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Investigational therapies targeting quorum-sensing for the treatment of *Staphylococcus aureus* infections

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Abstract

Introduction—Antibiotic resistance is a serious global health concern for developed and developing nations. Methicillin-resistant *Staphylococcus aureus* (MRSA) represents a particularly severe public health threat that is associated with high morbidity and mortality. The lack of novel antibiotics has led scientists to explore therapies targeting bacterial virulence mechanisms and virulence regulators, including those controlling cell-cell communication.

Areas covered—We discuss the role of quorum-sensing in *S. aureus* infections and components of the system that are being targeted using novel investigational drugs. In particular, the role of the accessory gene regulator (Agr) system in virulence regulation of *S. aureus* pathogenesis is examined. Finally, we present and compare natural and synthetic compounds that have been found to interfere with Agr functionality.

Expert opinion—There is a great need to develop new therapeutic methods to combat *S. aureus* infections. These include anti-virulence therapies that target key global regulators involved with the establishment and propagation of infection. Several molecules have been found to interfere with *S. aureus* virulence regulation, especially those targeting the Agr quorum-sensing signaling molecule. These preliminary findings warrant further investigation and validation, with the goal of

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refining a compound that has broad-spectrum inhibitory effects on most *S. aureus* strains and Agr sub-types.

Keywords

Staphylococcus aureus, *Staphylococcus epidermidis*, Quorum-sensing; Quorum-sensing blockers

1. Introduction

In 2014, the World Health Organization prioritized antimicrobial resistance as a serious threat to global health, with an emphasis on the growing resistance to common antibacterial therapies [1]. Methicillin-resistant *Staphylococcus aureus* (MRSA) infections remain a major public health concern in the U.S. and other developed countries, claiming responsibility for more than 11,000 deaths and resulting in an annual economic burden estimated at \$13.6 billion in the U.S. alone [1, 2]. Of particular concern is the increasing prevalence of highly virulent and transmissible community-associated MRSA (CA-MRSA) infections in healthy individuals outside of the hospital setting [3]. With the last novel class of antibiotic drugs discovered in the 1980s, there has been a void of new therapeutic approaches over the past quarter century to respond adequately to the widespread development of antibiotic resistance. As such, clinicians and scientists are searching for new ways to combat MRSA, which, in particular, includes the development of anti-virulence therapies.

The numerous and frequently multi-functional virulence determinants that *S. aureus* produces, such as exotoxins, proteases, superantigens, and adhesins, make *S. aureus* pathogenesis particularly complex and provide the pathogen with a series of mechanisms to evade elimination by human immune defenses [4, 5]. As a first step during infection, adhesins ascertain tissue attachment [6]. Later on, several secreted *S. aureus* enzymes, including proteases, elastases, and lipases, are important for spreading infection by breaking down physical impediments and acquiring nutrients from the degradation of host tissues. Many exotoxins of the *S. aureus* virulence arsenal, such as the widespread toxins, α -toxin and phenol-soluble modulins, or the more strain-specific superantigens such as toxic shock syndrome toxin (TSST), provide multiple mechanisms to evade immune defenses [7]. To name but a few selected mechanisms, certain *S. aureus* secreted toxins interfere with proper complement activation and neutrophil chemotaxis [5]. The secretion of superantigens is associated with improper antibody production, resulting in reduced opsonization, host immunosuppression, and the lack of protective memory against *S. aureus* infections [8]. Other toxins such as the phenol-soluble modulins (PSMs) lyse phagocytes after ingestion, thus evading pathogen elimination even if opsonophagocytosis occurs [9, 10]. Moreover, structures at the surface of *S. aureus* reduce the efficiency of human antimicrobial peptides and contribute to biofilm-like aggregation phenotypes that confer broadly protective, non-specific resistance to immune defense mechanisms [11, 12].

Quorum-sensing (QS), the ability of microorganisms to sense the bacterial cell density (or “quorum”) and respond with gene expression changes to adapt to changing environmental conditions arising as a consequence from the increased number of bacteria and decreased

availability of nutrients, was discovered in 1979 in *Vibrio fischeri* [13]. It has since been the subject of substantial investigation in many species, in particular as QS regulators have been found to control virulence mechanisms in many bacterial pathogens [14, 15]. However, our notion of the role of QS has broadened to include mechanisms other than those involved with infection, such as those bacteria may use generally to adapt to a changing host environment [16, 17]. Many bacteria have several QS systems, but the majority of *S. aureus* virulence factors that are important for the establishment of infection are under the control of one primary system called Agr for accessory gene regulator [15]. For those reasons, the Agr system has been frequently proposed as a target for novel drugs and several Agr-targeted therapeutic strategies are currently being developed.

2. *S. aureus* Agr quorum-sensing

The Agr QS system in staphylococci consists of two transcriptional units, RNAII and RNAIII, with four genes (*agrA*, *agrB*, *agrC*, *agrD*) contained within RNAII. The transcriptional units are transcribed in opposing directions, with RNAII transcribed as a single message in the order: *agrBDCA*. AgrD contains the propeptide sequence for the system's QS signal, autoinducing peptide (AIP), which is secreted from the cell with the help of AgrB. AgrB, a membrane-associated export protein, processes AgrD into AIP. AgrB accomplishes this by binding AgrD and inducing a thiolactone (or, rarely, lactone) ring structure between a conserved cysteine (or serine) and the C-terminal amino acid [18–21]. A type I signal peptidase, SpsB, cleaves the N-terminal leader on AgrD, completing AIP biosynthesis, before its extracellular release [22]. AIP (seven to nine aminoacyl residues in length) activates AgrC, its membrane-bound histidine kinase receptor, by binding at the AgrC N-terminal membrane-spanning domain [20, 23, 24]. Activation of ligand-independent AgrC dimers by AIP, even at very low concentrations, induces *trans*-autophosphorylation [25] and subsequent activation of the second component of the two-component signal transduction system, the response regulator, AgrA. Once activated, AgrA binds the *agr* promoter regions (P2 for RNAII and P3 for RNAIII), with a preference for binding P2 at low concentrations [26], perpetuating activation of its own expression. Hence, the resulting positive feedback loop is capable of substantially and quickly altering gene expression with varying cell densities, distinguishing the *agr* operon as a classic QS system [27] (Fig. 1). Variability of the *agr* operon across *S. aureus* strains is common, resulting in the classification of four Agr types (I–IV) [28, 29] each characterized by varying magnitude and timing of Agr activation in addition to differences in the amino acid sequences of the AIP, which sets them apart and leads to competitive cross-inhibition [30].

3. *S. aureus* Agr regulation

The *S. aureus* Agr regulon is one of the best-studied bacterial two-component regulation systems. Regulation of Agr expression is under the control of multiple repressors and inducers, some of which regulate each other to provide additional regulation loops. One inducer of optimal Agr expression is the staphylococcal accessory regulator A (SarA), a regulator primarily in charge of controlling expression of secreted proteins such as exoproteases, and member of a whole family of homologous gene regulatory systems with different regulatory tasks [31]. Furthering the relationship of Agr regulation with the

metabolic state of *S. aureus* infection, σ^B , a known regulator of biofilm formation, has been strongly associated with Agr regulation [32, 33]. Additionally, an oxidation sensing mechanism inherent in the Agr system was found to control the interaction of AgrA and DNA through disulfide bond formation between cysteine residues during oxidative states [34]. Finally, some other *S. aureus* two-component systems (TCSs), Sae, ArlRS, and SrrAB, have varying relationships with respect to Agr's activity [35–39]. The timing of Agr activation is indicative of its purpose in *S. aureus* infection (see part 4 in this review). Acting concomitantly with other regulatory proteins and responding to changing environmental conditions, the Agr system contributes to the progression of *S. aureus* infection by coordinating bacterial growth repression with virulence factor enhancement [40, 41].

Beyond its role in auto-feedback up-regulation of Agr through promotion of RNAII synthesis, AgrA enhances *S. aureus* pathogenesis through the up-regulation of RNAIII-dependent virulence genes and phenol soluble-modulin (PSM) cytolysin genes [40, 42]. AgrA regulates PSM expression independently of RNAIII through direct binding to the *psma* and *psm β* promoters [40]. Seven PSM peptides have been characterized in *S. aureus* (PSM α 1–4, PSM β 1–2, δ -toxin), which are ~20–45 amino acids in length [43]. Some PSMs, mostly those encoded in the *psma* operon, can activate and kill neutrophils [9, 44]. Furthermore, some aspects of bacterial physiology appear to be regulated independently of RNAIII [40]. However, a majority of Agr-dependent regulation of *S. aureus* virulence occurs through interactions of the regulatory RNA, RNAIII, with several target genes [42]. In addition, the *hld* gene, which encodes the PSM δ -toxin, is part of RNAIII. Aside from the production of δ -toxin, RNAIII has been associated with the up-regulation of several leukocidins, enterotoxins, and α -toxin, as well as degradative exoenzymes such as serine and cysteine proteases and lipases. Furthermore, RNAIII down-regulates surface-binding proteins and protein A (an immune evasion molecule), in accordance with the notion that surface binding proteins are important for the establishment of infection when bacterial cell densities are still low. However, when analyzing clinical strains it could not be confirmed that surface binding proteins are always down-regulated by Agr [45]. Moreover, metabolic enzymes have been found to be down-regulated by RNAIII, alluding to a balance between up-regulation of toxins and reduced metabolic activity, a notion supported by RNAIII-independent down-regulation of metabolic enzymes [27, 40]. It is believed that RNAIII mainly affects gene expression through an antisense base pairing mechanism [46] and via the transcriptional repressor protein gene Rot [46–48].

Biofilm formation has been strongly associated with Agr function across several *S. aureus* strains, with down-regulation leading to excessive biofilm thickness and lack of structuring, and up-regulation inducing dispersal [45, 49–53]. The detergent-like PSM peptides are the Agr-controlled direct facilitators of those effects [49, 51]. Another important QS system that is widespread in bacteria and also occurs in staphylococci is LuxS/AI-2. In *S. epidermidis*, LuxS influences infection through the down-regulation of the polysaccharide intercellular adhesin (PIA) exopolysaccharide important for biofilm formation. Furthermore, this system appears to act congruently with Agr in the regulation of biofilm formation by up-regulating the PSM peptides that promote biofilm structuring and detachment [54].

