

BREAKING BACTERIAL CODE: QUORUM SENSING DISRUPTION AS A NEXT-GENERATION ANTIMICROBIAL APPROACH

*Vaishnavi Sonar¹, Sujata Dudhe²,
Paresh Sonawane¹, Laxmikant Borse³*

¹ Department of Quality Assurance, Sandip Institute of Pharmaceutical Science, Mahiravani, Nashik-422213.

² Assistant Professor, Department of Pharmaceutical Chemistry, Sandip Institute of Pharmaceutical Science, Mahiravani, Nashik-422213.

³ Principal, Sandip Institute of Pharmaceutical Science, Mahiravani, Nashik-422213, India

ABSTRACT:

Quorum sensing (QS) is a technique of cell-to-cell communication used by bacterial pathogens to control virulence, biofilm production, and antibiotic tolerance and thus contributes to long-standing and intractable infections. Studies on QS pathways are important in development of new therapeutic interventions against the backdrop of growing antimicrobial resistance. This review recounts the molecular QS phenomenon in the Gram-positive and Gram-negative bacteria and dwells upon the heterogeneity of autoinducers, receptors, chassis and regulatory networks. There is a critical examination of the pathogenic importance of QS, particularly in the propagation of biofilm-associated infections and multidrug-resistant infections. Potential solutions in the form of strategies to interfere with bacterial communication, or quorum quench (QQ) are described to include enzymatic degradation of the signals, inhibitors of signal biosynthesis, utilisation of signal recep-

tor antagonists and natural product quorum sensing Inhibitors (QSIs).

The promising emerging directions of therapy are hyper-specific anti-virulence strategies, the development of nanotechnology, and the combination with traditional antibiotics. The study also involves future directions of CRISPR-based editing of QS genes, multi-omics tools to discover pathways, and non-medical applications of CRISPR as biotechnology and agriculture. Relating knowledge of mechanism and therapeutic research, with the help of this review one could see the possibility of destroying the networks involved in bacterial communication as a new method of treating infectious diseases and reducing antibiotic resistance.

Keywords: Quorum Sensing, Quorum Quenching, CRISPR, Autoinducers, AHL, AIP, LuxR

INTRODUCTION

Pathogenesis of bacteria:

Bear in mind that the pathogenicity, or capacity of a microbe to produce disease, is a balance between four large factors: the predisposition of the host, i.e., its stored immune system; the character of the visitor that enters in, or becomes congested within us; the genetic map that the pathogen brings with it; and the particular program that it executes while infecting. It starts with a molecular movement from the host to the microbe. From genetic and molecular data, we can determine which traits are linked with virulence or defence mechanisms that enable a germ to persist. Even with this understanding, the exact processes by which bacteria become established in the host are not always very well understood; some of these processes may be unique to a particular bacterial genus that infects humans, others may be more general across the microbial world [1].

Microbes are all around and they are unavoidable, some of them can cause illness, others are protective or beneficial to the human body, with the most common bacterial infections being spread by direct contact, contaminated water, air, food or by living vectors like insects and animals. For instance, when the causative bacteria from the hospitals go outside and get transmitted around, it can result in higher morbidity and mortality among the weakened bodies [2].

Quorum Sensing Concept and Discovery:

Quorum sensing (QS) refers to the cell-to-cell communication; typically employed by bacterial patho-

ADDRESS FOR CORRESPONDENCE:

Sujata Prashik Dudhe
Department of Pharmaceutical Chemistry
Sandip Institute of Pharmaceutical Science, Mahiravani,
Nashik-422213, India
phone: 9970891908
email: sujata.p.dudhe@gmail.com

