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MINIREVIEW

Regulatory Mechanisms of the LuxS/AI-2 System and Bacterial Resistance

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ABSTRACT The quorum-sensing (QS) system is an intercellular cell-cell communication mechanism that controls the expression of genes involved in a variety of cellular processes and that plays critical roles in the adaption and survival of bacteria in their environment. The LuxS/AI-2 QS system, which uses AI-2 (autoinducer-2) as a signal molecule, has been identified in both Gram-negative and Gram-positive bacteria. As one of the important global regulatory networks in bacteria, it responds to fluctuations in the numbers of bacteria and regulates the expression of a number of genes, thus affecting cell behavior. We summarize here the known relationships between the LuxS/AI-2 system and drug resistance, discuss the inhibition of LuxS/AI-2 system as an approach to prevent bacterial resistance, and present new strategies for the treatment of drug-resistant pathogens.

KEYWORDS LuxS/AI-2 system, bacterial resistance, biofilm formation, efflux pump

The quorum-sensing (QS) system is an intercellular cell-cell communication mechanism that controls the expression of genes involved in a variety of cellular processes and that plays critical roles in the adaption and survival of bacteria in their environment (1). For intra- and interspecific communication, bacteria use chemical signals and their corresponding receptors (2). When an extracellular threshold concentration is reached, these molecules bind to their receptors, thereby activating the QS system. With the discovery of autoinducer-2 (AI-2) and its corresponding synthase, LuxS, the first possible interspecies QS system was found, as the synthase is widespread among the bacterial kingdom in both Gram-positive and Gram-negative bacteria (3). The LuxS/AI-2 QS system modulates various cellular processes involved mainly in the regulation of virulence factors, bacterial luminescence, sporulation, motility, toxin production, biofilm formation, and drug resistance (4, 5).

The emergence of antibiotic-resistant bacterial pathogens was reported in a major health challenge worldwide (6). Recently, some studies have shown that the QS system may be involved in bacterial resistance (7–9). Consequently, inhibition of bacterial QS has become a new and promising antibacterial strategy, which not only prevents the development of bacterial resistance but also eliminates the virulence factor gene expression related to population density (10–12).

LUXS/AI-2 QS SYSTEM

The LuxS/AI-2 system, coexisting in both Gram-negative and Gram-positive bacteria, is a QS regulatory mechanism that enables bacteria to make collective decisions with respect to the expression of a specific set of genes by secreting and detecting the signal molecule AI-2, a furanosyl-borate-diester as it has been identified in *Vibrio harveyi*, as a furan molecule existing in *Escherichia coli* (13, 14). The synthesis of AI-2 involves the

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conversion of *S*-adenosylhomocysteine (SAH) to homocysteine either by a one-step reaction using the enzyme SAH hydrolase (SahH) or a two-step reaction that requires the SAH nucleosidase (Pfs) and LuxS, which catalyzes the cleavage of the thioether linkage of SRH to produce 4,5 dihydroxy-2,3-pentanedione (DPD), which can rearrange to *R*- or *S*-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R- or S-THMF), both better known as AI-2 (15–19). The discovery that the AI-2 signal molecule produced by one bacterial species can be sensed by bacteria of different species led to the proposition that AI-2 is probably a universal signaling molecule that functions in interspecies communication (20). LuxS, the AI-2 synthase, has been identified in a wide range of Gram-negative and Gram-positive bacteria (1). LuxS not only participates in the production of AI-2 signaling molecules but also plays an important role in activating the methyl cycle as part of the bacterial central metabolism (18, 19). LuxS is mainly responsible for the hydrolysis of *S*-adenosyl homocysteine to *S*-adenosylmethionine (SAMe) as a methyl donor, which is an important pathway for bacteria to recover methyl groups and plays an important role in bacterial vitamin synthesis and polyamine formation (21). Challan Belval et al. showed that the *luxS* mutation can also cause changes in the extracellular concentration of *S*-ribosyl homocysteine (SAMe with LuxS function) (18). The biological importance of the LuxS/AI-2 QS system has been demonstrated by numerous experimental evidences, which showed that LuxS/AI-2 is involved in physiological processes such as biofilm formation, conjugation, virulence, and antibiotic resistance (22–24).