4. Role of Agr in *S. aureus* infection

Several studies have been conducted examining the role of Agr during *S. aureus* infection. Most of them led to the conclusion that Agr is important for acute virulence, while the absence of Agr functionality may be beneficial in specific types of *S. aureus* infections, such as in chronic, biofilm-associated infection, and in bacteremia. Furthermore, the roles of Agr during *S. aureus* infection appear to be not only specific to the type of infection, but also the infecting strain.

For example, in the highly virulent CA-MRSA strain USA300, it has been found that Agr regulation led to strong expression of virulence factors, such as toxins and exoenzymes, and increased virulence in a skin abscess animal model [45]. Immunization against the *agr*-controlled toxin, α -toxin, led to reduced formation of skin abscesses [55], a common form of CA-MRSA infection. Similarly, *agr*-deleted *S. aureus* strains exhibited decreased infectivity in necrotizing pneumonia [56–58] and endocarditis [59] animal models. Contrastingly, there have been several clinical studies, as reviewed by Painter et. al. [60], showing an association of Agr dysfunction and increased pathogen success during bacteremia. Furthermore, serum components sequester AIP, leading to a downregulation of Agr in human blood [61]. Interestingly, Agr functionality appears to play a role in gastrointestinal transmission after bacteremic infection [62].

The increased capacity that *agr* mutants show in the production of excessively thick, albeit unstructured biofilms [51–53, 63] suggests that mutations leading to a defective Agr system may play a role in the colonization and success of persistent *S. aureus* infection. It is known that *agr* mutations can accumulate in *S. aureus* through *in vitro* passage and exhibit reduced production of virulence factors [64]. In screens for Agr dysfunction in clinical isolates, it has been discovered that Agr-defective strains are associated with hospitalization, passage among family members [65], and chronicity of infection [66]. The association of a defective Agr system with an increased incidence of chronic endocarditis [67] further indicates that there appears to be a benefit to Agr dysfunction for *S. aureus* survival in specific infections. Furthermore, antibiotic selection pressure appears to favor loss of Agr functionality [68] and may explain the decreased efficacy of certain antibiotic therapies against specific *S. aureus* infections. Of note, the survival benefit of reduced or absent Agr activity may be lost when introduced into naïve hosts [69] and may explain why HA-MRSA strains have not succeeded in community settings.

5. Targeting *S. aureus* Agr quorum-sensing

Notwithstanding the fact that there may be limitations to the application of Agr-inhibiting drugs in certain *S. aureus* infections, in which dysfunction of Agr appears to be beneficial to bacterial survival, there is growing interest in using naturally occurring AIPs and the development of chemically modified AIPs (Tab. 1, 2) and Agr-inhibiting molecules (Tab. 3) as a potential way of simultaneously inhibiting the production of the considerable number of Agr-controlled virulence genes.

5.1 *S. aureus* AIPs

There are four subgroups of Agr in *S. aureus*, each producing a specific AIP with a different amino acid sequence (Tab. 1), but a conserved thiolactone ring structure. The AIPs generally inhibit Agr functionality of different subgroups, while they work as stimulating agonists on the AgrC histidine kinase of their own subgroups [29]. Exceptions are the AIPs of subgroups I and IV, which are not cross-inhibiting owing to their pronounced similarity. While the size of the C-terminal thiolactone ring is conserved, *S. aureus* AIPs vary mostly in the constitution and length of the N-terminal amino acid residues constituting the N-terminal “tail” [27]. However, besides conservation of the cysteine residue necessary to form the AIP thiolactone-bonded ring, there is variability at every other residue among the four AIPs. Based on the naturally occurring situation of AIP cross-inhibition, natural and modified AIPs have been intensively studied as potential Agr inhibitors.

5.1.1 AIP I—AIP I has been subjected heavily to structural modifications in an effort to understand which determinants of AIP I contribute to the agonistic and antagonistic actions towards different Agr groups [70–72]. Notably, substitution of the aspartate residue at position 5 with an alanine leads to a loss of the full cognate activation activity of AIP I and makes it a potent inhibitor for all Agr groups [70, 71]. Furthermore, this phenotype is preserved in the absence of the exocyclic tail and thus, such a truncated AIP may serve as a putative general candidate for a therapeutic against Agr-dependent staphylococcal disease [71]. However, in addition to thiolactone structures being naturally unstable, AIPs are prone to proteolytic degradation and therefore have a relatively short half-life (~4 h) *in vitro* [73]. One general method to improve peptide stability is by moving the side chain of the amino acids from the α -carbons to the nitrogens of the peptide backbone. This creates a new peptoid molecular species, which often retains the phenotype of the original peptide but has increased stability and resistance to proteolysis. Interestingly, peptoid analogues of AIP I were recently generated [74], but information regarding their abilities to inhibit Agr QS has not been presented. However, one AIP peptoid analogue was found to mildly induce biofilm production in *S. aureus*, a phenotype associated with the inhibition of Agr.

5.1.2 AIP II—Similar to the studies performed with AIP I, an AIP II alanine screen revealed amino acid residues that are important for the activation and inhibition of Agr. In particular, when the asparagine of AIP II was substituted with an alanine at position 3, AIP II lost its cognate activation activity but gained inhibitory activity toward all *S. aureus* Agr groups [75, 76]. Furthermore, a truncated analogue of AIP II was inhibitory toward all four Agr groups [71]. A six-fold increase in inhibitory activity towards Agr group I was observed with a substitution of the serine with a glycine at position 7 of this analogue, but inhibitory activity towards Agr group II was unaltered [77]. Although no data on Agr groups III or IV were provided, these findings show that AIP II is very amenable to structural alterations that can create potent inhibitory analogues.

5.1.3 AIP III—An N-methyl and peptoid screen of full-length AIP III revealed analogues showing varying degrees of inhibition and activation across the Agr groups [78]. Most notably, AIP III nF5, with the phenylalanine residue transferred to the nitrogen at side chain position 5, showed strong inhibition directed only towards Agr group IV, while Agr group II

and group III-specific inhibitors were also described in that study. In a different study by the same group, an alanine screen of AIP III was performed [79], revealing a global Agr inhibitor that carries an alanine substitution for the aspartate residue at position 4 of AIP III [79]. Remarkably, this mutant (AIP III D4A) displayed greater AgrC inhibition towards all four Agr groups than the aforementioned truncated AIP I D2A analogue [71]. Specifically, Agr group I, II and IV inhibition by this analogue was enhanced ~10-fold whereas Agr group III inhibition was enhanced ~50-fold. AIP III double and triple amino acid replacement derivatives were also generated, but the overall potencies of those analogues against different Agr groups were similar to that of AIP III D4A. The potent phenotype of AIP III D4A was confirmed in a series of hemolysis and TSST-1 inhibition experiments in addition to an established, cell-based Agr reporter assay [24, 71, 77]. When AIP III D4A was immobilized on planar and complex objects with a rapidly dissolving carboxymethylcellulose (CMC) polymer, rapid release of that AIP analogue from CMC films was reported, which promoted strong inhibition of Agr group III activity [80]. Although a comparison with other strong AIP inhibitory analogues was not performed, this preliminary study emphasizes practical applicability for an AIP analogue in interfering with Agr activity.

5.1.4 AIP IV—Removing the exocyclic tail of AIP IV created an inhibitor of all four Agr groups, but inhibition of Agr groups I and IV was less pronounced than that of Agr groups II and III [71]. While only few studies have investigated AIP IV as a potential inhibitor of Agr, an interesting study by Yerushalmi et al. showed that Agr group IV inhibition can be enhanced in the presence of an AIP IV macromolecule [81]. In that report, the authors conjugated up to 40 AIP IV molecules onto a polymer backbone scaffold using AIP IV monomers modified with a norleucine residue in place of the carboxyl methionine residue. This modified AIP IV (AIP IV') is indistinguishable in terms of activity from the original molecule. Only macromolecules grafted with 20 to 40 AIP IV' molecules inhibited Agr activity in *S. aureus* strain RN9371 (Agr group IV). Moreover, fluorescently-labeled AIP IV' macromolecules were shown to bind to the surface of bacteria suggesting that the mechanism of action is likely due to competitive inhibition of AIP binding to AgrC.

5.2 Non-*S. aureus* AIPs

Genome sequencing of clinical *S. epidermidis* isolates revealed the existence of three Agr groups (I – III) in most important coagulase-negative staphylococcal species [82]. Similar to *S. aureus*, cross-interference also exists between these three *S. epidermidis* Agr groups [83], which is most likely attributable to their differing amino acid sequences (Tab. 2). Using a reporter-based assay, strong cross-interference was observed between the *S. epidermidis* Agr groups I and II and between groups I and III but, interestingly, not between Agr groups II and III. This may be due to the fact that the AIPs of *S. epidermidis* Agr groups II and III are much longer and similar to each other compared to that of group I. Cross-inhibition between *S. epidermidis* and *S. aureus* Agr groups has also been evaluated; *S. epidermidis* AIP I was shown to inhibit δ -toxin production as a readout of Agr functionality in the supernatants of *S. aureus* Agr group I cultures [84]. Interestingly, removing the N-terminal aspartate residue from the natural *S. epidermidis* AIP I sequence selectively inhibited Agr *S. aureus* Agr

group I strain without activating the *S. epidermidis* Agr system. The inhibitory activity of this truncated AIP I towards other *S. aureus* agr groups remains to be elucidated.