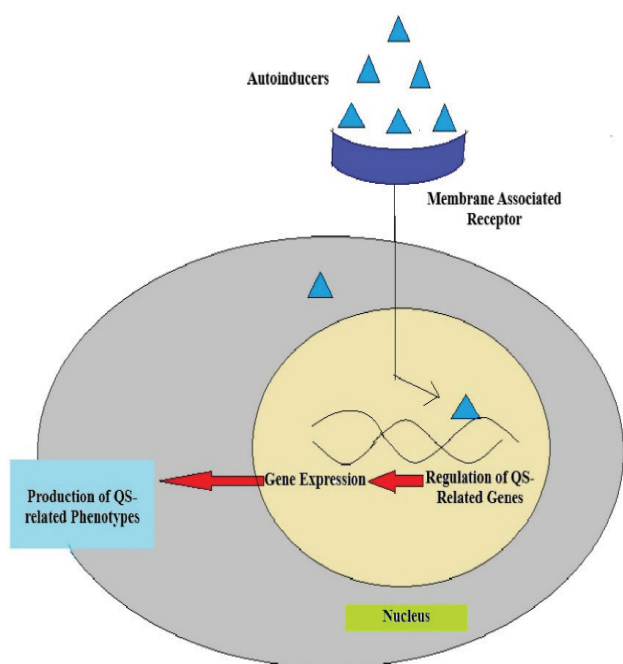


Figure 1 QS General mechanism in bacteria [5]

gens in order to coordinate the expression of a range of common traits including the production of multiple virulence factors, biofilm, and swarming motility, when the population densities of individuals attain a population level [3].

Bacteria are one of the simplest types of unicellular organisms, each being able to grow, divide, and react with its environment on its own. However, even being unicellular, bacteria can organize themselves and even share work with other cells. A complex process called quorum sensing (QS) is responsible in the attainment of such intercellular coordination. The result of this collaboration in organization can be seen when bacteria achieve a high density and create a thin gel-like material known as a biofilm.

In QS, intracellular signal transducers react to external stimuli; the extracellular signal may be diffusible components, which act directly on transcriptional regulators or via sensor kinases. The signaling molecules are produced by bacterial cells into the surrounding, and once their concentration reaches a certain level they bind to a specific receptor in the membrane, which causes a change in gene expression. The traditional activation of activity of genes in quorum-sensing (QS) leads to the increased synthesis of proteins partially contributing in the production of signalling-molecules (see Fig. 1). This enhanced expression of protein sets up a positive feedback loop

forming the basis to the ubiquitous description of QS components as being autoinducers [4].

The QS process was initially explained in the bioluminescent marine bacterium; *Vibrio fischeri*. Here, a luxI / luxR type QS system operates: luxI encodes for an enzyme, the autoinducer synthase, to synthesize autoinducer, whereas luxR encodes for a receptor protein LuxR. During low density of microbial cells, n-acyl homoserine lactone (AHL) is produced in response to luxI gene expression. This AI (autoinducer) spreads in the medium and thus its concentration gets raised. When the threshold concentrations of AI are achieved, they interact to bind LuxR to produce a cytoplasmic AI-R complex which is a transcriptional activator which can bind DNA. This complex induces transcription of lux operon (luxCDABEG) expression and leads to an increase of the level of transcription of messenger-RNA encoding bioluminescence in the cell. At the threshold level, AI molecules regulate their own virulence factors and other virulence factors resulting in QS phenomenon [6].

It is also estimated that a large proportion (70 to 80 %) of all microbial infections are biofilm-based, and these complexes remain central to pathogenesis. The exogenous stressors are withstood through the biofilms, which serve as a barrier to receiving antibiotics and antiseptics [7].

Gram positive bacteria utilize the oligopeptides to express genes through the auto-inducer mechanisms. After release by the cell, these molecules are received by the membrane receptors of the same bacteria, a cascade of signal transduction then occurs and leads specifically to the activation of transcription of a certain gene. On the other hand, the Gram-negative bacteria regulate gene expressions density-dependently. They also release other self-activating molecule by a stimulation of Lux operon that also controls production of the major enzymes found in quorum sensing signals. These difference in controlling the gene action occur owing to both the number density and the physiological condition of the bacteria [8].

2. Mechanisms of QS

2.1 Autoinducers-Signal Molecules

QS allows the communication of bacterial cells by identifying autoinducers and secreting them. Autoinducers (AIs) are small signaling molecules which are generated at basal levels in the stationary phase of bacterial growth. These molecules serve as a population density marker and after a specific growth level

is attained, they control the expression of the corresponding genes. The signal molecules employed by a Gram-positive bacterium will be peptide derivatives in contrast to fatty acid derivatives by a Gram-negative bacterium. Most of the bacteria are capable of using both the types of AIs to control the expression of target gene [5].