LUXS/AI-2 QS SYSTEM CONTRIBUTES TO ANTIBIOTIC RESISTANCE

The modulation of antibiotic bacterial resistance by the LuxS/AI-2 system is complex (25–27). As summarized in Fig. 1, the LuxS/AI-2 system contributes to antibiotic resistance through effects on efflux pumps, mobile genetic elements, the VraSR two-component system, and the folate synthesis pathway. The fact that the LuxS/AI-2 system coordinates bacterial biofilm formation further contributes to bacterial resistance. Each of these aspects will be discussed separately. Table 1 lists the antimicrobials mentioned in this review, as well as their major mechanisms of action.

LuxS/AI-2 affects drug resistance through efflux pumps. Overexpression of multidrug (MDR) efflux pumps is considered one of the main mechanisms of bacterial resistance (28–30). The expression of the efflux system is regulated in multiple levels, involving local and global transcriptional regulation, as well as posttranscriptional and posttranslational regulation (31). Studies have shown that overexpression of the QS regulator SdiA leads to an increased expression of the AcrAB efflux pump, in addition to participate in the MDR efflux pump system in *E. coli* (32). We recently showed that the LuxS/AI-2 QS system affects the expression of the efflux pump SatAB, further affecting the resistance to quinolone antibiotics in *Streptococcus suis* (33). This study also showed that the reduced resistance of the *luxS* gene deletion mutant strain to the quinolone antibiotics norfloxacin and enrofloxacin was achieved through the *luxS* gene affecting the expression levels of the efflux pump genes *SatA* and *SatB*. Mou et al. (34) showed no significant difference in *cmeB* efflux gene expression levels in the *luxS* mutant compared to the wild type in *Campylobacter jejuni*. However, there is a decrease in *cmeR* efflux gene expression in the *luxS* mutant, resulting in fewer CmeR proteins, thereby reducing CmeABC inhibition. This may in turn lead to an increase in efflux expression and function. Despite the lack of changes in *cmeB* expression, the *luxS* deletion may trigger expression of other efflux systems associated with CmeR regulatory factors (34). In addition, bacterial signaling molecules need to be exported and released outside the cell to be recognized by other bacteria. In Gram-negative bacteria, the signaling molecule AHL is actively transported across the cell membrane by the MexAB-OprM efflux pump (35). Our previous studies have brought evidence that the signal molecule AI-2 is involved in the resistance to quinolone antibiotics (33). Once the AI-2 produced by the *luxS* gene is excreted from the cells, it binds to the corresponding receptors and regulates the overexpression of efflux pump SatAB involved in bacterial resistance in *S. suis*. Further research in this area will reveal in depth