The question of whether AIP from *S. epidermidis* Agr group I strains could inhibit *S. aureus* Agr groups II – IV was addressed in a follow-up study [85]. Unmodified *S. epidermidis* AIP I inhibited *S. aureus* Agr group III with greatest potency. *S. aureus* Agr groups I and II were moderately inhibited by this AIP, whereas Agr group IV was non-responsive to inhibition. Conversely, only *S. aureus* AIP IV, but not AIPs I – III, interfered with Agr of the group I *S. epidermidis* test strain. Cross-inhibition experiments between *S. epidermidis* Agr groups II and III with all *S. aureus* Agr groups have yet to be performed.

S. intermedius, a pathogen of animals and occasionally humans, produces an AIP cyclized with a lactone rather than thiolactone ring (Tab. 2). This unique AIP is formed due to the presence of a serine in place of the conserved cysteine residue in the peptide sequence [86]. Interestingly, replacing the thiolactone with a lactone ring in *S. epidermidis* AIP I abolished cognate Agr activity [84], indicating that usually, the thiolactone that is characteristic for all other staphylococcal AIPs is needed for activity. Therefore, the fact that the *S. intermedius* AIP can signal via a lactone-cyclized AIP is very intriguing. Notably, *S. intermedius* AIP displays inhibitory activity towards *S. aureus* Agr groups I through III. However, *S. aureus* agr group IV was least responsive to inhibition, an observation previously documented for the *S. epidermidis* AIP I [84].

5.3 Non-staphylococcal QS signals

Quorum sensing in many Gram-negative bacterial species is dependent on N-acylhomoserine lactone (AHL) QS signals. For instance, the biofilm-producing *Pseudomonas aeruginosa*, a bacterial pathogen commonly found in the lungs of cystic fibrosis patients [87] produces a short- and long-chain AHL, N-butyryl homoserine lactone (C₄-HSL) and N-(3-oxododecanoyl)-L-homoserine lactone (3-O-C₁₂-HSL) (Fig. 2), respectively [88, 89]. Sub-inhibitory concentrations of 3-O-C₁₂-HSL have been reported to inhibit Agr QS in *S. aureus* [90], although bactericidal activity was observed with higher concentrations of 3-O-C₁₂-HSL. Modifications to 3-O-C₁₂-HSL yield analogues with enhanced Agr inhibitory activity, but these molecules, such as C14-TOA 17, do not show reduced toxicity towards *S. aureus* [91]. An important finding from this study was that the derivatives, including 3-O-C₁₂-HSL, are not competitive inhibitors of AgrC, indicating that the mechanism of Agr inhibition must lay downstream of the signaling pathway.

Cyclic dipeptides (or diketopiperazines) are produced and used as QS molecules by a variety of bacteria [92–94]. Intriguingly, two cyclic dipeptides, cyclo(L-Tyr-L-Pro) and cyclo(L-Phe-L-Pro) (Fig. 2) produced by the human vaginal isolate *Lactobacillus reuteri* RC-14, show antagonistic activities towards all four *S. aureus* Agr groups [95]. Although the mechanism of action is unknown, it is thought that the cyclic dipeptides may compete for the AgrC binding pocket or interact with molecular targets downstream of the Agr signaling pathway. Cross interference between *P. aeruginosa* and *L. reuteri* with *S. aureus* would lend support to the idea that cross-species interference serves as a means for survival when occupying the same ecological niche. Agr inhibition by *L. reuteri* in particular may limit the pathogenesis of menstrual toxic shock syndrome by TSST-producing *S. aureus*.

However, this idea is not as easily explained in the case of the marine bacterium, *Photobacterium*. While it may seem unusual that *S. aureus* would ever be in the same environment as *Photobacterium*, studies show that this bacterium produces a group of *S. aureus* Agr-inhibitory molecules, known as solonamides (Fig. 2), which dramatically reduce the transcription of both *hla* and *rnaIII* but increase the expression of *spa* without affecting bacterial growth [96]. Moreover, of the two solonamides produced, solonamide B inhibited all Agr specificity groups, in addition to inhibiting the hemolytic activity and PSM production in the highly virulent CA-MRSA lineage USA300 [97]. Furthermore, supernatants collected from *S. aureus* cultures incubated in the presence of solonamide B induced significantly less lysis of human neutrophils compared to supernatants collected from *S. aureus* cultures incubated without solonamide B. Interestingly, *Photobacterium* produces another set of Agr-inhibitory molecules called ngercheumicins [97]. Although the ngercheumicins interfere with the expression of virulence genes controlled by Agr, the effect was generally less potent when compared to solonamide B. Both solonamide B and the ngercheumicins share structural resemblance to AIPs (Fig. 2), such as a cyclic ring and an exocyclic tail, suggesting that their mechanisms of inhibition may be mediated by interaction with AgrC [97].

Similarly, a number of compounds that interfere with Agr have been detected in the culture supernatants from a variety of fungi, such as ambuic acid (Fig. 2) isolated from a screen of 51 fungal strains [98], polyhydroxyanthraquinones produced by *Penicillium restrictum* [99], and unidentified compounds produced by several actinomycetes [100]. Furthermore, flavonoids and polyphenols, which are examples of non-toxic metabolites produced by plants and herbs, have Agr-inhibitory activity in *S. epidermidis* [101] and *S. aureus* [102–105]. Lastly, the antibiotic oxacillin, was demonstrated to have Agr-inhibiting activity when used at sub-inhibitory concentrations [106]. Interestingly, only a decrease in PSM production was observed but not of known Agr-regulated toxins such as α -toxin. The molecular mechanisms underlying these interferences are not known.

5.4 Monoclonal antibodies

A monoclonal antibody recognizing AIP IV was generated against a synthetic AIP IV molecule, AP4 hapten 5 [107]. When the isolated monoclonal antibody, AP4–24H11, was incubated with an Agr group IV strain (RN4850) and, to a lesser extent, group I strain (Wood 46), α -toxin production was inhibited, suggesting that AP4–24H11 interferes with the Agr QS system. Furthermore, co-injection of AP4–24H11 with strain RN4850 significantly abolished the severity of dermonecrosis in a mouse model of skin and soft tissue infection (SSTI). Lastly, passive immunization with AP4–24H11 afforded 100% protection in mice that received a lethal intra-peritoneal injection with RN4850 compared with a majority of mice that succumbed to death when given a control mAb. The results from this study indicate that there is promise in the therapeutic use of antibodies against staphylococcal infection, similarly to what has been shown for antibodies targeting α -toxin [55]. On the other hand, the study of AP4–24H11 helped identify epitopes for the generation of virus-like particles (VLP) with specificity towards AIP IV [108]. Importantly, active immunization with AIP IV-specific VLPs effectively reduced the severity of dermonecrosis in a murine model of SSTI, similar to passive immunization with AP4–24H11 [107]. While

both AP4–24H11 and AIP IV-targeted VLPs are efficacious against staphylococcal infection *in vivo*, their exclusive specificity for the interaction with AIP IV limits their utility against *S. aureus* infections by other Agr groups [109].

5.5 AgrA

Several small molecules that are proposed to bind to the phosphoryl-binding pocket on the N-terminal domain of the cytoplasmic response regulator AgrA were recently described [110]. As no crystal structure information for the N-terminal domain of AgrA (AgrA_N) is available, the authors used the crystal structure of a similar transcriptional regulator from *Aquifex aeolicus* as a model for discovering those molecules using *in silico* modeling. The findings from this study are especially interesting, because a series of naphthalene derivatives and biaryl compounds were found to inhibit *maIII* and *psma* transcription in *S. aureus* without affecting bacterial growth. The authors also demonstrated a reduction in α -toxin expression in the culture supernatants of compound-treated *S. aureus* USA300 cultures, and, accordingly a reduction in hemolytic activity towards rabbit erythrocytes [110]. Interestingly, an FDA-approved non-steroidal anti-inflammatory drug, diflunisal, was highlighted in their screen (Fig. 2).

On the other hand, the crystal structure of the AgrA C-terminal LytTR DNA binding (AgrA_C) domain is resolved [111]. With this information, three compounds were identified from a library of small, low-complexity ligands that interfered with the ability of AgrA_C to bind to DNA as demonstrated by electrophoretic mobility shift assays. However, evidence that these molecules modulated the transcription of *agr*-controlled genes was not presented.

The molecule savirin (*S. aureus* virulence inhibitor) (Fig. 2), was identified to be a potent modulator of Agr-regulated toxin gene transcription and expression across all four *S. aureus* Agr groups without affecting cell viability [112]. Moreover, savirin is the first ever described molecule to display *S. aureus*-specific Agr inhibition, inasmuch as Agr activity of the *S. epidermidis* strain 12228 (*agr* group I) was not affected. Importantly, no resistance was developed by *S. aureus* after multiple passages with savirin. As far as we know, the mechanism of inhibition by savirin is mediated solely by interfering with AgrA binding to DNA [112].

5.6 In vivo interference

Although a number of effective inhibitors of the *S. aureus* Agr system have been described thus far, only a few of those inhibitors have been assessed for efficacy in animal models of *S. aureus* disease. The efficacy of a heterologous AIP to protect from disease caused by a different *S. aureus* Agr group was demonstrated in a mouse model of SSTI [73, 75]. In that study, a significant reduction in the size and severity of dermonecrosis was found in mice co-injected with 5 or 10 μ g of a synthetic AIP II and an *S. aureus* Agr group I strain, compared to mice injected with bacteria alone.

Similarly, a significant reduction in abscess size and dermonecrosis was observed in mice when 10 μ g of savirin was co-injected with the USA300 strain LAC compared to control mice [112]. Delayed administration of savirin reduced the severity of dermonecrosis, but not total abscess size, and promoted bacterial clearance from the skin and spleen. Moreover, in a

murine air pouch infection model, a reduction in bacterial load was observed in the air pouch and kidneys when savirin was administered at the same time as the bacteria [112]. These impressive findings would place savirin as one of the most promising molecules for therapeutic development targeted at Agr QS to date.