Bacterial sporulation, biofilm formation, pathogenicity production, and interaction connections that involve interspecific competition, cooperation, and paternity recognition are all regulated by quorum sensing (QS) [9].

Acyl Homoserine Lactone-AHL (Gram negative bacteria)

Quorum sensing (QS) signaling molecules, which are referred to as autoinducers vary across different bacteria species. Acyl-homoserine lactones (AHLs) are the most prevalent autoinducer secreted by gram-negative bacteria, while oligopeptides referred to as autoinducing peptides (AIPs) are most commonly secreted by gram-positive bacteria [10].

Acyl Homoserine Lactones (AHL) molecule is composed of a homoserine-lactone strand and fatty acid acyl chain (C4 to C18) (Fig.2). The AHL molecules may differ in 3- hydroxy, 3-oxo, methyl or varying levels of unsaturation based on the organisms. The LuxI-type AHL synthases are the first component in AHL signal which synthesizes the AHL molecules. In the event of AHL molecules synthesis, the molecules can be passively and actively transported in and out of the cells. The second mode of action of AHL signalling is through LuxR-type receptor proteins, which recognize AHL signal molecules and in turn, trigger the expression or repression of target genes in a QS-dependent manner. The expression of genes mediated by QS is therefore controlled by LuxR-like DNA-binding transcription factors [11].

A group of autoinducers known as acyl homoserine lactones (AHLs) is formed by around fifty Gram-negative bacteria, many of which are pathogens of clinical interest. Their synthesis combines three structural motifs: a homoserine lactone ring formed by S-adenosylmethionine, a central amide group and a variation of the chain with different length and level of oxygenation dependent on the bacteria species. Within some strains, the chain ends with a 3-oxo group; in other strains, such as *V. fischeri* and *P. aeruginosa*, it ends in a 3R-hydroxyl functional group. Aliphatic chains with 4-18 carbon atoms have essential hydro-

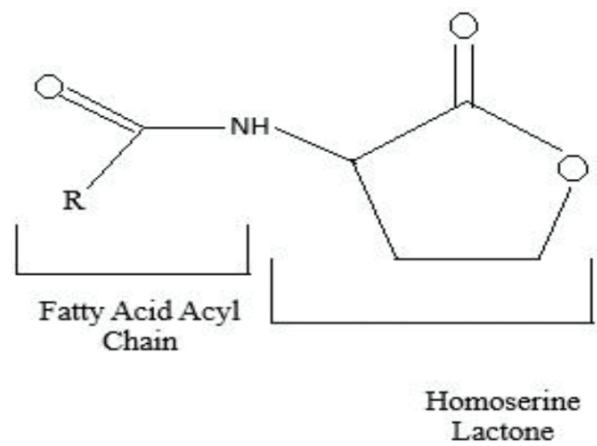


Figure 2 AHL structure [12]

phobic characteristics that allow penetration of the various cell membranes and subsequent binding with the hydrophobic pocket of proteins as receptors.

Lactone, amide, and 3-oxygens functionalities, in particular, promote formation of robust hydrogen bonding networks of the receptor active site. In spite of the richness of AHL structure which differs between different species of bacteria, some analogues are shared between many organisms, such as between *Vibrio fischeri* and *Erwinia carotovora*, serving as a means of communication between bacteria which cross species boundaries, i.e. overcoming the classical species barrier [13].

Autoinducing peptides-AIP (Gram positive bacteria)

The Autoinducer Peptides (AIPs) are the primary communication mechanism of Gram-positive bacteria, which are small peptides that are frequently exposed to chemical changes. Histidine kinases are two-component membrane-bound receptors that detect these peptides [10].

Autoinducing Peptides, or AIPs, are referred to as AIP-I-II-III-IV (Fig.3). They are all more hydrophobic in their N-terminal-to-C-terminal order in their peptide architecture, but peptides might differ in their amino acid sequence. The hydrophobic side chains of the amino acids at the C-terminal locations of the sequence are present in the AIPs. AIP linear peptide analogs or the hydrolysis of the thioester moiety deactivate its activities. It was discovered that an ester moiety in the form of a thioester macrocyclic ring might prevent the deactivation of signaling. AIP loses its signaling when N-terminal exocyclic structures are eliminated [14,15].

