or sB-induced regulatory protein. RsaA has a typical sigma B factor promoter which detects its corresponding genes [41]. RsaA base pair with mRNAs repressed by sB like citM and involves in the encoding of Mg-citrate transport systems. sB-dependent sRNAs are the most conserved regions in *S.aureus*. It has been reported that among the three dependent sRNAs, two of them are expected to involve in the regulation of small, highly basic peptides [42].

Production of virulence factors has been regulated by sigma B factor under the stress-dependent activation process. SigB gets activated in the normal stress conditions, also during the growth phase transitions and in different physiological and biochemical changes in *S.aureus* [43]. Thus playing a major role for regulating several others downstream genes. Whereas rsaA are also regulated by a Sigma B-dependent promoter [44].

6. Regulatory sRNA network

Several sRNAs uses Hfq or ProQ chaperones to anneal with their respective mRNAs targets. *Hfq*, a RNA chaperone comprises a six-ring hexamer fostering annealing of RNAs by aligning to their distal and proximal surfaces [45]. The major function of small RNAs regulation is the suppression by base-pairing with the mRNA RBS to inhibit the initiation of translation. sRNA binding blocks binding of small ribosomal subunit [46]. They also regulate both positively and negatively

various mechanisms involved in regulating gene expression [47]. The different mechanism includes the processing and stability of transcript process [48], transporting and localization of ribosomes, antisense sponging interactions and termination of transcription process [49, 50].

It has been reported that both small RNA and transcriptional mechanisms work together within interleaved feedback and feed-forward loops and regulate the expression of genes. 108 sRNAs were identified using RNA-seq analysis in the model organism *E.coli* [51], and similarly, around 1600–1900 sRNA-mRNA interactions were identified using interactome profiling analysis [52].

Therefore, it is hardly comparable with the 3446 sRNA-mRNA interactions being regulated by the 217 transcription factors with the chromosome [53]. Several transcriptional regulatory networks have an sRNA that integrates with the extra post-transcriptional networks. Small RNAs act similar to transcription factors as a regulatory centre and unevenly controls various RNAs targets. sRNAs are involved in antibiotic sensitivity by mRNAs interactions which take place in drug import, efflux pump regulation, cell membrane synthesis and enhancing antibiotic resistance pattern [54, 55].

7. sRNA expressions in infections

In *S.aureus*, some of the known sRNAs with their targets involve regulating major biochemical pathways that are further responsible for producing virulence factors [56]. *Staphylococcus aureus* sRNAs were identified using different techniques in various strains, and their expression profiling during the course of infections in humans was studied. Functions of around 250 sRNAs expressed under different conditions are yet unknown. But the expression profiling of *RNAIII* in clinical isolates from nasal cystic fibrosis patients was studied. In most of the clinical isolates of acute infections, *RNAIII* has been expressed in in-vivo conditions [57, 58]. Therefore these data infers that *RNAIII* majorly involves in the regulation of virulence factors and production of *agr*-defective mutants [59]. However, there has been a difference in the variation of agr-defective mutants in healthy and infected patients. Thus, agr regulation occurs during acute infections, whereas the agr mutants expression can only be observed during the stages of the chronic or dormant infection [60].

Expression profiling of five different small RNAs like *RNAIII*, *RsaE*, *RsaH*, *RsaG* and *RsaA* in *S. aureus* strains isolated from three conditions, including cutaneous infections, chronic cystic fibrosis and commensal nasal colonization [61]. Expression patterns of five small RNAs were strain-specific and do not have any correlations with respect to the variations of the infectivity pattern or colonization. However, it has been observed that there was a uniform expression pattern among the commensal strains in comparison to the infectious strains. Therefore, these results show that *S.aureus* was mainly a commensal strain and became an opportunistic pathogen [62, 63].

S. aureus regulatory RNA, SSR42, which modulates the expression of approximately 80 mRNA species, including several virulence factors, in *S.aureus* strains UAMS-1 and USA300 (LAC) during stationary-phase growth. Mutagenesis studies revealed that SSR42 codes for an 891-nucleotide RNA molecule and that the full-length transcript mediates the molecule's regulatory effects. Western blotting and functional assays indicated that the regulatory effects of SSR42 correlate with biologically significant changes in corresponding protein abundances. Further, in *S.aureus* strain LAC, SSR42 is required for wild-type levels of erythrocyte lysis, resistance to human polymorphonuclear leukocyte killing, and pathogenesis in a murine model of skin and soft tissue infection (**Figure 3**) [65].