The AHL derivative C14-TOA 17 (Fig. 2) afforded protection in mice against *S. aureus*-mediated arthritis [91]. In this study, mice were given a daily dose of 10 mg/kg of C14-TOA 17 for 14 days post intravenous infection with *S. aureus* strain Newman. A significant reduction in the frequency and severity of arthritis, as determined by visible erythema and/or swelling of at least one joint, was observed in C14-TOA 17-treated mice compared with PBS-treated control mice. Interestingly, C14-TOA 17 did not reduce bacterial load in the kidneys, which potentially is due to the observations that Agr dysfunction is associated with pathogen success in bacteremia and hematogenous seeding [67].

5.7 luxS/AI-2

The red seaweed, *Delisea pulchra* (Rhodophyta) produces a number of halogenated furanones [113], which are structurally similar to AHLs and were shown to interfere with biofilm formation, virulence factor production, and pathogenesis in a number of Gram-negative [114–117] and Gram-positive bacteria that possess functional AHL-dependent QS systems [118–121]. Although *S. epidermidis* does not use AHL QS, furanone derivatives and sulphur analogues of furanones (thiophenones) were shown to inhibit biofilm formation by *S. epidermidis* at sub-inhibitory concentrations [122, 123]. In contrast, different furanone derivatives enhance biofilm formation of both *S. epidermidis* and *S. aureus* [124]. The differences in phenotypes reported by these two studies could be due to the subtle modifications of the furanones used in each study (Fig. 2) [125]. Clearly, the precise mechanisms underlying the interference of AHLs with staphylococcal biofilm formation still need to be elucidated. Notably, bacterial resistance has been shown to develop towards furanones as a consequence of their bactericidal activity [126].

5.8 “RNAIII-inhibiting peptide” (“RIP”)

Reports about “RIP” (for RNAIII Inhibitory Peptide), which allegedly caused RNAIII-dependent toxin and biofilm inhibition, surfaced in the mid-1990’s [127]. Notably, this peptide was claimed to inhibit Agr, not directly RNAIII, as the name would suggest. Much controversy surrounds RIP as the reported activities of RIP have never been independently reproduced in other laboratories [128]. The most paradoxical effect allegedly induced by RIP is the inhibition of biofilm formation, a phenomenon known to contradict many studies that demonstrate increased biofilm formation in the absence of Agr activity [49–51]. Despite this, in numerous studies RIP was reported to limit biofilm-related catheter infections *in vivo* [129, 130], a mechanism potentially related to amphipathic detergent-like activity that many peptides exhibit.

6. Conclusions

Given the lack of antibiotic development over the past thirty years and the increasing number of resistant bacteria, it is imperative that new approaches be explored to combat this

growing health concern. In particular, the development of therapeutic methods to target bacterial virulence factors has gained popularity. Anti-virulence therapies provide a novel mode of action to combat infection, differing from the classic targeting of bacterial structural components or replicating machinery and potentially decreasing the development of resistance owing to their non-bactericidal mechanism.

In particular, anti-virulence therapies that target bacterial QS systems have been explored, as they often comprise the regulation of key virulence determinants. In the case of *S. aureus*, Agr is the best-studied QS and virulence regulating system. It has a demonstrated key role in many acute forms of *S. aureus* disease, why the more recent past has seen a considerable interest in targeting this system for anti-virulence drug development.

The response regulator AgrA and Agr AIP-signaling stand out as the most promising targets for anti-Agr drug development to date; several drugs that target AgrA have been developed, which have the advantage of blocking Agr-dependent virulence development in all Agr groups. Targeting AIPs carries the advantage of influencing QS extracellularly, and potentially avoiding complications of cellular uptake and degradation that intracellular therapies, such as those targeting AgrA, may encounter. On the other hand, the stability of AIPs and their hypervariable nature provide their own set of complications to effectively inactivate all Agr groups. Alteration of specific AIP components/residues has not yet ensured complete QS disruption for all Agr groups. Nevertheless, there are promising results indicating that QS-targeted anti-virulence therapies may soon become a new clinical option for combating *S. aureus* infection.

7. Expert opinion

Targeting the Agr QS of *S. aureus* is not without challenges. The inhibitory activities of Agr-inhibiting compounds have generally been tested against only one staphylococcal strain. Therefore, it is unknown whether the compounds target a sufficient number of Agr subgroups or other staphylococcal species, such as *S. epidermidis*. Recent reports on the beneficial role of *S. epidermidis* as part of the human microflora in limiting overgrowth of the more pathogenic *S. aureus* [131] may suggest that Agr inhibition specifically of *S. aureus*, but not *S. epidermidis*, represents a valuable strategy for the development of Agr inhibitors. Moreover, with biofilm formation representing the most important virulence determinant of *S. epidermidis* [132], and biofilms being negatively impacted by Agr, the development of Agr inhibitors against *S. epidermidis* infection appears counterproductive, a situation likely similar for most coagulase-negative staphylococcal species. The same limitation for the use of Agr inhibitors applies to chronic, biofilm-associated infection by *S. aureus* and most likely, cases of *S. aureus* bacteremia [60]. The latter may potentially be linked to observations indicating that the production of fewer pro-inflammatory signals, as in strains with low-activity or dysfunctional Agr, helps staphylococci to evade recognition and elimination by the innate immune system [133]. Thus, attenuating Agr activity would only be beneficial in some cases of acute staphylococcal infection, in which loss of toxin production severely hampers the progression and severity of disease. Notwithstanding those caveats about the limitations of applicability of Agr inhibitors, much progress has been made recently in the identification of synthetic and natural products inhibiting Agr, especially

those interfering with AgrC AIP receptor recognition and activity of the response regulator AgrA.

Owing to the inhibitory overall effect that Agr has on biofilm development, all Agr-inhibiting compounds that are meant to repress key Agr-dependent virulence factors, such as PSMs and α -toxin, are also prone to increase biofilm formation as an undesirable side effect. However, potential side effects are not always jointly investigated with the desirable phenotypes, in particular using dedicated animal infection models. Furthermore, insufficient information on the stability, chemical structure, basis of the Agr inhibition, in-vitro and in-vivo toxicity, induction of bacterial resistance, and the bactericidal activity towards different *S. aureus* Agr groups, other staphylococci, and other microorganisms, make it difficult to ascertain the merit of novel Agr-inhibiting compounds. Thus, there is a clear need for researchers to provide more of these types of data.

Lastly, although several compounds show promising therapeutic treatment of different types of acute staphylococcal disease in animal models of infection, such as SSTIs, many of these drugs have not reached Phase I trials. Thus, pre-clinical pharmacology and toxicology data are regrettably scarce.

In conclusion, selectively inhibiting the QS systems of *S. aureus* is warranted, especially in an era of heightened bacterial resistance to multiple antibiotics. However, the biggest challenge that these compounds face, in addition to those mentioned above, will be their safety for use in humans and how it will be best to administer the drugs for a maximum therapeutic effect against the many facets of acute staphylococcal disease, without upsetting the delicate balance of the human resident microflora.

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Acronyms

Agr	Accessory gene regulator
AgrA	Response regulator
AgrB	Membrane-associated export protein, processes AgrD into AIP
AgrC	Membrane-bound histidine kinase receptor
AgrD	Propeptide gene for AIP
AHL	Acyl-homoserine lactone
AIP	Auto-inducing peptide
hla	Gene coding for α -toxin

HA/CA-MRSA	Hospital-associated/Community-associated methicillin-resistant <i>Staphylococcus aureus</i>
hld	Gene coding for δ -toxin
HSL	Homo-serine lactone
mAb	Monoclonal antibody
PIA	Polysaccharide intercellular adhesin
PSM	Phenol-soluble modulins
QS	Quorum-sensing
RIP	RNA-inhibiting peptide
spa	Gene coding for protein A
SSTI	Skin and soft tissue infections
TCS	Two-component system
TSST	Toxic shock syndrome toxin
VLP	Virus-like particles

Bibliography

Papers of special note have been highlighted as either of interest (*) or of considerable interest (**) to readers.

1. WHO. Antimicrobial Resistance Global Report on Surveillance 2014. 2014.
2. CDC. Antibiotic resistance threats in the United States, 2013, 2013.
3. DeLeo FR, Otto M, Kreiswirth BN, Chambers HF. Community-associated methicillin-resistant *Staphylococcus aureus*. Lancet 2010;375:1557–68. [PubMed: 20206987]
4. Lowy FD. *Staphylococcus aureus* infections. N Engl J Med 1998;339:520–32. [PubMed: 9709046]
5. Rooijackers SH, van Kessel KP, van Strijp JA. Staphylococcal innate immune evasion. Trends Microbiol 2005;13:596–601. [PubMed: 16242332]
6. Foster TJ, Hook M. Surface protein adhesins of *Staphylococcus aureus*. Trends Microbiol 1998;6:484–8. [PubMed: 10036727]
7. Otto M *Staphylococcus aureus* toxins. Curr Opin Microbiol 2014;17:32–7. [PubMed: 24581690]
8. Schlievert PM. Role of superantigens in human disease. J Infect Dis 1993;167:997–1002. [PubMed: 8486972]
9. Wang R, Braughton KR, Kretschmer D, et al. Identification of novel cytolytic peptides as key virulence determinants for community-associated MRSA. Nat Med 2007;13:1510–4. [PubMed: 17994102]
10. Surewaard BG, de Haas CJ, Vervoort F, et al. Staphylococcal alpha-phenol soluble modulins contribute to neutrophil lysis after phagocytosis. Cell Microbiol 2013;15:1427–37. [PubMed: 23470014]
11. Otto M Bacterial evasion of antimicrobial peptides by biofilm formation. Curr Top Microbiol Immunol 2006;306:251–8. [PubMed: 16909925]
12. Otto M Staphylococcal biofilms. Curr Top Microbiol Immunol 2008;322:207–28. [PubMed: 18453278]

