

# Chapter 15

## Combating Staphylococcal Infections Through Quorum Sensing Inhibitors



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**Abstract** *Staphylococcus aureus* is a clinically important pathogen mainly causing hospital borne infections. These bacterial infections range from mild skin infections to serious health threats like endocarditis, osteomyelitis, and pneumonia. Few strains have developed resistance against antibiotics used to treat *S. aureus* infections and are termed as Methicillin Resistant *S. aureus* strains. The pathogen releases Auto Inducing Peptides to establish cell density dependent inter-cell communication, also known as quorum sensing (QS). QS results in the expression of accessory gene regulator system. It causes successful biofilm formation and enhanced expression of toxins. QS mediated biofilm formation provides an additional resistance against the antibiotics used. An innovative therapeutic approach has been studied vastly in last decade to deal with severe infections using specific QS inhibitors (QSIs). This chapter comprehensively describes the QSIs studied to control the infections caused by *S. aureus* strains.

**Keywords** Agr system · *Staphylococcus aureus* · Biofilm · Inhibitors · Quorum sensing · RAP/TRAP

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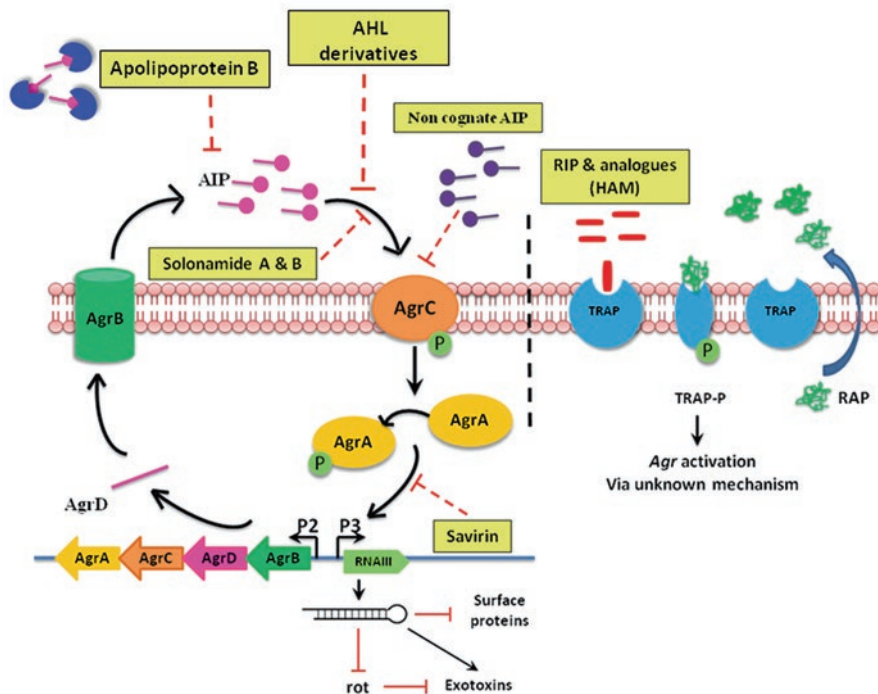
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## 15.1 Introduction