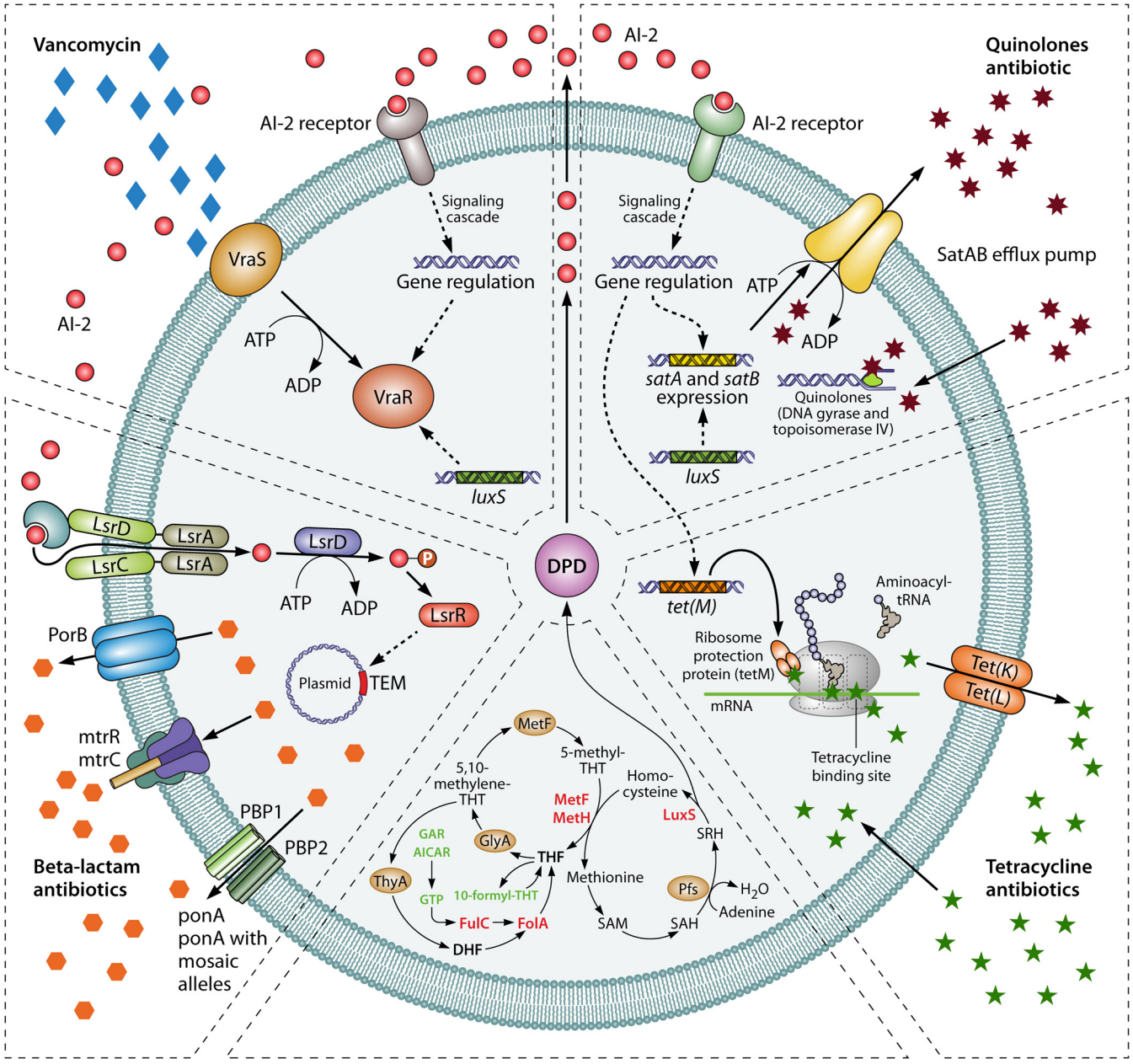

FIG 1 Regulation mechanism between LuxS/AI-2 and bacterial resistance. Abbreviations: AI-2 autoinducer-2; LuxS, AI-2 synthase; LrsC, response regulator; MetF, 5,10-methylenetetrahydrofolate reductase; MetE, methionine synthase; MetH, B_{12}-dependent homocysteine-5-methyltetrahydrofolate methyltransferase; THF, tetrahydrofolate; GlyA, serine hydroxymethyltransferase; ThyA, thymidylate synthase; AICAR, 5-aminoimidazole-4-carboxamine ribonucleotide; DHF, dihydrofolate; FolC, dihydrofolate synthase; FolA, dihydrofolate reductase; SAM, *S*-adenosylmethionine; SAH, *S*-adenosylhomocysteine; Pfs, *S*-adenosylhomocysteine nucleosidase; SRH, *S*-ribosylhomocysteine; LuxS, *S*-ribosylhomocysteinase; DPD, 4,5-dihydroxy-2,3-pentanedione (the precursor of AI-2).

the role of the LuxS/AI-2 system in controlling the effects of these efflux pumps on bacterial resistance (33). The ability of the QS system to regulate MDR efflux pumps represents a potential target for antibiotic resistance (36).

LuxS/AI-2 affects drug resistance through mobile genetic elements. Mobile genetic elements such as plasmids, integron gene cassettes and transposable elements play an important role in bacterial resistance (37). Plasmid-mediated resistance can cause horizontal transfers among different bacteria, leading to the spread of bacterial resistance, which can cause serious public health problems (38). Extended-spectrum beta-lactamase (ESBL) is a type of lactamase encoded in plasmids that hydrolyzes most of the beta-lactam antibiotics such as penicillin, cephalosporin, and aztreonam (39, 40).