13. Nealson KH, Hastings JW. Bacterial bioluminescence: its control and ecological significance. *Microbiol Rev* 1979;43:496–518. [PubMed: 396467]
14. Passador L, Cook JM, Gambello MJ, et al. Expression of *Pseudomonas aeruginosa* virulence genes requires cell-to-cell communication. *Science* 1993;260:1127–30. [PubMed: 8493556]
15. Recsei P, Kreiswirth B, O'Reilly M, et al. Regulation of exoprotein gene expression in *Staphylococcus aureus* by agr. *Mol Gen Genet* 1986;202:58–61. [PubMed: 3007938] *First report on Agr controlling toxin and exoprotein expression in *S. aureus*.
16. Cassat J, Dunman PM, Murphy E, et al. Transcriptional profiling of a *Staphylococcus aureus* clinical isolate and its isogenic agr and sarA mutants reveals global differences in comparison to the laboratory strain RN6390. *Microbiology* 2006;152:3075–90. [PubMed: 17005987]
17. Yao Y, Vuong C, Kocianova S, et al. Characterization of the *Staphylococcus epidermidis* accessory-gene regulator response: quorum-sensing regulation of resistance to human innate host defense. *J Infect Dis* 2006;193:841–8. [PubMed: 16479519]
18. Zhang L, Lin J, Ji G. Membrane anchoring of the AgrD N-terminal amphipathic region is required for its processing to produce a quorum-sensing pheromone in *Staphylococcus aureus*. *J Biol Chem* 2004;279:19448–56. [PubMed: 15001569]
19. Thoendel M, Kavanaugh JS, Flack CE, Horswill AR. Peptide signaling in the staphylococci. *Chem Rev* 2011;111:117–51. [PubMed: 21174435]
20. Ji G, Beavis RC, Novick RP. Cell density control of staphylococcal virulence mediated by an octapeptide pheromone. *Proc Natl Acad Sci U S A* 1995;92:12055–9. [PubMed: 8618843]
21. Otto M, Sussmuth R, Jung G, Gotz F. Structure of the pheromone peptide of the *Staphylococcus epidermidis* agr system. *FEBS Lett* 1998;424:89–94. [PubMed: 9537521]
22. Kavanaugh JS, Thoendel M, Horswill AR. A role for type I signal peptidase in *Staphylococcus aureus* quorum sensing. *Mol Microbiol* 2007;65:780–98. [PubMed: 17608791]
23. Lina G, Jarraud S, Ji G, et al. Transmembrane topology and histidine protein kinase activity of AgrC, the agr signal receptor in *Staphylococcus aureus*. *Mol Microbiol* 1998;28:655–62. [PubMed: 9632266]
24. Lyon GJ, Mayville P, Muir TW, Novick RP. Rational design of a global inhibitor of the virulence response in *Staphylococcus aureus*, based in part on localization of the site of inhibition to the receptor-histidine kinase, AgrC. *Proc Natl Acad Sci U S A* 2000;97:13330–5. [PubMed: 11087872] **Detailed study on the design of AIP analogues with inhibiting activity toward all *S. aureus* Agr groups.
25. George Cisar EA, Geisinger E, Muir TW, Novick RP. Symmetric signalling within asymmetric dimers of the *Staphylococcus aureus* receptor histidine kinase AgrC. *Mol Microbiol* 2009;74:44–57. [PubMed: 19708918]
26. Koenig RL, Ray JL, Maleki SJ, et al. *Staphylococcus aureus* AgrA binding to the RNAIII-agr regulatory region. *J Bacteriol* 2004;186:7549–55. [PubMed: 15516566]
27. Novick RP. Autoinduction and signal transduction in the regulation of staphylococcal virulence. *Mol Microbiol* 2003;48:1429–49. [PubMed: 12791129]
28. Jarraud S, Lyon GJ, Figueiredo AM, et al. Exfoliatin-producing strains define a fourth agr specificity group in *Staphylococcus aureus*. *J Bacteriol* 2000;182:6517–22. [PubMed: 11053400]
29. Ji G, Beavis R, Novick RP. Bacterial interference caused by autoinducing peptide variants. *Science* 1997;276:2027–30. [PubMed: 9197262] **Groundbreaking study describing cross-inhibitory activity of *S. aureus* Agr AIPs.
30. Geisinger E, Chen J, Novick RP. Allele-dependent differences in quorum-sensing dynamics result in variant expression of virulence genes in *Staphylococcus aureus*. *J Bacteriol* 2012;194:2854–64. [PubMed: 22467783]
31. Cheung AL, Nishina KA, Trottona MP, Tamber S. The SarA protein family of *Staphylococcus aureus*. *Int J Biochem Cell Biol* 2008;40:355–61. [PubMed: 18083623]
32. Lauderdale KJ, Boles BR, Cheung AL, Horswill AR. Interconnections between Sigma B, agr, and proteolytic activity in *Staphylococcus aureus* biofilm maturation. *Infect Immun* 2009;77:1623–35. [PubMed: 19188357]

33. Shaw LN, Aish J, Davenport JE, et al. Investigations into sigmaB-modulated regulatory pathways governing extracellular virulence determinant production in *Staphylococcus aureus*. J Bacteriol 2006;188:6070–80. [PubMed: 16923874]
34. Sun F, Liang H, Kong X, et al. Quorum-sensing agr mediates bacterial oxidation response via an intramolecular disulfide redox switch in the response regulator AgrA. Proc Natl Acad Sci U S A 2012;109:9095–100. [PubMed: 22586129]
35. Fournier B, Klier A, Rapoport G. The two-component system ArlS-ArlR is a regulator of virulence gene expression in *Staphylococcus aureus*. Mol Microbiol 2001;41:247–61. [PubMed: 11454217]
36. Giraudo AT, Calzolari A, Cataldi AA, et al. The *sae* locus of *Staphylococcus aureus* encodes a two-component regulatory system. FEMS Microbiol Lett 1999;177:15–22. [PubMed: 10436918]
37. Giraudo AT, Cheung AL, Nagel R. The *sae* locus of *Staphylococcus aureus* controls exoprotein synthesis at the transcriptional level. Arch Microbiol 1997;168:53–8. [PubMed: 9211714]
38. Throup JP, Zappacosta F, Lunsford RD, et al. The *srhSR* gene pair from *Staphylococcus aureus*: genomic and proteomic approaches to the identification and characterization of gene function. Biochemistry 2001;40:10392–401. [PubMed: 11513618]
39. Yarwood JM, McCormick JK, Schlievert PM. Identification of a novel two-component regulatory system that acts in global regulation of virulence factors of *Staphylococcus aureus*. J Bacteriol 2001;183:1113–23. [PubMed: 11157922]
40. Queck SY, Jameson-Lee M, Villaruz AE, et al. RNAIII-independent target gene control by the agr quorum-sensing system: insight into the evolution of virulence regulation in *Staphylococcus aureus*. Mol Cell 2008;32:150–8. [PubMed: 18851841] *Reveals RNA-independent target gene regulation by Agr (with PSMs as main such targets).
41. Cheung AL, Bayer AS, Zhang G, et al. Regulation of virulence determinants in vitro and in vivo in *Staphylococcus aureus*. FEMS Immunol Med Microbiol 2004;40:1–9. [PubMed: 14734180]
42. Novick RP, Ross HF, Projan SJ, et al. Synthesis of staphylococcal virulence factors is controlled by a regulatory RNA molecule. Embo j 1993;12:3967–75. [PubMed: 7691599] *Reveals the gene regulatory function of RNAIII in the Agr system.
43. Peschel A, Otto M. Phenol-soluble modulins and staphylococcal infection. Nat Rev Microbiol 2013;11:667–73. [PubMed: 24018382]
44. Cheung GY, Rigby K, Wang R, et al. *Staphylococcus epidermidis* strategies to avoid killing by human neutrophils. PLoS Pathog 2010;6:e1001133. [PubMed: 20949069]
45. Cheung GY, Wang R, Khan BA, et al. Role of the accessory gene regulator agr in community-associated methicillin-resistant *Staphylococcus aureus* pathogenesis. Infect Immun 2011;79:1927–35. [PubMed: 21402769]
46. Boisset S, Geissmann T, Huntzinger E, et al. *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. Genes Dev 2007;21:1353–66. [PubMed: 17545468]
47. McNamara PJ, Milligan-Monroe KC, Khalili S, Proctor RA. Identification, cloning, and initial characterization of rot, a locus encoding a regulator of virulence factor expression in *Staphylococcus aureus*. J Bacteriol 2000;182:3197–203. [PubMed: 10809700]
48. Said-Salim B, Dunman PM, McAleese FM, et al. Global regulation of *Staphylococcus aureus* genes by Rot. J Bacteriol 2003;185:610–9. [PubMed: 12511508]
49. Periasamy S, Joo HS, Duong AC, et al. How *Staphylococcus aureus* biofilms develop their characteristic structure. Proc Natl Acad Sci U S A 2012;109:1281–6. [PubMed: 22232686]
50. Vuong C, Gerke C, Somerville GA, et al. Quorum-sensing control of biofilm factors in *Staphylococcus epidermidis*. J Infect Dis 2003;188:706–18. [PubMed: 12934187]
51. Wang R, Khan BA, Cheung GY, et al. *Staphylococcus epidermidis* surfactant peptides promote biofilm maturation and dissemination of biofilm-associated infection in mice. J Clin Invest 2011;121:238–48. [PubMed: 21135501]
52. Vuong C, Kocianova S, Yao Y, et al. Increased colonization of indwelling medical devices by quorum-sensing mutants of *Staphylococcus epidermidis* in vivo. J Infect Dis 2004;190:1498–505. [PubMed: 15378444]