Quorum sensing (QS) is reported to be critical for various human pathogens for example *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Pseudomonas aeruginosa*, *Serratia pneumonia*, *Yersinia pestis*, *Brucella abortus* and *Burkholderia pseudomallei* (Swift et al. 2001; Williams 2002). The successful establishment of disease is governed through the pathogen's ability to invade and forms biofilm in the host. This also facilitates the pathogen to avoid antibiotic mediated killing in vivo. There are various methods to prevent the formation and disrupt the pathogenic biofilms in the host for examples nanoparticles, azithromycin, etc (Agarwala et al. 2014; Gui et al. 2014; Wadhvani et al. 2016; Ahiwale et al. 2017). Interfering bacterial QS through QS inhibitors (QSIs) is a novel therapeutic approach to curb the bacterial infection (Kumar et al. 2015). *Staphylococcus aureus* is the major cause of nosocomial infections in USA and developing countries as well. *S. aureus* secretes few virulence factors which are under the control of *agr* operon. The *agr* operon encodes AgrB (membrane bound peptidase), AgrD (precursor of AIP), AgrC (membrane bound histidine kinase), and AgrA (response regulator). The promoter region P2 governs polycistronic operon *agrBDCA* while the adjacent promoter P3 encodes mRNA for  $\delta$ -hemolysin and pleiotropic regulator of other virulence genes (Bronesky et al. 2016). AgrB cleaves AgrD into a thiolactone intermediate which is secreted to undergo subsequent cleavage to yield mature AIP. AIP is then sensed by the receptor histidine kinase AgrC, thus phosphorylating itself and response regulator. The activated response regulator AgrA binds to the P2 and P3 to enhance the expression through these promoters. RNA III encoded RNA which acts as an antisense and interferes with the translation of 'repressor of toxin' Rot, an inhibitor of  $\alpha$ -hemolysin. Another QS system RAP/TRAP consists of two proteins, which are RAP (**R**NAIII-**a**ctivating **p**rotein) and TRAP (**t**arget **R**NAIII-**a**ctivating **p**rotein). RAP activates the production of toxins by phosphorylating the histidine amino acid of TRAP, when RAP reaches a certain threshold concentration (Balaban et al. 2001). Emergence of multidrug resistance in *S. aureus* strains is a serious public health issue (Kalia 2014a, 2015). Therefore, an alternative approach of targeting the QS molecules of bacteria is a viable option to effectively treat the infections (Koul and Kalia 2017) (Fig. 15.1).

## 15.2 QS Inhibitors in Controlling *S. aureus* Infections

QSIs are antimicrobial compounds that interfere with the ability of bacteria to communicate in a colony (Kalia and Purohit 2011, Kumar et al. 2013). They should be specific for the protein to be targeted to avoid killing of host and its microbiome. Specific QSIs have been researched for their application in the prophylaxis of *S. aureus* borne infections. Structurally QSIs can belong to different categories of macromolecules for examples, peptides, sugar, amides or their analogues. Following, we have discussed the inhibitors showing significant potency against the infection caused by methicillin resistant *S. aureus* (Table 15.1).



**Fig. 15.1 Inhibition of Agr and RAP/TRAP systems of *S. aureus* using quorum sensing inhibitors.** Left panel shows Agr inhibition while Right panel shows RAP/TRAP inhibition. Red Dotted Lines indicate specific inhibition steps in the pathways caused by the inhibitors

### 15.3 Savirin Inhibits Growth via Interaction with AgrA

High throughput screening of small molecule inhibitors led to the discovery of the Savirin (*Staphylococcus aureus* virulence inhibitor), which specifically inhibits the agr mediated signaling in *S. aureus* without affecting the growth of skin commensal *S. epidermidis*. Chemically, Savirin is 3-(4-propan-2-ylphenyl) sulfonyl-1H-triazolo (1,5-a) quinazolin-5-one (Sully et al. 2014). Apart from its molecular weight, the lipophilic nature of savirin makes it an interesting drug candidate for treatment of *S. aureus* infections (Lipinski et al. 2001). *S. aureus* possess a two-component system (TCS) which comprises of AgrC, histidine kinase and AgrA, response regulator. The extracellular autoinducing peptide (AIP) binds to the transmembrane protein AgrC, which in turn phosphorylates AgrA. Thus activated AgrA binds to promoter P2 and P3 encodes AgrB, AgrD, AgrC, AgrA and RNAIII respectively. RNA levels of RNAIII increases dramatically upon AgrA binding (Koenig et al. 2004). RNAIII primarily functions as an antisense and is earlier reported to inhibit transcription of repressor of toxins such as rot (Boisset et al. 2007). RNAIII mediated inhibition of rot expression thus increases transcription of downstream virulence factors such as  $\alpha$ -hemolysin (Yarwood and Schlievert 2003; Le and Otto 2015). Recently, it was