There are two main QS systems in Gram-positive bacteria (Fig. 4). After being ribosomally generated

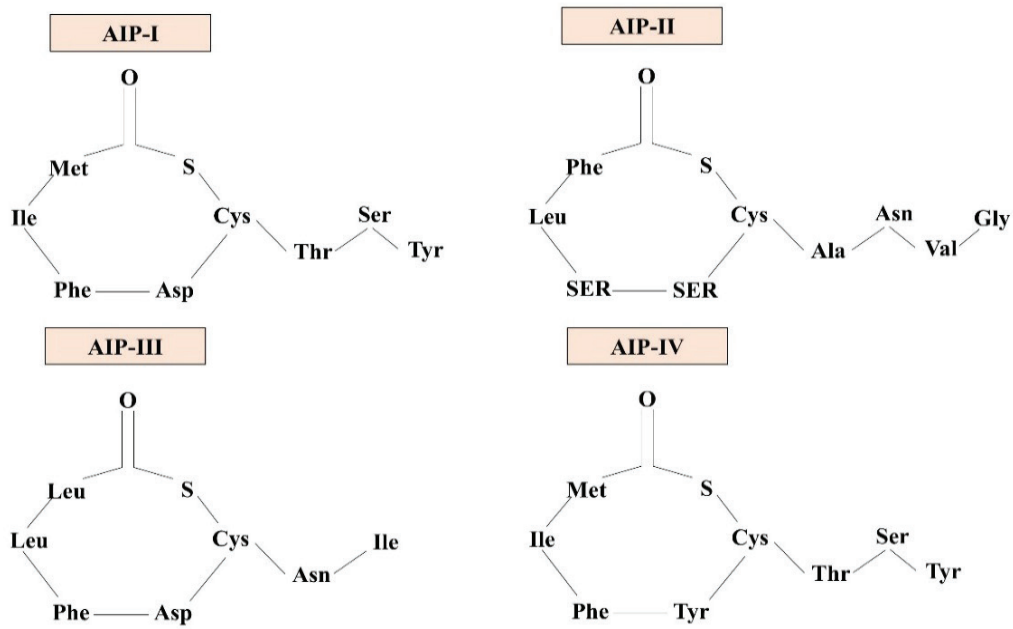


Figure 3 AIP-I, II, III, IV Structure found in Staphylococcus aureus [5].

as pro-peptides of the first pathway, AIPs undergo post-translational modification. In order to evolve into AIPs, they undergo multiple cleavages by secreted proteases after being released by specific ABC transporters. Certain cell surface receptor kinases phosphorylate a conserved His residue when they detect AIPs at a threshold concentration. By transferring the phosphoryl group to an Asp residue, the

active kinase then activates an intracellular regulator receptor downstream. Lastly, the secretion mechanism of AIP and the elections of certain target genes are controlled by the activated intracellular regulatory receptor. Because it contains two essential components—the intracellular regulating receptor and the His kinase at the membrane—this system is frequently referred to as a two-component pathway [16].