TABLE 1 Antibiotics involved in the regulation of LuxS/AI-2 QS system and their resistance mechanisms

Antibiotic class	Example(s)	Major mechanism of action	Major mechanism of resistance
Nucleic acid synthesis inhibitors			
Quinolones	Norfloxacin, enrofloxacin	Inhibit DNA replication by complexing with DNA and DNA gyrase or topoisomerase IV	Target enzymes (DNA gyrase and topoisomerase IV) of changes and efflux pump protein expression
Sulfonamides	Trimethoprim-sulfamethoxazole	Ultimately prevent THF[a] synthesis by inhibiting dihydrofolate reductase (DHFR) and dihydropteroate synthetase (DHPS)	Acquired resistance genes located on mobile genetic elements; and mutations on the chromosomal DHFR and DHPS gene
Protein synthesis inhibitors			
Tetracyclines	Tetracycline, chlorotetracycline	Bind to the 16S rRNA of the 30S ribosomal subunit, prevent-ing aminoacyl-tRNA from binding to the ribosomal A site	Acquisition of mobile genetic elements with resistance genes, efflux, ribosomal protection, and enzymatic inactivation
Cell wall synthesis inhibitors			
β-Lactams	Penicillin, cephalosporin, aztreonam	Prevent transpeptidation of peptidoglycan by inhibiting transpeptidases (penicillin-binding proteins [PBPs])	Reduced access to the PBPs, reduced PBPs binding affinity, expression of enzymes that bind and hydrolyze β-lactams.
Glycopeptides	Vancomycin	Bind to the terminal D-Ala–D-Ala residues of peptidoglycan subunits, preventing transpeptidation	Alteration of the peptidoglycan synthesis pathway, specifically D-Ala–D-Ala to either D-Ala–D-Lac or D-Ala–D-Ser

[a]Tetrahydrofolate (THF) is a cofactor required for the *de novo* biosynthesis of purines and of thymidine.

TEM-type ESBL includes TEM-1 and TEM-2 (41). Xue et al. (42) showed that exogenous AI-2 increased the antibiotic resistance of clinical *E. coli* strains isolated from cow's papillitis by upregulating the expression of TEM-type enzyme in an LsrR-dependent manner. Transposons are a group of mobile genetic elements that are defined as a DNA sequence (43). Because of its ability to move between bacterial chromosomes, plasmids, and phages, resistance on the transposon is more easily transmitted and disseminated horizontally (44, 45). The antibiotic resistance gene *tet*(M) is located on the Tn*916* family of junctional transposons (46). Our previous studies have shown that exogenously added AI-2 affects the resistance of *S. suis* to tetracycline through an upregulation of *tet*(M) gene expression. Although the specific signaling mechanism needs to be further studied, LuxS/AI-2 appears to be the main target for preventing the spread of bacterial resistance.

LuxS/AI-2 affects drug resistance through the VraSR two-component regulatory system. The two-component signal transduction system is widely distributed in bacteria. The VraSR two-component system is an important regulatory system in *Staphylococcus aureus*, allowing bacteria to sense changes in the external environment and to adjust their response to maintain its homeostasis (47, 48). The VraSR two-component system consists of a histidine kinase sensor protein (VraS) and an effector regulatory protein (VraR) (49, 50). VraS autophosphorylates *in vitro* and rapidly transfers phosphate groups to VraR, which selectively dephosphorylates VlaS-mediated signaling pathways (51). Mutation or increased expression of the VraSR two-component system is one of the mechanisms of resistance to vancomycin in *Staphylococcus aureus* (48). Xue et al. (52) have shown that the loss of *S. aureus luxS* gene leads to a decrease in susceptibility to cell wall synthesis inhibitor antibiotics accompanied by upregulation of the VraSR two-component system. This revealed that the *luxS* gene may regulate bacterial resistance through a VraSR two-component regulatory system (52). In the presence of exogenous AI-2, the susceptibility of the *luxS* deletion mutant to cell wall synthesis inhibitors was restored, demonstrating that LuxS is involved in the antibiotic susceptibility of *S. aureus*, which may be primarily due to AI-2 signaling (52). In addition, as a two-component regulatory system, VraSR is able to detect conditions that may disrupt bacterial cell wall synthesis and regulate cell wall biosynthesis pathways (51, 53). Higher VraSR levels in the *luxS* deletion strain indicate that cells can respond to cell wall structure damage more rapidly than the wild type when exposed to cell wall synthesis inhibitor antibiotics (52). Therefore, the LuxS/AI-2 system affects the resistance of *S. aureus* to cell wall synthesis inhibitors through a VraSR two-component regulatory system.