**Table 15.1** Natural and synthetic inhibitors of *S. aureus* quorum sensing

Compound Name	Molecular Details	Target proteins/ systems	Mechanism of action	References
Savirin	(3-(4-propan-2-ylphenyl) sulfonyl-1H-triazolo [1,5-a] quinazolin-5-one)	AgrA, the <i>agr</i> response regulator	Inhibitor of AgrA-DNA interaction and thus inhibiting RNAIII synthesis.	Sully et al. (2014)
Solonamide A	A cyclodepsipeptide consist of a 3-hydroxyhexanoic acid and four amino acids	AgrC, the <i>agr</i> signal receptor	Antagonist of AgrC.	Mansson et al. (2011)
Solonamide B	A cyclodepsipeptide consist of a 3-hydroxyoctanoic acid and four amino acids	AgrC, the <i>agr</i> signal receptor	Antagonist of AgrC.	Mansson et al. (2011)
RNAIII-inhibiting peptide	A heptapeptide (YSPXTNF-NH <sub>2</sub> )	RNAII and RNAIII, biofilms	Inhibits TRAP phosphorylation of RAP/TRAP QS system and <i>agr</i> expression.	Gov et al. (2001)
Apolipoprotein B	4536 amino acid protein	AIP	Sequesters AIP	Elmore et al. (2015)
Hamamelitannin and its analogues	Ester of D- hamamelose (2-hydroxymethyl-D-ribose) with 2 molecules of gallic acid (2',5-di-O-galloyl-Dhamamelose)	Inhibits <i>agr</i> expression by blocking TRAP	Non peptide analogue of RIP	Brackman et al. (2016), Vermote et al. (2017)
Non cognate AIP	7–9 amino acid residues in length with 5 membered ring; C-terminus forms the thiolactone bond with conserved central cysteine.	Blocking AgrC receptor	Inhibition of <i>agr</i> by non cognate binding of AgrC-AIP	Tal-Gan et al. (2013a), Vasquez et al. (2017)
TMA, TOA and TTA derivatives of AHL	Modifications in 3-oxo-12-HSL structure	Inhibitor <i>agr</i> signalling	Inhibition of AgrC-AIP interaction	Murray et al. (2014), Zapotoczna et al. (2017)

shown that total activity of  $\alpha$ -hemolysin was significantly reduced in savirin-treated bacterial supernatants of MRSA isolated from different sites of infection. Since histidine kinase domain of AgrC is conserved in *S. aureus* and *S. epidermidis* (a skin commensal), therefore AgrA was selected as a target for drug development using high throughput screening. C-terminal DNA binding domain of AgrA was used to identify the drug candidates using swissdock, an online server. The study reveals that savirin binds to the CTD of AgrA from *S. aureus* (SA\_AgrA) however was

unable to interact with AgrA from *S. epidermidis* (SE\_AgrA). DNA binding domain of SA\_AgrA and SE\_AgrA differs in two positions (229, Tyr to Phe) and (227, His to Asn), which significantly reduces the binding affinity of Savirin (Sully et al. 2014). The crystal structure of LytTR domain of the SA\_AgrA was analyzed in a DNA unbound form. At the same time, screening a library of small molecules reveals that the AgrA-DNA interactions might destabilize by targeting an exposed hydrophobic cleft with a small molecule (Leonard et al. 2012). Mechanistic studies involving a novel reporter strain of SA\_AgrA activation and electromobility shift assays have demonstrated the efficacy of savirin, both in vivo and in vitro, by inhibiting the binding function of SA\_AgrA with DNA in *S. aureus*. These evidences suggested that savirin impedes the function of SA\_AgrA, thus preventing the transcription from *agrBDCA* promoter P2 and RNAlII promoter P3 and other *agr*-regulated virulence genes. The clinical isolate of *S. aureus* was studied for few generations for the emergence of resistance to savirin both in vivo and in vitro. Even the persistent exposure to the drug savirin could not lead to the emergence of resistant strain (Sully et al. 2014). Unlike conventional antibiotics, savirin is highly specific and does not foster stress responses and disrupt membrane integrity (Defoirdt et al. 2013). Moreover, AgrA has similar sequence in all four *S. aureus agr* groups, making it more desirable therapeutic target (Wang and Muir 2016).