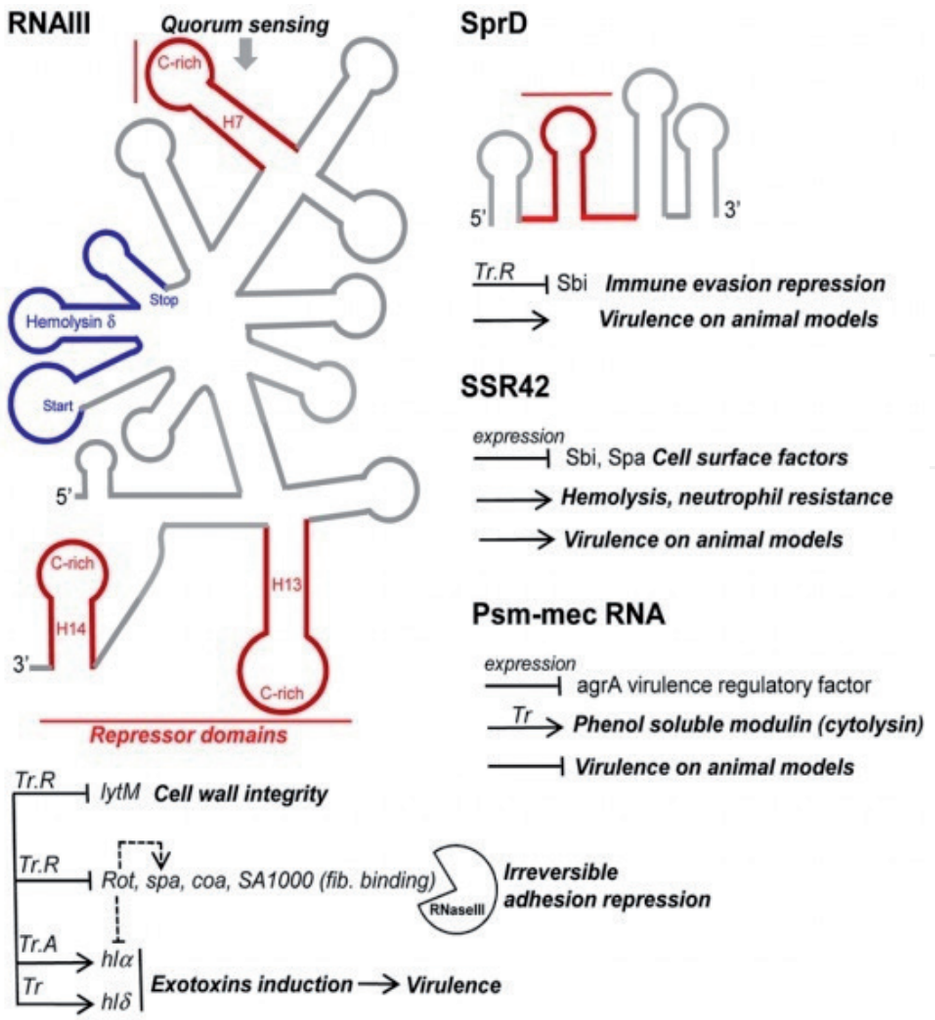

Figure 3.
S. aureus *sRNAs from the RNome implicated in bacterial virulence. Multitasking RNAIII is the effector of quorum sensing to perceive population density and regulates multiple targets involved in peptidoglycan synthesis, adhesions, exotoxins production and virulence. RNAIII internally encodes hemolysin represented in blue color. It contains three repressor domains which are represented in red color, containing accessible UCCC motifs that interact with antisense pairings, with the ribosome binding sites of numerous target mRNAs for translational repression (Tr.R), some triggering endoribonuclease III (RNase III) cleavages to induce target mRNA degradations and irreversible gene expression decay. Translation of at least two exotoxins is activated by RNAIII, one encoded (hld), and another (hla) by translation activation (Tr.A). SprD is expressed from the genome of a converting phage and interacts, by antisense pairings, with the 59 part of the sbi mRNA encoding an immune evasion molecule. SprD possesses an important role in* S. aureus *virulence, but the mechanism of its control is yet to be elucidated, with Sbi being only one player among others. The 891-nucleotide long SSR42 affects extracellular virulence expression, hemolysis, neutrophil virulence, and pathogenesis and contains a putative internal ORF. The mechanisms of target regulation remain to be elucidated. The SCCmec-encoded psm-mec RNA suppresses agrA translation and attenuates MRSA virulence, acting as a dual-function RNA regulator [64].*

8. Pathogenicity Island encoded RNAs

SCCmec (Staphylococcal Cassette Chromosome mec) is responsible for the regulation of antibiotic resistance genes, particularly for the methicillin resistance genes in facultative *S.aureus*. Thus it helps the pathogen to adapt under different stress conditions for survival in the hosts. Elements involved in these processes are genomic islands, transposons, plasmids, and the pathogenicity islands (PIs) acquired horizontally and encode various virulence factors like toxins and cell attachment factors, superantigens factors, invasion factors and two-component system [66]. Apart from the protein-coding genes, it pathogenicity islands also codes for phage-related genes and involves sRNA [67]. Several sRNAs are found in

numerous copies distributed encircling the *S.aureus* genome and also some additional copies are present in the plasmids. Multiple copies are present due to either repeated events of gene duplication or horizontally gene transfer [68].