LuxS/AI-2 affects drug resistance by inhibiting the folate synthesis pathway. Folic acid refers to substances such as tetrahydrofolate and its derivatives, which are important cofactors for mediating carbon transfer and participate in many important reactions in organisms (54). Studies have shown that specific target binding-like interaction with LuxR may contribute to transcriptional activation and that sulfonamides compete with dihydropterylic acid synthetase for binding, which inhibits the biosynthesis of folate and causes toxicity (55). Yu et al. (56) showed that the presence of exogenous AI-2 increased the sensitivity of avian pathogenic *Escherichia coli* strain to trimethoprim-sulfamethoxazole (SXT) in the folate synthesis-dependent pathway, but does not rely on the LsrR-dependent pathway. The addition of the exogenous AI-2 precursor molecule DPD triggers product feedback inhibition and then reduces the expression of *luxS* and a number of other products of LuxS, such as homocysteine (56). Homocysteine is a substrate for methionine synthase E (MetE) and methionine synthase H (MetH), which are important enzymes in the folate synthesis pathway (57). Substrate inhibition caused by a decrease in homocysteine downregulates the expression of *metE* and *metH*, which in turn leads to a decrease in the intermediate metabolite tetrahydrofolate (THF), an important substrate for the synthesis of purines and pyrimidines (58). In THF metabolism, purines and pyrimidines are two important intermediate metabolites for the resynthesis of THF, and *folA* and *folC* are two important folate synthase-encoding genes (59). In the absence of SXT, AI-2 downregulates the transcrip-

tional levels of the folate synthase-encoding genes *folA* and *folC* only by the folate pathway (56). However, in the presence of SXT, exogenous AI-2 enhances the growth inhibition of the APEC strain by SXT by downregulating the transcriptional level of the folate-related gene (56). Further information is provided on the potential drug targets for prophylactic and adjuvant antibiotic treatment.

LuxS/AI-2 affects drug resistance through biofilm formation. Bacterial biofilms, which are surface-attached communities of bacterial cells composed of polymers produced by the microorganisms themselves embedded in an extracellular polymeric matrix, are a cause of multidrug resistance (60). The ability of *S. suis* to form biofilm was significantly increased when a small amount of AI-2 was added during growth, whereas deleting the *luxS* gene leads to a decreased ability to form a biofilm (4, 61–63). These observations suggest that the LuxS/AI-2 QS system modulates the formation of bacterial biofilms. Biofilm formation by *Helicobacter pylori* decreases its susceptibility to antibiotics, and antibiotic resistance mutations in *H. pylori* are more frequently generated in biofilms than in planktonic cells (64). The *luxS* gene is the only known QS gene found in the genome sequence of *H. pylori* (65). Some reports indicated that *H. pylori* produces extracellular signaling molecules associated with AI-2 and that AI-2 production is dependent on *luxS* function (66). Bacterial biofilm is associated with increased antibiotic resistance and is involved in many persistent diseases (67). The main mechanisms of bacterial biofilm resistance are QS, activation of efflux pumps, the formation of biofilms, and the production of inactive enzymes and antibiotic-modifying enzymes (68). Alone, each of these mechanisms only partially accounts for the increased antimicrobial recalcitrance observed in biofilms. However, the influence of LuxS/AI-2 on biofilm formation is likely a combination of various mechanisms and environmental changes.

TARGET THE LUXS/AI-2 SYSTEM: NEW ANTIBACTERIAL STRATEGY FOR BACTERIAL RESISTANCE

Inhibition of bacterial QS system represents a novel antibacterial strategy, which not only prevents the development of bacterial resistance but also eliminates the density-induced control of bacterial virulence factors that contributes to serious infections (69). Several strategies developed to prevent bacterial resistance by inhibiting QS systems are summarized in Fig. 2, while an overview of LuxS/AI-2 QS system inhibition strategies is summarized in Table 2.