## 15.4 Solonamide A and B Act As Antagonist of AgrC

Two of the most important strains of MRSA are “hospital acquired” or HA-MRSA and “community acquired” or CA-MRSA (Gordon and Lowy 2008). In general, HA-MRSA is an opportunistic pathogen unable to infect healthy individuals while in recent years, the most common strain of CA-MRSA, USA300 has emerged as a serious concern due to its capability of infecting healthy individuals (Loughman et al. 2009). Increased resistance to different antibiotics in MRSA has led to the development of new therapeutic strategies. The *agr* system regulates the expression of virulence gene in *S. aureus* (Gordon and Lowy 2008). Therefore, anti-virulence therapy has received an appreciable interest for combating *S. aureus* infections (Wright and Sutherland 2007). Recently, two novel compounds were isolated from the marine bacterium *Photobacterium halotolerans* (strain S2753) named Solonamides A and B. They impede *agr* QS of *S. aureus* and subsequently disrupt the expression of virulence gene. Based on NMR data, solonamides structure was characterized as cyclodepsipeptides consisting of a 3-hydroxy fatty acid and four amino acids (phenylalanine, alanine and two leucines). It was also found that solonamide A contains a 3-hydroxyhexanoic acid (Hha), whereas solonamide B is made up of 3-hydroxyoctanoic acid (Hoa) (Mansson et al. 2011). In vitro, it was shown that signals downstream to the *agr* sensing system upregulates the expression of  $\alpha$ -hemolysin encoded by *hla* and downregulates the expression of cell surface protein such as protein A encoded by *spa* at the beginning of the stationary growth phase in *S. aureus* (Vuong et al. 2000). Northern blot analysis examined the amount of mRNA isolated from

strain of CA-MRSA, USA300 and *S. aureus* 8325–4 after the treatment with solonamide, verified the interference of these compounds in virulence gene expression. Solonamide B minimizes the expression of *hla* and *rnaIII* and increases the expression of *spa*. Whereas solonamide A has been shown to increase the expression of *spa* however there were minor reduction in *hla* and *rnaIII* expression in USA300 and 8325–4 strains (Mansson et al. 2011). The primary host defense in opposition to *S. aureus* infections are neutrophils and therefore, lysis of neutrophils is crucial for the virulence of these strains. The PSMs and  $\alpha$ -hemolysin are two major virulence factors; both are remarkable at killing immune cells and responsible for an increased virulence of CA-MRSA (Bubeck Wardenburg et al. 2007; Wang et al. 2007). It was reported that solonamide B reduces the expression of virulence factors such as phenol soluble modulins, the PSMs and  $\alpha$ -hemolysin in USA300 strain. Additionally the toxicity of supernatants was shown to be minimized when tested against human neutrophils. AgrA, the response regulator of *agr* QS system directly controls the expression of PSMs. Apart from disrupting expression of genes via RNAIII, solonamide B also affects expression of PSMs through AgrA (Nielsen et al. 2014). The QS signal molecules of *S. aureus* are the cyclic thiolactone peptides generally known as auto-inducing peptides (AIPs). AIPs activate the *agr* QS system and thus controlling the virulence gene expression via the effector molecule RNAIII (Novick and Geisinger 2008). Depending on the strain, there are four distinct types of AIPs in which AIP of one type specifically binds to its cognate receptor agrC (*agr* signal receptor) but shows antagonistic activity in strains harboring other types of AIPs (George and Muir 2007). It was suggested that solonamides are the competitive inhibitors of the *agr* system as they have structures similar to the AIPs. Solonamide is a lactone whereas AIP is a thiolactone. However in recent studies, AIP analogues harboring lactone instead of thiolactone have been found to act as competitive inhibitors. It was also found that both solonamides contain hydrophobic phenylalanine and leucine residues that are crucial for the impediment of the *agr* response (Mayville et al. 1999; Mansson et al. 2011). Moreover, Baldry and colleagues chemically synthesized the solonamide analogues to improve its anti-virulence candidacy (Baldry et al. 2016). These findings suggest that inhibition via solonamides is probable alternative therapeutic approach to treat MRSA infections.

## 15.5 Apolipoprotein Act As Sequester of AIP

In recent times serum lipoproteins (LP) have emerged as a molecule having a dual role of contributing to cholesterol homeostasis as well as host innate defense. It has been established that very low levels of serum lipoprotein (hypolipoproteinemia) is related to increased bacterial infection in critically ill patients (Han 2010; Femling et al. 2013). In this respect Apolipoprotein B (apoB100), a 4536 amino acid protein is essential for the formation of these LPs (LDLs, VLDLs, Chylomicrons, etc.). Recent studies have shown that apoB100 disrupts virulence factor expression of *S. aureus* thus limiting its pathogenesis (Hall et al. 2013). It is done by binding of