However, the sRNAs expressed from *S.aureus* Pathogenicity Islands (SaPIs) were involved in the regulation of gene expression present on the regions of cognate PIs (**Table 1**). Therefore it forms the functional linkage between the PIs and the genome of the organisms. Expression of SprD (Small Pathogenicity islands D) by PIw involves repression of sbi mRNAs during the initiation process of translation, which encodes an immune evasion molecule [69]. A central hairpin of SprD binds with the sbi mRNA RBS and thus prevents the initiation of the translation process. SprD sRNA has a prominent effect on virulence factors, it involves different pathways for regulating staphylococcal infectivity by altering the expression patterns of SprD. Several other sRNAs are also responsible for pathogenicity through regulatory networks by either direct or indirect way and other translational process regulatory networks. However, from the recently determined sRNAs, 4 are present in PIs and other 6 are in the SCCmec mobile element, with 54 to 400 nucleotides long in size [70].

Teg152 and SprF are two sRNAs that are completely complementary to other two sRNAs SprA1 and SprG. In type I TA (Toxins-Antitoxins) modules, the pairing of SprA1 with Teg152 and SprG with SprF sRNAs takes place. SprA and SprG encodes smaller hydrophobic peptides [53]. SprA1 is a multifunctional sRNA with pressumed antisense function. It's 3-end pairs with 39-UTRs region of three different mRNA targets. The independent transcriptional regulation is responsible for synthesizing appropriate expression levels of sRNAs for effective functional regulation [71].

Group	Examples of virulence factors	PAI
Iron uptake system	FyuA, acrobactin, Sit, Pit2ABCD	HPI, SPI-1, PPI-1, SHI-2,3, PAII-CFT073, PAI III, IV
Adhesins	Type 4 pili, P-pili, S- and P-fimbriae, sap adhesins, Hek adhesins, AfasE-III, Iha, TcpA	Major PAI,PAI I, II CFT 073,PAI I-IV, PAI-I AL863, TAI, VPI-I
Pore forming toxins	Listreiolysin, alpha-haemolysin, RTX-like exotoxins	aLIPI-I, PAI-I536
Second Messenger pathway toxins	CNF-I	PAI-I C5, PAI II J96
Protein causing apoptosis	SipB	SPI-I
Superantigens	TSST-I, ET	SAPI I, SAP I2, SAPIbov, etd
Secreted lipases	PlcA, plcB, SmlC	LIPI I, LIPI II
Secreted protease	EspC, SigA, Pic, ShetA1, Mop,	SHI-I, EspC, PAI-I, VPI-I, BFPAI
O antigens	GtrA, GtrB, Gtr	SHI-O
Proteins transported by type I, II, III, IV and V protein secretion system	Alpha -Hemolysin, EspI, EspC, SigA, Cag, Tir, EspB, G, F, map, SptP, Sae, SopD, SopE, SopE2, PipB, SifA, SifC, EspC, CagA	SHI-I, PAI-I, II536, PAI-I, PAI-II96, LPA, EspC, PAI, SPI-I, SPI-3, SPI-5, LEE, cag, PAI
Antibiotic resistance phenotypes	Pse-I, FloR, AadA2, Sull, TerR, G	SGI-I

Table 1.
Groups of virulence factors encoded by PAI (pathogenicity islands).

9. Phenotypes associated with sRNA expressions

The expression pattern of sRNAs is different in normal compared with SCV (small colony variants) phenotypes of *S.aureus* clinical isolates from the osteomyelitis patients [72]. Different characteristics of SCV strain are slow growth, low pigment production, lower hemolytic activity, lower susceptibility pattern to aminoglycosides, low production of toxins and improved intracellular persistence [73]. Usually, the normal phenotypes are considered as virulent strain and SCVs are considered as persister cells. RNA III expression is a phenotypic-specific, as it is detected in normal phenotypes but not in SCV phenotypes [74]. The absence of