Inhibition of signal molecule synthesis. In QS systems, the synthesis of signal molecules plays an essential role in the communication between cells (70). Among them, the signal molecule AI-2 is an important molecule for signal exchange between different bacterial species (71). The precursor *S*-ribosyl homocysteine (SRH) of AI-2 is formed by the action of 5′-methylthioadenosine/*S*-adenosylhomocysteine nucleosidase (MTAN) on SAH (72). The inhibition of MTAN results in an accumulation of 5′-methylthioadenosine (MTA) and SAH and consequently the inhibition of AI-2 production (73). The production of AI-2 is significantly reduced in MTAN knockout strains or in the presence of tight-binding inhibitors of MTAN (74). Since MTAN is not expressed in humans, it provides a potential target for antibacterial drug design for QS signaling. 5′-Methylthioadenosine phosphorylase (MTAP) plays a role in the polyamine pathway by circulating MTA and maintaining SAM (75). Its transition state structure is used to direct the synthesis of MT-DADMe-ImmA, a picomolar inhibitor that blocks QS in *Vibrio cholerae* without altering the rate of bacterial growth (73). Transition state analog inhibitors have shown promise as anticancer agents and antibacterial agents (73). These results indicate that MTAN inhibition is a possible drug strategy since it provides a "single injection" target for LuxS/AI-2-controlled bacteria. Guillermo et al. first showed that hydroxylated pyrrolidine represents a SAH/MTA inhibitor and speculated that these compounds might be transitional analogs (76). However, inhibition of Pfs is fatal to cells because the accumulation of MTA and SAH is toxic to cells (77, 78). Excess MTA levels in cells inhibit growth processes and DNA synthesis by indirectly preventing the synthesis of polyamines involved in these important processes (79, 80). Few studies

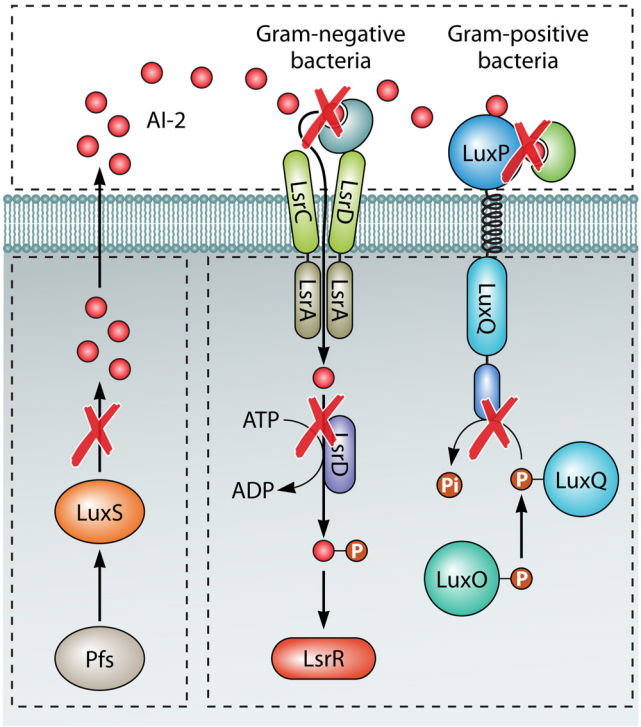

FIG 2 Strategies for QS interception. (1) Signal-generating enzymes (LuxS and Pfs). (2) Signal sequestration and degradation outside the cell. (3) Receptors and transducers in the signal transduction cascade.

have focused on the inhibition of *S*-ribosylhomocysteinase (LuxS). LuxS inhibition should not affect the processes essential for growth and survival (81–83). This enzyme is a DPD synthetase and is involved in the detoxification of SAH; it plays a minor role in the sulfur cycle pathway (84, 85). Han and Lu (86) used phage display technology to screen for a phage-encoded peptide that specifically interacts with the LuxS enzyme in *S. suis*. This LuxS peptide inhibitor (TNRHNPHHLHHV) showed partial inhibition of enzyme activity (86).

TABLE 2 Overview of LuxS/AI-2 QS inhibition strategies

Inhibitor	Action mode	Biological effect	Reference
Inhibition target: signal generators			
MT-DADMe-ImmA (transition state analog)	Pfs inhibitor	Inhibit AI-2 production	73
Hydroxylated pyrrolidines derivatives	Inhibitors of SAH/MTA nucleosidase.	Inhibit AI-2 production	76
TNRHNPHHLHHV (peptide)	Interact with the LuxS enzyme	Inhibit enzyme activity	86
Inhibition target: signal molecule			
Ex vivo addition of LsrK and ATP	Phosphorylation and degradation of AI-2	Inhibits bioluminescence in *V. harveyi* and lsr expression in *E. coli* and *S.*Typhimurium	90
Imidazole	A furan carbocyclic analog of AI-2	Inhibiting AI-2 function	93
Inhibition target: signal receptor/transduction			
D-Galactose	Inhibitor AI-2 activity	Targeting AI-2 activity for prevention biofilm formation.	99
Small peptide, 5906	Prevents LuxS homodimer formation	Inhibits LuxS activity by binding specifically to LuxS	100