apoB100 to AIPs and thus disrupting *agr* mediated virulence. While in human intestinal enterocytes, a truncated form of apoB100 is produced, which is apoB48. It is studied that enteral feeding in critically ill patients leads to reduced risk of infection as compared to parenteral feeding, which suggests the importance of apoB48 in host innate immune response, however the mechanism is unknown (Kattelman et al. 2006). This led to the development of new quorum quenching inhibitor i.e. apoB48 to control *agr* mediated *S. aureus* QS by Bradley and colleague. It was seen that apoB48 and apoB100 antagonizes *agr* signalling with similar  $IC_{50}$  of 3.5 and 2.3 nM, respectively. The  $IC_{50}$  values were found to be below the reported  $EC_{50}$  (28 nM) for activation of *agr* system via AIP1. This could provide effective protection against *S. aureus* infections. In vivo studies also showed that exogenous apoB48 treated mice infected with *S. aureus* USA300 strain had decreased bacterial burden at site of infection as compared to untreated mice. This data makes apoB48 an important inhibitor of *agr* signalling mediated QS in vivo and providing protection against *S. aureus* infection (Elmore et al. 2015). Thus apolipoprotein can prove to be a global inhibitor of QS and warrants more research for its use as therapeutic agent.

## 15.6 Non Cognate AIP

QS in *S. aureus* is controlled by the chromosome locus named *agr* (Accessory Gene Regulator). It is an operon system, genes of which encode for and also sense a small peptide autoinducer named AIP (Autoinducing Peptide) (Novick and Geisinger 2008). AIP consists of 7–9 amino acid residues and harbours a five membered ring wherein the C-terminal forms the thiolactone bond with cysteine (central position). This arrangement is crucial for AIP's activity (Ji et al. 1997; Mayville et al. 1999; McDowell et al. 2001). A conserved hydrophobic patch in the C-terminus and few specific contacts aid in binding of AIP to its cognate receptor, AgrC via the hexahelical transmembrane (TM) sensor domain. Thus resulting in activation of downstream signalling cascade (Lyon et al. 2002; Wright et al. 2004; Geisinger et al. 2008). The *agr* locus possesses polymorphism within a single species. This polymorphism is due to the variability in the regions of RNAII, AgrB, AgrD and AgrC, giving rise to four allelic variants of *S. aureus*. This hypervariability guides the generation of four different types of AIPs (I-IV) on the basis of the strain (Ji et al. 1997; Jarraud et al. 2000). Generally, only the cognate interaction of AIP with AgrC guides the expression of *agr* operon whilst the non-cognate interactions of the same lead to the inhibition of the expression, thus causing the inhibition of QS. Owing to this property of inhibition of QS by non-cognate AIPs Lyon and McDowell research groups independently designed hybrid AIPs by altering length or amino acid sequence, by introducing truncations and structural substitutions. The hybrid AIPs thus created have the property to act as universal inhibitors of all the AgrC and thus outcompeting all types of AIP (Lyon et al. 2000, 2002; McDowell et al. 2001). Based on the Structure activity relationship (SAR) studies conducted on AIP-I, II and III, a few important points have been revealed. Modifications of



these can convert the AIPs to global *agr* inhibitors, for instance, a 16-membered macrocycle important for binding. Playing with the size and stoichiometry of this ring is deleterious to AIP activity (McDowell et al. 2001; Johnson et al. 2015). Second, C-terminal end of AIPs have hydrophobic residues which are important for effective binding to AgrC. Point mutations on alanine at these particular positions destroy the potency of the AIPs (McDowell et al. 2001; Tal-Gan et al. 2013b). Lastly, structural modification of AIPs plays a detrimental role in its activity. Owing to this the second residue within the macrocycle and the exocyclic tail are required for AgrC activation. Modification and truncations of these sites lead to loss of its potency (Tal-Gan et al. 2013a). Owing to its peptidic backbone and its consequent higher immunogenicity and lack of stability in vivo, elaborate research is underway to make the peptidomimetics corresponding to these AIPs. For this purpose, modifications in AIP-III by replacement of amino acid residues with corresponding peptoids or N-methyl mimics has produced new QSIs (Tal-Gan et al. 2014). Further research is needed to completely turn them into peptidomimetics, which would help to bring them in clinical trials.