53. Vuong C, Saenz HL, Gotz F, Otto M. Impact of the agr quorum-sensing system on adherence to polystyrene in *Staphylococcus aureus*. J Infect Dis 2000;182:1688–93. [PubMed: 11069241] *First report showing that Agr has an overall negative rather than positive impact on biofilm formation.
54. Li M, Villaruz AE, Vadyvaloo V, et al. AI-2-dependent gene regulation in *Staphylococcus epidermidis*. BMC Microbiol 2008;8:4. [PubMed: 18182108]
55. Kennedy AD, Bubeck Wardenburg J, Gardner DJ, et al. Targeting of alpha-hemolysin by active or passive immunization decreases severity of USA300 skin infection in a mouse model. J Infect Dis 2010;202:1050–8. [PubMed: 20726702]
56. Montgomery CP, Boyle-Vavra S, Daum RS. Importance of the global regulators Agr and SaeRS in the pathogenesis of CA-MRSA USA300 infection. PLoS One 2010;5:e15177. [PubMed: 21151999]
57. Bubeck Wardenburg J, Patel RJ, Schneewind O. Surface proteins and exotoxins are required for the pathogenesis of *Staphylococcus aureus* pneumonia. Infect Immun 2007;75:1040–4. [PubMed: 17101657]
58. Heyer G, Saba S, Adamo R, et al. *Staphylococcus aureus* agr and sarA functions are required for invasive infection but not inflammatory responses in the lung. Infect Immun 2002;70:127–33. [PubMed: 11748173]
59. Cheung AL, Eberhardt KJ, Chung E, et al. Diminished virulence of a sar-/agr- mutant of *Staphylococcus aureus* in the rabbit model of endocarditis. J Clin Invest 1994;94:1815–22. [PubMed: 7962526]
60. Painter KL, Krishna A, Wigneshweraraj S, Edwards AM. What role does the quorum-sensing accessory gene regulator system play during *Staphylococcus aureus* bacteremia? Trends Microbiol 2014;22:676–85. [PubMed: 25300477]
61. Peterson MM, Mack JL, Hall PR, et al. Apolipoprotein B Is an innate barrier against invasive *Staphylococcus aureus* infection. Cell Host Microbe 2008;4:555–66. [PubMed: 19064256]
62. Kernbauer E, Maurer K, Torres VJ, et al. Gastrointestinal dissemination and transmission of *Staphylococcus aureus* following bacteremia. Infect Immun 2014.
63. Periasamy S, Joo HS, Duong AC, et al. How *Staphylococcus aureus* biofilms develop their characteristic structure. Proc Natl Acad Sci USA 2012;109:1281–86. [PubMed: 22232686] *Describes the mechanistic details underlying the impact of Agr on biofilm formation.
64. Somerville GA, Beres SB, Fitzgerald JR, et al. In vitro serial passage of *Staphylococcus aureus*: changes in physiology, virulence factor production, and agr nucleotide sequence. J Bacteriol 2002;184:1430–7. [PubMed: 11844774]
65. Shopsin B, Drlica-Wagner A, Mathema B, et al. Prevalence of agr dysfunction among colonizing *Staphylococcus aureus* strains. J Infect Dis 2008;198:1171–4. [PubMed: 18752431]
66. Gagnaire J, Dauwalder O, Boisset S, et al. Detection of *Staphylococcus aureus* delta-toxin production by whole-cell MALDI-TOF mass spectrometry. PLoS One 2012;7:e40660. [PubMed: 22792394]
67. Fowler VG, Jr., Sakoulas G, McIntyre LM, et al. Persistent bacteremia due to methicillin-resistant *Staphylococcus aureus* infection is associated with agr dysfunction and low-level in vitro resistance to thrombin-induced platelet microbicidal protein. J Infect Dis 2004;190:1140–9. [PubMed: 15319865] *Describes that Agr dysfunction is associated with increased pathogen success during *S. aureus* bacteremia.
68. Abdelnour A, Arvidson S, Bremell T, et al. The accessory gene regulator (agr) controls *Staphylococcus aureus* virulence in a murine arthritis model. Infect Immun 1993;61:3879–85. [PubMed: 8359909]
69. Shopsin B, Eaton C, Wasserman GA, et al. Mutations in agr do not persist in natural populations of methicillin-resistant *Staphylococcus aureus*. J Infect Dis 2010;202:1593–9. [PubMed: 20942648]
70. McDowell P, Affas Z, Reynolds C, et al. Structure, activity and evolution of the group I thiolactone peptide quorum-sensing system of *Staphylococcus aureus*. Mol Microbiol 2001;41:503–12. [PubMed: 11489134]
71. Lyon GJ, Wright JS, Muir TW, Novick RP. Key determinants of receptor activation in the agr autoinducing peptides of *Staphylococcus aureus*. Biochemistry 2002;41:10095–104. [PubMed: 12146974] *Study elucidating structural details of AIP-AgrC interaction.

72. Scott RJ, Lian LY, Muharram SH, et al. Side-chain-to-tail thiolactone peptide inhibitors of the staphylococcal quorum-sensing system. *Bioorg Med Chem Lett* 2003;13:2449–53. [PubMed: 12852941]
73. Wright JS, 3rd, Jin R, Novick RP. Transient interference with staphylococcal quorum sensing blocks abscess formation. *Proc Natl Acad Sci U S A* 2005;102:1691–6. [PubMed: 15665088]
74. Fowler SA, Stacy DM, Blackwell HE. Design and synthesis of macrocyclic peptomers as mimics of a quorum sensing signal from *Staphylococcus aureus*. *Org Lett* 2008;10:2329–32. [PubMed: 18476747]
75. Mayville P, Ji G, Beavis R, et al. Structure-activity analysis of synthetic autoinducing thiolactone peptides from *Staphylococcus aureus* responsible for virulence. *Proc Natl Acad Sci U S A* 1999;96:1218–23. [PubMed: 9990004] **Detailed structure-function analysis of Agr AIPs.
76. Novick RP, Geisinger E. Quorum sensing in staphylococci. *Annu Rev Genet* 2008;42:541–64. [PubMed: 18713030]
77. George EA, Novick RP, Muir TW. Cyclic peptide inhibitors of staphylococcal virulence prepared by Fmoc-based thiolactone peptide synthesis. *J Am Chem Soc* 2008;130:4914–24. [PubMed: 18335939]
78. Tal-Gan Y, Stacy DM, Blackwell HE. N-Methyl and peptoid scans of an autoinducing peptide reveal new structural features required for inhibition and activation of AgrC quorum sensing receptors in *Staphylococcus aureus*. *Chem Commun (Camb)* 2014;50:3000–3. [PubMed: 24509706]
79. Tal-Gan Y, Stacy DM, Foegen MK, et al. Highly potent inhibitors of quorum sensing in *Staphylococcus aureus* revealed through a systematic synthetic study of the group-III autoinducing peptide. *J Am Chem Soc* 2013;135:7869–82. [PubMed: 23647400] *Systematic screen for Agr inhibitors based on mimetics of AIP III.
80. Broderick AH, Stacy DM, Tal-Gan Y, et al. Surface coatings that promote rapid release of peptide-based AgrC inhibitors for attenuation of quorum sensing in *Staphylococcus aureus*. *Adv Healthc Mater* 2014;3:97–105. [PubMed: 23813683]
81. Melamed Yerushalmi S, Buck ME, Lynn DM, et al. Multivalent alteration of quorum sensing in *Staphylococcus aureus*. *Chem Commun (Camb)* 2013;49:5177–9. [PubMed: 23628938]
82. Dufour P, Jarraud S, Vandenesch F, et al. High genetic variability of the agr locus in *Staphylococcus* species. *J Bacteriol* 2002;184:1180–6. [PubMed: 11807079]
83. Olson ME, Todd DA, Schaeffer CR, et al. *Staphylococcus epidermidis* agr quorum-sensing system: signal identification, cross talk, and importance in colonization. *J Bacteriol* 2014;196:3482–93. [PubMed: 25070736]
84. Otto M, Sussmuth R, Vuong C, et al. Inhibition of virulence factor expression in *Staphylococcus aureus* by the *Staphylococcus epidermidis* agr pheromone and derivatives. *FEBS Lett* 1999;450:257–62. [PubMed: 10359085]
85. Otto M, Echner H, Voelter W, Gotz F. Pheromone cross-inhibition between *Staphylococcus aureus* and *Staphylococcus epidermidis*. *Infect Immun* 2001;69:1957–60. [PubMed: 11179383] *First study describing inter-species cross-inhibition in staphylococci based on Agr AIPs.
86. Ji G, Pei W, Zhang L, et al. *Staphylococcus intermedius* produces a functional agr autoinducing peptide containing a cyclic lactone. *J Bacteriol* 2005;187:3139–50. [PubMed: 15838041]
87. Lyczak JB, Cannon CL, Pier GB. Establishment of *Pseudomonas aeruginosa* infection: lessons from a versatile opportunist. *Microbes Infect* 2000;2:1051–60. [PubMed: 10967285]
88. Winson MK, Camara M, Latifi A, et al. Multiple N-acyl-L-homoserine lactone signal molecules regulate production of virulence determinants and secondary metabolites in *Pseudomonas aeruginosa*. *Proc Natl Acad Sci U S A* 1995;92:9427–31. [PubMed: 7568146]
89. Pearson JP, Gray KM, Passador L, et al. Structure of the autoinducer required for expression of *Pseudomonas aeruginosa* virulence genes. *Proc Natl Acad Sci U S A* 1994;91:197–201. [PubMed: 8278364]
90. Qazi S, Middleton B, Muharram SH, et al. N-acylhomoserine lactones antagonize virulence gene expression and quorum sensing in *Staphylococcus aureus*. *Infect Immun* 2006;74:910–9. [PubMed: 16428734]