Inhibition of signaling molecules. Inactivation or denaturation of the signal molecule itself, which can be achieved by various mechanism, is the most basic way to use the QS system to prevent bacterial resistance and to study new antibacterial strategies (87). Some microorganisms have the ability to metabolize AI-2 and consequently to inhibit QS function. Signal molecule degradation can be achieved by adding LsrK (AI-2 kinase) and ATP into the bacterial culture, in which AI-2 is phosphorylated outside the cell; the bacterial cross talk controlled by AI-2 is then significantly reduced (88, 89). *In vitro*-phosphorylated AI-2 quenched the QS response in *E. coli*, *Salmonella* Typhimurium, and Haber's bacillus (90). Phosphorylated AI-2 is more hydrophilic and is thought to fail to cross the cell membrane and act as a QS signal (89). This strategy might be particularly effective in mixed infections because LsrK can phosphorylate DPD (the precursor molecule of AI-2) (91). Moreover, it may be effective regardless of the AI-2 structure and transport/sensor mechanism used by different bacterial QS systems (92). Yu et al. (93) and others reported that exogenous imidazole, a furan carbocyclic analogue of AI-2, reduced the antibiotic resistance of clinical *E. coli* strains to β-lactam antibiotics by inhibiting the function of AI-2.

Inhibit signal molecule conduction or binding to receptors. In the activated methyl cycle, *S*-adenosylmethionine acts as a methyl donor, resulting in the accumulation of the toxic intermediate *S*-adenosylhomocysteine (SAH) in bacterial cells (94). The LuxS enzyme plays a role in the detoxification of SAH with homocysteine and DPD (19, 95). DPD is a highly active pre-AI-2 molecule. Destruction of the activated methyl cycle by inactivation of *luxS* may result in a series of chemical reactions (96). Therefore, further complementary studies were performed using synthetic DPD. Recently study showed a correlation between threshold DPD concentration and antibiotic susceptibility in *Streptococcus anginosus* (97, 98). These results are consistent with other studies on DPD and AI-2 and show the importance of achieving appropriate AI-2 threshold levels in bacterial populations (97). AI-2 does not have a specific structure; rather, it represents a class of molecules. The precursor DPD of AI-2 is cyclized in solution to form various isomers. The most significant inhibitory effect of propyl and butyl-DPD relates to *Salmonella* Typhimurium QS (99). Ryu et al. showed that D-galactose, as an inhibitor of AI-2 activity, inhibits biofilm formation by periodontal pathogens (99). The D-galactose-binding protein shows high sequence similarity to ribose-binding protein (RbsB), a known AI-2 receptor of *Actinobacillus* sp. (99). Sun et al. (100) identified the small peptide 5906 that inhibits *Edwardsiella tarda* LuxS activity by specifically binding LuxS in a manner that may prevent the formation of a functional LuxS homodimer. Furthermore, the AI-2 activity of *Aeromonas hydrophila* and *Vibrio harveyi* can be inhibited, and fish supplemented with DH5α/p5906 exhibit enhanced resistance to both bacteria. The results indicate that 5906 or an analog/derivative thereof can be used to develop a broad-spectrum antimicrobial agent for the prevention and control of bacterial diseases in fish (100). Bacterial QS responses are not necessarily triggered by AI-2 produced by organisms of the same species, genus, or even classes (20). AI-2-mediated QS typically occurs in bacterial communities composed of many different types of microorganisms. Several studies have demonstrated the QS phenotype in multimicrobial communities, including LuxS/AI-2, mediates activity between normal microflora and pathogens (101). Since LuxS/AI-2 regulates pathogen virulence in multimicrobial communication networks, disrupting signaling in these networks provides another goal for QS quenchers (102).

CONCLUSION

The LuxS/AI-2 QS system plays a key role in antibiotic resistance in bacteria. The LuxS/AI-2 system controls the expression of a variety of genes and then regulates the cellular activities of bacteria to adapt to different environments (97, 103–105). Since QS controls the expression of many virulence factors and drug-resistant genes in bacteria, any process that blocks QS signaling molecules or receptor-recognizing signaling molecules can attenuate the virulence and resistance gene expression of bacterial QS-dependent genes (106–109). The concerns regarding the rising in antibiotic resis-

tance require an alternative approach to antibacterial therapy. Extensive AI-2 communication between bacteria makes it a possible therapeutic target. Therefore, understanding the role of AI-2 QS in antibiotic susceptibility is of great interest.

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