Recently a group of scientists created a focussed library of 63 peptidomimetic by using standard Fmoc Solid Phase Peptide Synthesis (SPPS) method for evaluating AgrC inhibition in four groups of *S. aureus*. These were the simplified peptidomimetics of the previously reported truncated native AIP, *t*-AIP-II (Lyon et al. 2002; George et al. 2008). Out of these, three peptidomimetics namely *n*7FF, *n*8FF, and *n*7OFF inhibited AgrC activity in the clinically relevant group I: *S. aureus* strain with potencies similar to that of the parent peptide minus their shortcomings like solubility and stability (Vasquez et al. 2017). However, further research is required to test these non cognate AIPs as therapeutic agents to control infections by methicillin resistant *S. aureus* strains.

## 15.7 Analogues of Signal Molecules

Acyl homoserine lactone (AHL) is a class of QS molecule produced by gram negative bacteria and shows polymorphisms even in the same genera (Huma et al. 2011; Kalia 2014b). Two AHL compounds are produced by *P. aeruginosa*, which are short chain N-butanoyl-L-homoserine lactone (C4-HSL) and long chain N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL). These compounds regulate virulence and the generation of secondary metabolites. However only 3-oxo-C12-HSL acts on gram positive bacteria by inhibiting their growth. The 3-oxo-C12-HSL is earlier reported to have a killing effect on *S. aureus* (Kaufmann et al. 2005; Qazi et al. 2006). While at subinhibitory concentrations it hinders the release of *S. aureus* exotoxins ( $\alpha$ -hemolysin,  $\delta$ -hemolysin and toxic shock syndrome toxin) and thus acts as a quorum quenching agents (Qazi et al. 2006; Kalia et al. 2011). 3-oxo-C12-HSL undergoes intramolecular changes to give acid product 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl)pyrrolidine-2,4-dione [(S)-5-hydroxyethyl-3-decanoyltetramic acid;8 5-HE-C10-TMA, 5] (Kaufmann et al. 2005). This belongs to TMA family of compounds which have



antibacterial activity. Lately Murray and colleagues designed a series of 3-oxo-C12-HSL, TMA, and TOA analogues. This was done by bringing about systematic modifications on the parent compound 3-oxo-12-HSL focusing on (I) homoserine lactone, (II) 3-oxo substituent, (III) acyl side chain and (IV) amide structural units. HSL analogue namely 3-oxo-C12-HSL **1** having modifications in the homoserine lactone ring inhibited AgrC with an  $IC_{50}$  of  $22 \pm 6 \mu\text{M}$ . TMA analogues (namely **3–13**) created by varying the 3-acyl chain length **3–8**, stereochemistry **9**, and substitution at the 5-position of the heterocyclic ring **12** and **13** were tested for their inhibitory activity against *agr*. It was observed that compound **4** 5-HE-C8-TMA has good inhibitory activity ( $42 \pm 13 \mu\text{M}$ ) against *agr*. It also fully abolished the expression of *agr*-mediated exotoxin  $\alpha$ -hemolysin at  $100 \mu\text{M}$ . This makes it a good candidate for future therapeutics however research should be focused on increasing its stability (Murray et al. 2014). Next in line are the TOA compounds (namely **14–18**) synthesized by bringing about variations in TMA structure wherein the ring nitrogen was replaced by oxygen. Upon evaluation of these TOAs against *S. aureus* growth and *agr* inhibition, it was found that C-14 TOA **17** was the most effective having an  $IC_{50}$  of  $3 \pm 1 \mu\text{M}$  which is approximately 8 times lower than the MIC ( $25 \mu\text{M}$ ). Another compound C-12 TOA **16** was found to be most potent than any other compound in preventing AIP mediated activation of AgrC by maintaining allosteric interaction with AgrC. Finally C-14 TOA **17** also reduced *S. aureus* colonization of human nasal passage. C-14 TOA **17** also showed its potency in mouse model system without any toxicity to host (Murray et al. 2014). Recently Zapotoczna and colleagues tested antibacterial and anti-biofilm potential along with a new sulphur-containing analogue (3-tetradecanoylthiotetronic acid; C14-TTA) towards MRSA and MSSA strains of *S. aureus*. Their potential clinical use as catheter lock solution was also examined using in vitro and in vivo models of IVC infection (Zapotoczna et al. 2015). Evaluation of biofilm killing activity of these compounds 5HE-C14-TMA killed over 50% of both MSSA and MRSA biofilms at  $128 \mu\text{g/ml}$  with full abolishment at  $512\text{--}1024 \mu\text{g/ml}$ . Similar results were obtained in in vivo rat model for IVC infections. However the efficacy of C14-TOA and C14-TTA were far less in killing MSSA and MRSA biofilms. Taking into account of all these observations 5HE-C14-TMA proves to be a compound of therapeutic value against *S. aureus* biofilms (Zapotoczna et al. 2017).