91. Murray EJ, Crowley RC, Truman A, et al. Targeting *Staphylococcus aureus* quorum sensing with nonpeptidic small molecule inhibitors. *J Med Chem* 2014;57:2813–9. [PubMed: 24592914]
92. Holden MT, Ram Chhabra S, de Nys R, et al. Quorum-sensing cross talk: isolation and chemical characterization of cyclic dipeptides from *Pseudomonas aeruginosa* and other gram-negative bacteria. *Mol Microbiol* 1999;33:1254–66. [PubMed: 10510239]
93. Park DK, Lee KE, Baek CH, et al. Cyclo(Phe-Pro) modulates the expression of ompU in *Vibrio spp.* *J Bacteriol* 2006;188:2214–21. [PubMed: 16513751]
94. Degrassi G, Aguilar C, Bosco M, et al. Plant growth-promoting *Pseudomonas putida* WCS358 produces and secretes four cyclic dipeptides: cross-talk with quorum sensing bacterial sensors. *Curr Microbiol* 2002;45:250–4. [PubMed: 12192521]
95. Li J, Wang W, Xu SX, et al. *Lactobacillus reuteri*-produced cyclic dipeptides quench agr-mediated expression of toxic shock syndrome toxin-1 in staphylococci. *Proc Natl Acad Sci U S A* 2011;108:3360–5. [PubMed: 21282650] *Describes the Agr-inhibiting potential of cyclic dipeptide compounds from a non-Staphylococcus Gram-positive species, potentially limiting the pathogenesis of toxic shock syndrome in a probiotic fashion.
96. Mansson M, Nielsen A, Kjaerulff L, et al. Inhibition of virulence gene expression in *Staphylococcus aureus* by novel depsipeptides from a marine photobacterium. *Mar Drugs* 2011;9:2537–52. [PubMed: 22363239]
97. Nielsen A, Mansson M, Bojer MS, et al. Solonamide B inhibits quorum sensing and reduces *Staphylococcus aureus* mediated killing of human neutrophils. *PLoS One* 2014;9:e84992. [PubMed: 24416329]
98. Nakayama J, Uemura Y, Nishiguchi K, et al. Ambuic acid inhibits the biosynthesis of cyclic peptide quormones in gram-positive bacteria. *Antimicrob Agents Chemother* 2009;53:580–6. [PubMed: 19015326]
99. Figueroa M, Jarmusch AK, Raja HA, et al. Polyhydroxyanthraquinones as quorum sensing inhibitors from the guttates of *Penicillium restrictum* and their analysis by desorption electrospray ionization mass spectrometry. *J Nat Prod* 2014;77:1351–8. [PubMed: 24911880]
100. Desouky SE, Nishiguchi K, Zendo T, et al. High-throughput screening of inhibitors targeting Agr/Fsr quorum sensing in *Staphylococcus aureus* and *Enterococcus faecalis*. *Biosci Biotechnol Biochem* 2013;77:923–7. [PubMed: 23649251]
101. Moran A, Gutierrez S, Martinez-Blanco H, et al. Non-toxic plant metabolites regulate *Staphylococcus* viability and biofilm formation: a natural therapeutic strategy useful in the treatment and prevention of skin infections. *Biofouling* 2014;30:1175–82. [PubMed: 25397362]
102. Qiu J, Jiang Y, Xia L, et al. Subinhibitory concentrations of licochalcone A decrease alpha-toxin production in both methicillin-sensitive and methicillin-resistant *Staphylococcus aureus* isolates. *Lett Appl Microbiol* 2010;50:223–9. [PubMed: 20025648]
103. Qiu J, Wang D, Xiang H, et al. Subinhibitory concentrations of thymol reduce enterotoxins A and B and alpha-hemolysin production in *Staphylococcus aureus* isolates. *PLoS One* 2010;5:e9736. [PubMed: 20305813]
104. Qiu J, Li H, Meng H, et al. Impact of luteolin on the production of alpha-toxin by *Staphylococcus aureus*. *Lett Appl Microbiol* 2011;53:238–43. [PubMed: 21671964]
105. Luo M, Qiu J, Zhang Y, et al. alpha-cyperone alleviates lung cell injury caused by *Staphylococcus aureus* via attenuation of alpha-hemolysin expression. *J Microbiol Biotechnol* 2012;22:1170–6. [PubMed: 22713997]
106. Rudkin JK, Edwards AM, Bowden MG, et al. Methicillin resistance reduces the virulence of healthcare-associated methicillin-resistant *Staphylococcus aureus* by interfering with the agr quorum sensing system. *J Infect Dis* 2012;205:798–806. [PubMed: 22301683]
107. Park J, Jagasia R, Kaufmann GF, et al. Infection control by antibody disruption of bacterial quorum sensing signaling. *Chem Biol* 2007;14:1119–27. [PubMed: 17961824] **Describes the potential of monoclonal antibodies toward AIPs to inhibit Agr.
108. O'Rourke JP, Daly SM, Triplett KD, et al. Development of a Mimotope Vaccine Targeting the *Staphylococcus aureus* Quorum Sensing Pathway. *PLoS One* 2014;9:e111198. [PubMed: 25379726]

109. Kirchdoerfer RN, Garner AL, Flack CE, et al. Structural basis for ligand recognition and discrimination of a quorum-quenching antibody. *J Biol Chem* 2011;286:17351–8. [PubMed: 21454495]
110. Khodaverdian V, Pesho M, Truitt B, et al. Discovery of antivirulence agents against methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2013;57:3645–52. [PubMed: 23689713]
111. Leonard PG, Bezar IF, Sidote DJ, Stock AM. Identification of a hydrophobic cleft in the LytTR domain of AgrA as a locus for small molecule interactions that inhibit DNA binding. *Biochemistry* 2012;51:10035–43. [PubMed: 23181972]
112. Sully EK, Malachowa N, Elmore BO, et al. Selective chemical inhibition of agr quorum sensing in *Staphylococcus aureus* promotes host defense with minimal impact on resistance. *PLoS Pathog* 2014;10:e1004174. [PubMed: 24945495] **Study on the development of one of the most promising Agr inhibitors to date, savirin.
113. de Nys R, Steinber PD, Willemsen P, et al. Broad spectrum effects of secondary metabolites from the red alga *Delisea pulchra* in antifouling assays. *Biofouling* 1995;8:259–71.
114. Hentzer M, Givskov M. Pharmacological inhibition of quorum sensing for the treatment of chronic bacterial infections. *J Clin Invest* 2003;112:1300–7. [PubMed: 14597754]
115. Kim C, Kim J, Park HY, et al. Furanone derivatives as quorum-sensing antagonists of *Pseudomonas aeruginosa*. *Appl Microbiol Biotechnol* 2008;80:37–47. [PubMed: 18566810]
116. Janssens JC, Steenackers H, Robijns S, et al. Brominated furanones inhibit biofilm formation by *Salmonella enterica* serovar *Typhimurium*. *Appl Environ Microbiol* 2008;74:6639–48. [PubMed: 18791004]
117. Ren D, Sims JJ, Wood TK. Inhibition of biofilm formation and swarming of *Escherichia coli* by (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone. *Environ Microbiol* 2001;3:731–6. [PubMed: 11846763]
118. Jones MB, Jani R, Ren D, et al. Inhibition of *Bacillus anthracis* growth and virulence-gene expression by inhibitors of quorum-sensing. *J Infect Dis* 2005;191:1881–8. [PubMed: 15871122]
119. Lonn-Stensrud J, Petersen FC, Benneche T, Scheie AA. Synthetic bromated furanone inhibits autoinducer-2-mediated communication and biofilm formation in oral streptococci. *Oral Microbiol Immunol* 2007;22:340–6. [PubMed: 17803632]
120. He Z, Wang Q, Hu Y, et al. Use of the quorum sensing inhibitor furanone C-30 to interfere with biofilm formation by *Streptococcus mutans* and its *luxS* mutant strain. *Int J Antimicrob Agents* 2012;40:30–5. [PubMed: 22578766]
121. Ren D, Sims JJ, Wood TK. Inhibition of biofilm formation and swarming of *Bacillus subtilis* by (5Z)-4-bromo-5-(bromomethylene)-3-butyl-2(5H)-furanone. *Lett Appl Microbiol* 2002;34:293–9. [PubMed: 11940163]
122. Lonn-Stensrud J, Naemi AO, Benneche T, et al. Thiophenones inhibit *Staphylococcus epidermidis* biofilm formation at nontoxic concentrations. *FEMS Immunol Med Microbiol* 2012;65:326–34. [PubMed: 22443118]
123. Lonn-Stensrud J, Landin MA, Benneche T, et al. Furanones, potential agents for preventing *Staphylococcus epidermidis* biofilm infections? *J Antimicrob Chemother* 2009;63:309–16. [PubMed: 19098295]
124. Kuehl R, Al-Bataineh S, Gordon O, et al. Furanone at subinhibitory concentrations enhances staphylococcal biofilm formation by luxS repression. *Antimicrob Agents Chemother* 2009;53:4159–66. [PubMed: 19620329]
125. Yujie L, Geng X, Huang YC, et al. The effect of brominated furanones on the formation of *Staphylococcus aureus* biofilm on PVC. *Cell Biochem Biophys* 2013;67:1501–5. [PubMed: 23979982]
126. Maeda T, Garcia-Contreras R, Pu M, et al. Quorum quenching quandary: resistance to antivirulence compounds. *ISME J* 2012;6:493–501. [PubMed: 21918575]
127. Balaban N, Novick RP. Autocrine regulation of toxin synthesis by *Staphylococcus aureus*. *Proc Natl Acad Sci U S A* 1995;92:1619–23. [PubMed: 7533297]
128. Novick RP, Ross HF, Figueiredo AMS, et al. Activation and Inhibition of the Staphylococcal AGR System. *Science* 2000;287:391.