Protein/Gene	Functions	References
FLIPr	Protein that inhibits leucocyte response mediated by activation of FPR-like protein 1. FPR is a high affinity receptor for N-formly-met-leu-phe signaling tripeptide.	[76]
CHIPS	Binds C5aR and the formyl peptide receptor FPR	[77]
Capsule	Polysaccharide capsule prevents phagocytosis and adherence	[78]
SCIN	Staphylococcal complement inhibitor interacts with C3 convertase, C4b2a and C3bBb	[79]
Ecb	Extracellular complement binding protein blocks C3 and C5 convertase	[80]
Efb	Extracellular fibrinogen binding protein, blocks complement and binding of neutrophils to fibrinogen, and platelet aggregation	[81]
Protease V8 (SspA)	Inhibition to complement pathway	[82]
Aureolysin (Aur)	Inhibition to complement pathway	[83]
Staphopain (SepA, SspB)	Cysteine protease cleaving CXCR2 chemokine receptor	[84]
Protein A	Interacts with Fc region of IgG	[85]
Sbi	Interacts with Fc region of IgG	[85]
Dismutases (SodA, SodM)	Conversion of superoxide to hydrogen peroxide	[86]
Catalase (KatA)	Conversion of hydrogen peroxide to water and oxygen	[86]
Staphyloxanthins	Antioxidant carotenoids	[87]
DNAses	Clears DNA in neutrophils extracellular traps, NETs	[88]
Dlt operon	Addition of D-alanyl esters to teichoic acids to protect against alpha defensins	[89]
Phenol soluble modulins	Small amphipathic alpha helical peptides	[90]
Alpha toxins, hla	Pore forming toxin, lyses human leucocyte, epithelial and endothelial cells, platelets	[91]
Panton-valentine leucocidin (PVL)	Pore forming bi-complement leucocidin	[91]
Gamma- haemolysin (HlgAB, HlgCB)	Pore forming bi-complement leukocidin	[91]
Coagulase	Activate prothrombin to induce blood coagulation	[92]
Von- willebrand factor binding protein	Activate prothrombin to induce blood coagulation	[93]
Staphylokinase	Plasminogen activator to form the active protease	[94]

Table 2.
Factors used by S.aureus *to counter host defense mechanism.*

RNAIII sRNA in SCV phenotypes may be the reason for the reduced production of toxins and less virulence. Several PI encoded sRNAs' expression pattern is switched off in the SCV phenotypes during the late growth phase. Also, the less expression profile of SprS in the SCV phenotypes may also be responsible for their less pathogenicity in comparison to the normal phenotypes [75] (**Table 2**).

It has been reported that there is an up-regulation of Sau-13 in normal phenotypes, whereas it is down-regulated in the SCV phenotypes. Sau-13 involve in ion transport and other metabolism by its antisense function against the precursor phoB. But Sau-66 sRNA up-regulated in SCV phenotypes only and down-regulated in normal phenotypes [95]. Sau-66 has antisense region on a gene encoding protein which is involved in folate biosynthesis. Sau-66 has a major impact on the formation of thymidine autotrophs in SCV phenotypes in purine biosynthesis pathway because folate is a carbon donor [96].

10. sRNAs as antimicrobial drug targets

The evolution of CA-MRSA (Community Associated-Methicillin Resistance *Staphylococcus aureus*) strains are major threats to healthcare. Currently available narrow-spectrum antibiotics target only particular functions of bacteria such as synthesis of peptidoglycans, DNA replication, and protein synthesis. Hence, a broad spectrum of antibiotics can target different cellular pathways, thus reducing the resistance pattern among the pathogens. Other methods to reduce antimicrobial resistance are by targetting the production of virulence factors causing the host damage and disease [97].

Since most of the currently used antibiotics bind to the ribosomal RNA, this influences the designing of new multi-targeted antibacterial drugs with respect to small RNAs. Riboswitches, which are termed as metabolites sensing mRNAs, are currently used as a structured receptor that binds with smaller metabolites with higher precision and thus regulates downstream genes. Riboswitches regulates 7 operons and 33 genes, which respond for intracellular concentration of SAM, TPP, FMN, Glc-6P, certain amino acids residues and 7- aminomethyl-7-deazaguanine (preQ1) [98]. Targeting any of these riboswitches would alter the gene's expression pattern even if the cells do not possess any natural compounds. Several synthetically designed analog of guanine upon binding with the purine riboswitches inhibits growth [99].

11. Conclusion

This review focuses on the functions of sRNA and their role in regulating genes in *S. aureus*. Combined application of High throughput screening (HTS), genomic analysis and phenotypic methods for the prediction and determination of sRNAs, functional proteins, RNA binding proteins and riboswitches would provide information on the mechanism of integration of proteins and regulatory RNAs into interwined regulatory networks responsible for adaptation to stress conditions and virulence production [100]. Further study is needed for the determination of signals that can initiate the regulation of sRNA transcription and their targets. Another point that needs to be focused on is host-virulence adaptation or interactions, then cell communication among the dense population of microorganisms and cell differentiation. The expression pattern of sRNAs will be different in a population, leading to adaptability in response to various environmental and stress variations. Furthermore, variations in sRNA expression and their regulatory networks

due to host-microbiome interactions also need to be studied. Metagenomic studies, HTS approach, RNA-seq analysis and transcriptomics studies could help understand the mechanisms by which the commensal pathogens cause infections and disease.

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Conflict of interest

The authors declare no conflict of interest.

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