## 15.8 RNAIII-Inhibiting Peptide (RIP) Binds to TRAP

The key feature in pathogenesis of *S. aureus* is the regulation of toxin production. *S. aureus* produces different toxins during its proliferation that can cause severe disease. At the initiation of growth, when the population of *S. aureus* is scarce, various molecules required for adhesion such as protein A, fibronectin binding-proteins and fibrinogen binding-proteins are expressed and help bacteria to colonize and attach to host cells. Whereas at early stationary phase of growth, bacteria are in greater density, produce toxic molecules such as hemolysins, enterotoxins and Toxic Shock Syndrome Toxin-1 (TSST-1) that help the bacteria to spread, survive and initiate the infection (Lowy 1998). There are two QS mechanisms in *S. aureus* which regulates

the production of toxin molecules in greater densities and adhesion molecules expression in lesser densities. The first one is RAP/TRAP QS system, made up of two components, RAP and TRAP (mentioned in introduction). RAP is a protein that activates the production of toxins by phosphorylating the histidine amino acid of TRAP, when RAP reaches a certain threshold concentration (Balaban et al. 2001). With an unknown mechanism, phosphorylation of TRAP causes increased cell attachment to the host and activation of *agr* QS system. The chromosomal locus, *agr* encodes RNAII and RNAIII transcripts. RNAII transcript encodes AgrA, AgrD, AgrC and AgrB, where propeptide AgrD is processed, and secreted in the form of an autoinducer AIP with the help of transmembrane protein, AgrB. In the mid exponential phase of growth, *agr* is activated which results in AIP secretion. The secreted AIP molecules then bind to the AgrC and causes AgrC phosphorylation. In turn, AgrA is activated which leads to RNAIII production. RNAIII upregulates the expression of toxins and downregulates the expression of cell surface proteins (Bronesky et al. 2016). In addition, AIP reduces the phosphorylation of TRAP and thus, leading to decreased cell adhesion (Balaban et al. 2001). RNAIII- inhibiting peptide (RIP) is a heptapeptide that can attenuate the virulence of *S. aureus*. YSPXTNF-NH<sub>2</sub> was identified as a sequence of RIP (Balaban et al. 1998). RIP acts as a competitor of RAP on activating TRAP and thus inhibits its phosphorylation, which leads to attenuation of transcription from RNAII and RNAIII promoters and thus inhibiting toxin production. Synthetic analogues of RIP, YSPWTNF was made and shown to effectively inhibits the RNAIII synthesis in vitro and reduces the *S. aureus* infections caused by different strains in vivo, including osteomyelitis, cellulitis, mastitis, septic arthritis and keratitis. Theoretically, RIP would lead to increase bacterial adhesion as it inhibits the RNAIII synthesis and RNAIII function is to decrease the cell surface adhesion molecules. But, by using atomic force and fluorescence microscopy, it was shown that RIP decreases attachment of bacterial cells to mammalian cells (HEP2) and to polystyrene. Thus, RIP can be used as a better therapeutic candidate for *S. aureus* infections (Gov et al. 2001). *S. aureus* infections connected to biofilm formation are commonly linked with the implantated medical devices (Costerton et al. 1999). After the removal of devices, the predominant species found on biofilms are *S. aureus* (Marr 2000). Biofilm is the structure formed due to QS or cell-cell communication and highly resistant to antibiotics. A novel way to treat biofilm related *S. aureus* infections is to use RNAIII inhibiting peptide, which disrupts the QS system and decreases bacterial adhesion. In an experiment, RIP was applied systematically and locally in a vascular-graft rat model, suggested that RIP completely inhibits the antibiotic-resistant *S. aureus* infections (Dell'Acqua et al. 2004). Therefore, RIP can thus be used as a coating material for various medical devices to be used during medical procedure. Moreover, antibiotics such as carbapenems (imipenem) and cephalosporins (cefazolin) in combination with RIP, inhibits the infection completely (Giacometti et al. 2003). Therefore, RIP can inhibit QS regulated toxin production and biofilm formation.