129. Cirioni O, Giacometti A, Ghiselli R, et al. RNAIII-inhibiting peptide significantly reduces bacterial load and enhances the effect of antibiotics in the treatment of central venous catheter-associated *Staphylococcus aureus* infections. *J Infect Dis* 2006;193:180–6. [PubMed: 16362881]
130. Balaban N, Cirioni O, Giacometti A, et al. Treatment of *Staphylococcus aureus* biofilm infection by the quorum-sensing inhibitor RIP. *Antimicrob Agents Chemother* 2007;51:2226–9. [PubMed: 17371825]
131. Naik S, Bouladoux N, Wilhelm C, et al. Compartmentalized control of skin immunity by resident commensals. *Science* 2012;337:1115–9. [PubMed: 22837383]
132. Otto M *Staphylococcus epidermidis*–the ‘accidental’ pathogen. *Nat Rev Microbiol* 2009;7:555–67. [PubMed: 19609257]
133. Cheung GY, Kretschmer D, Duong AC, et al. Production of an attenuated phenol-soluble modulins variant unique to the MRSA clonal complex 30 increases severity of bloodstream infection. *PLoS Pathog* 2014;10:e1004298. [PubMed: 25144687]
134. Kjaerulff L, Nielsen A, Mansson M, et al. Identification of four new agr quorum sensing-interfering cyclodepsipeptides from a marine Photobacterium. *Mar Drugs* 2013;11:5051–62. [PubMed: 24351904]

Article Highlights

- The quorum-sensing system Agr is the predominant and best-studied virulence regulator in *S. aureus*.
- Agr regulates a series of aggressive virulence determinants and contributes significantly to acute forms of *S. aureus* disease.
- Several investigational drugs interfering with Agr functionality have been recently developed, with the most promising targeting recognition of the extracellular quorum-sensing signal or the DNA-binding component of the Agr system signal transfer system.
- Targeting Agr must be evaluated with great caution in cases of *S. aureus* bacteremia and biofilm-associated infection, in which Agr has a role that is opposite to that in chronic infection.
- Agr is not likely a good drug target for infections by coagulase-negative staphylococci, whose virulence for the most part relies on the formation of biofilms.
- Drugs interfering with *S. aureus* Agr best not interfere with the Agr system of commensal staphylococci as beneficial parts of the human microflora.

Figure 1. The Agr QS system, Agr-dependent control of gene expression, and Agr inhibitors with established mechanisms.

The Agr RNAII operon, consisting of the *agrBDCA* genes and the adjacent RNAIII-encoding region (also containing the *hld* gene for δ -toxin) are shown at the bottom right. AgrD is exported by AgrB, which also introduces the thiolactone post-translational modification into the AgrD AIP. The mature AIP (after proteolytic cleavage of the N-terminal leader peptide) activates AgrC when a certain threshold concentration is reached. Activated AgrC in turn activates AgrA. The response regulator AgrA binds to and activates transcription from the Agr P2 and P3 promoters, as well as the promoters of the *psm* operons (RNAIII-independent Agr regulation). Microarray analyses suggest that some other, metabolic genes may also be under direct control of AgrA, but this needs further evaluation. The classic targets of Agr are under RNAIII-dependent control, comprising several toxins that are up- and several surface-binding proteins such as protein A, that are down-regulated. Agr inhibitors with clearly defined mechanisms of action include the monoclonal antibody (mAb) against AIP of group IV, AIP analogues that inhibit AgrC activation, and the AgrA inhibitor savarin. Some evidence suggests that ambuic acid may interfere with production of the AIP signal; and solonamides and ngercheumicins may also inhibit AgrC activation.

Figure 2. Structures of select Agr-inhibiting molecules.

Note for example that the *S. aureus* AIP I has considerable structural similarities to the solonamides and ngercheumicin. Diflusinal is one example of an Agr-inhibiting compound identified from a small peptide library screen. R, variable side chains; X, H or Br; Z, O (furanone) or S (thiofuranone).

Table 1.

S. aureus AIPs and modified constructs

Agr group	Name	Sequence	Mutation	Inhibition (IC ₅₀ , nM)				Ref.
				AgrC I	AgrC II	AgrC III	AgrC IV	
I	AIP I	YST(CDFIM)	None	-	25	3	-	[71]
	AIP I D5A	YST(CAFIM)	D to A substitution at position 5	5	8	0.3	3	[70, 71]
	tr ¹ AIP I	Ac ² (CDFIM)	No exocyclic tail	-	47	240	-	[71]
	tr AIP I D2A	Ac(CAFIM)	No exocyclic tail/D to A substitution at position 2	5	5	0.1	5	[71]
II	AIP II	GVNA(CSSLF)	None	40	-	1	86	[71]
	tr AIP II	Ac(CSSLF)	GVNA replaced by N-acetyl group	260	230	4	150	[71, 77]
	tr AIP II S6G	Ac(CGSLF)	No exocyclic tail/S to G substitution at position 6	40	194	N/A ³	N/A	[77]
	tr AIP II S7G	Ac(CSGLF)	No exocyclic tail/S to G substitution at position 7	77	238	N/A	N/A	[77]
III	AIP III	IN(CDFLL)	None	5.05	5.63	-	8.53	[79]
	AIP III D4A	IN(CAFLL)	D to A substitution at position 4	0.49	0.43	0.051	0.035	[79]
	tr AIP III	Ac(CDFLL)	No exocyclic tail	26.7	1.53	-	25.5	[79]
	tr AIP III D2A	Ac(CAFLL)	No exocyclic tail/D to A substitution at position 2	0.26	0.9	0.329	0.96	[79]
IV	AIP III N2A/D4A	AN(CAFLL)	N to A substitution at position 2, D to A substitution at position 4	0.33	0.21	0.066	0.022	[79]
	AIP IV	YST(CYFIM)	None	-	4	1	-	[71]
	tr AIP IV	Ac(CYFIM)	No exocyclic tail	930	8	10	4100	[71]

¹ truncated,² N-acetyl,³ not tested.

Table 2.

Select AIPs produced by other staphylococci

<i>S. epidermidis</i> Agr group	Name	Sequence	Ref.
I	AIP I	DSV(CASYF)	[82]
II	AIP II	NASKYNP(CSNYL)	[82]
III	AIP III	NAAKYNP(CASYL)	[82]
<i>S. intermedius</i> ¹	AIP	R ² IPT(STGFF)	[84]

¹Due to the presence of a Serine at position 5, this AIP is cyclized by a lactone ring.

²Variants have a K residue in place of the R residue.

Table 3.

Molecules found to interfere with *S. aureus* quorum sensing

Source	Molecule	<i>In vitro</i> effects	Mechanism of action	Ref.
Targeting Agr				
<i>Pseudomonas aeruginosa</i> (Gram-negative)	N-(3-Oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL)	Inhibits production of exotoxins and fibronectin-binding proteins but enhances protein A expression	May affect SarA functionality; potentially antagonizes membrane-associated regulators (e.g. sensor components of ArlRS, SaeRS and SsAB)	[90]
Synthetic	(S)-3-Decanoyl-5-(2-hydroxyethyl)tetramic acid (5-HE-C10-TMA 5)	Inhibits P3 activity	Prevents AgrC receptor dimerization or interference with the interactions between AgrC and AgrA (?)	[91]
Synthetic	3-Dodecanoyltetronic acid (C12-TOA, 16)	Inhibits P3 activity	Prevents AgrC receptor dimerization or interference with the interactions between AgrC and AgrA (?)	[91]
Synthetic	3-Tetradecanoyltetramic acid (C14-TOA 17)	Inhibits P3 activity	Prevents AgrC receptor dimerization or interference with the interactions between AgrC and AgrA (?)	[91]
<i>Lactobacillus reuteri</i> (Gram-positive)	Cyclic dipeptides: cyclo(L-Phe-L-Pro) and cyclo(L-Tyr-L-Pro)	Inhibits TSST-1, downregulates RNAPII and RNAPIII, SarA, and SaeRS	Competitive inhibitors of AIP-mediated P3 promoter activation, affecting <i>sarA</i> and <i>saeRS</i>	[95]
<i>Photobacterium</i> (Gram-negative)	Solonomide A/B, Ngercheumicins	Reduces <i>hla</i> , <i>malIII</i> and increases <i>spa</i> transcripts, reduces <i>S. aureus</i> mediated killing of human neutrophils	Competitive inhibitor of AgrC	[96, 97, 134]
Fungal strains	Ambuic acid	Inhibit hemolytic activity, RNAPIII transcription, AIP-I production	Prevents AIP biosynthesis (?)	[98]
<i>Penicillium restrictum</i>	Polyhydroxyanthraquinones	Inhibits <i>hld</i> expression and P3 activity	Unknown	[99]
<i>Actinomycetes</i> strains	Chromatography eluates	Inhibits P3 activity and hemolytic activity	Unknown	[100]
Chinese liquorice root (flavonoid)	Licochalcone A	Inhibits <i>sea</i> , <i>seb</i> and <i>agrA</i> expression	Unknown	[102]
A p-cymene-derived compound primarily found in thyme and oregano	Thymol	Inhibits <i>sea</i> , <i>seb</i> , <i>hla</i> and <i>agrA</i> expression	Unknown	[103]
Plant origin (flavonoid)	Luteolin	Inhibits <i>hla</i> and <i>agrA</i> expression	Unknown	[104]
<i>Cyperus rotundus</i> (Chinese medicinal herb)	Alpha-cyperone	Inhibits <i>hla</i> and <i>agrA</i> expression	Unknown	[105]
Antibiotic	Oxacillin	Pleiotropic effect on toxin expression	Unknown	[106]
NCI Library of small-molecule compounds	Naphtalene and biaryl compounds	Inhibits <i>hla</i> , <i>psma</i> , RNAPIII expression	Targets AgrA (N) phosphoryl binding pocket; may interfere with AgrA DNA binding activity	[110]
Maybridge Ro3 fragment library	9H-Xanthene-9-carboxylic acid	N/A	Interferes with AgrA DNA binding	[111]

Source	Molecule	<i>In vitro</i> effects	Mechanism of action	Ref.
			activity	
	2-(4-Methyl-phenyl)-1,3-thiazole-4-carboxylic acid	N/A	Interferes with AgrA DNA binding activity	[111]
NIH Small molecule repository	Savarin	Inhibits RNAPIII expression (only in <i>S. aureus</i> , not <i>S. epidermidis</i>), <i>psmA</i> promoter	Interferes with AgrA DNA binding activity	[112]
Targeting LuxS/AI-2				
Synthetic	Furanone 202	Inhibits biofilm formation by <i>S. epidermidis</i>	Unknown	[123]
Synthetic	(5 <i>Z</i>)-4-bromo-5-(bromomethylene)-3-butyl-2(5 <i>H</i>)-furanone	Inhibits RNAPIII expression, enhances biofilm formation	Leads to increased PIA production enhancing biofilm formation	[124]

¹N/A, not applicable (not tested).