## 15.9 Non-peptide Analogues of RIP

Hamamelitannin (HAM), condensed tannin is a natural product obtained from the bark of the plant witch hazel (*Hamamelis virginiana*). It is the ester of D-hamamelose (2-hydroxymethyl-D-ribose) with 2 molecules of gallic acid (2', 5-di-O-galloyl-Dhamamelose). Because gallic acid contains three phenolic functional groups, it is considered a polyphenol. Owing to studies on HAM in last decade, it emerged as a candidate of QSI of drug resistant Staphylococcal infection. It works by acting as non-peptide analogue of RIP and thus hinders biofilm formation. Non-peptide analogue of RIP also block the production of RNAPIII in vitro as well as in vivo by blocking TRAP phosphorylation and thus affects TRAP mediated *agr* expression (Gov et al. 2004; Kiran et al. 2008). A recent study conducted by Brackman and colleagues showed that HAM increased the antibiotic susceptibility of *S. aureus* biofilms. It was observed that HAM in combination with vancomycin resulted in enhanced killing of *S. aureus* Mu50 biofilm cells compared to vancomycin alone in in vitro models. Similar results were observed for a combination of HAM with clindamycin. The in vivo effect of combined treatment was seen in *C. elegans* model system. HAM and vancomycin together significantly ( $p < 0.01$ ) increased the survival of *S. aureus* Mu50 infected *C. elegans* model system (Brackman et al. 2011). They further elaborated their study to give the mechanistic view about the action of HAM by showing that this increase in susceptibility towards antibiotics is via affecting peptidoglycan biosynthesis and exogenous DNA (eDNA) release. Combination of HAM with other antibiotics such as vancomycin, cefazolin, cefalonium, cephalixin, cefoxitin, daptomycin, linezolid, tobramycin or fusidic acid also significantly increase the killing of biofilm cells for various *S. aureus* strains. Mutations in gene belonging to QS and RNA sequencing studies showed that HAM has specificity towards TRAP receptor (Brackman et al. 2016).

However the structure of HAM makes it very polar affecting its bioavailability. It is also more prone to oxidation and glucuronidation because of its aromatic hydroxy functional moieties. Formation of ester linkages in vivo also raise an issue related to its stability (Vermote et al. 2016). Based on these observations, Vermote and group worked on making analogues of HAM by improving its stability. Three modifications were made in the HAM structure. These were modification or elimination of the aromatic hydroxy groups, replacement of the ester groups with isosteric linker moieties and lastly removal of the anomeric hydroxy group. This resulted in developing of rigid and structurally well-defined tetrahydrofuran core (position 5). Further changes led to the development of 58 analogues of HAM. Out of these the ortho chloro derivative i.e. **38** came out to be the most potent analogue of HAM. The compound **38** in combination with vancomycin resulted in enhanced killing of *S. aureus* Mu50 biofilm cells. Also it had better stability in vivo and displayed no cytotoxicity towards host cells. Thus giving **38** a better hand over HAM for therapeutic use (Vermote et al. 2016). In a latest study by same group more analogues were created by making changes at C-2' position and conducting

**Structure Activity Relationship (SAR)** based studies. This led to the generation of 52 analogues of HAM focussing on benzamides with different substituents at different positions. Three derivatives namely **10u**, **15** and **25** showed promising results when tested for disruption of *S. aureus* biofilm cells in vitro and their susceptibility to vancomycin on these biofilm cells. These products warrant more study for their therapeutic use (Vermote et al. 2017).

## 15.10 Future Directions

Hospital borne infections are a nuisance to the medical industry. *Staphylococcus aureus* is the causative agent of diseases like endocarditis, osteomyelitis, and pneumonia. Using small molecule inhibitors to combat the infections is therapeutically effective approach in case of various pathogens like *Bacillus anthracis* (Dhasmana et al. 2014). As discussed in this review, various small molecule inhibitors have been tested against *S. aureus* which have proved their efficacy in various in vitro as well as in vivo model systems. However bacterial colonization takes places during the initial phases of disease establishment and hence the implication of QSIs becomes limiting. It is important to take precautionary measures in case of medical devices. These potent QSIs could be used as a coating material on these medical devices, which would help in reducing nosocomial infections by MRSA strains. Currently, there are fewer studies testing these inhibitors on various medical devices and this field should be explored further.

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