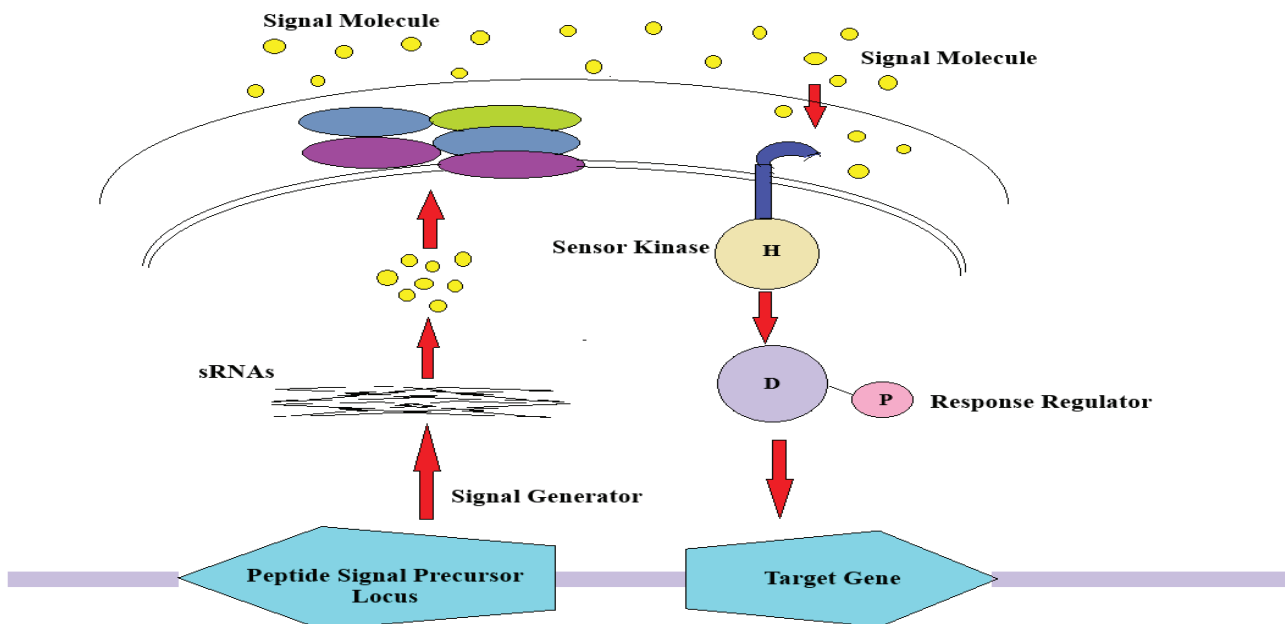


Figure 4 Two-component signal transduction and Gram-positive QS peptide signals, a general model. A response regulator protein regulates the transcription of downstream target genes, while the membrane-bound sensor kinase protein auto-phosphorylates to start the signal transduction [17].

Universal Signal AI-2

It has been reported that QS mechanisms are similar in Gram-positive and Gram-negative bacteria, but the specific autoinducers involved in these mechanisms can differ among different organisms. Saying so, there exists a group of QS systems, most noticeably DPD (dihydroxy pentanedione)/AI-2 which occurs in both Gram-positives and Gram negatives. The most versatile signaling mechanism employed by both Gram-positive and Gram-negative bacteria, DPD/AI-2, has been observed in over 50 percent of the QS-competent bacteria whose genome has been sequenced [18]. In contrast to AI (autoinducer)-1, AI-2, (quorum sensing) system occurs in both Gram-positive and Gram-negative bacteria and is believed to mediate cross-species communication [19]. In addition, autoinducer-2 (AI-2), also called furanosyl borate diester or tetrahydroxy-furan, is the common language system of both Gram-positive and Gram-negative species [9].

2.2 Receptors of QS

Signal transduction in the quorum sensing (QS) system relies heavily on receptors. Allosteric regulation takes place to control gene transcription when these receptors pick up an autoinducer. Therefore, one of the primary strategies for re-engineering bacterial behaviour is to block the activity of a receptor [20]. Bacterial group activities and population-level functions are systematized by QS (quorum-sensing) receptors and the signaling molecules that accompany them, which either directly or indirectly regulate gene expression [21].

LuxR:

With an N-terminal ligand binding domain and a C-terminal helix turn helix domain, LuxR type proteins are two domain proteins that bind DNA, typically as a homodimer via a recognition motif. It has been shown that repression is brought on by steric hindrance but that class I or II pathway proteins can induce transcriptional activation depending on protein.

The folding and ligand-binding characteristics of LuxR-type proteins are used to categorize them. AHL is necessary for Class I proteins to fold and bind them permanently. AHL can bind to the class II proteins, such as *V. fischeri* LuxR to organize, but the binding is reversible. Class III proteins that are independent of AHL to assemble into functional homodimers, such as *Erwinia* ExpR (the ortholog of SdiA) bind AHL reversibly. *Escherichia coli*'s SdiA proteins may need an endogenous ligand, 1-octanoyl-rac-glycerol, in order to be purified without the use of AHL, suggesting that they are class III [22].

1. LuxR-type (typical)

Gram-negative bacteria use the LuxI/R type QS protein receptor system to mediate the QS process. Auto-inducers N-acyl-L-homoserine lactones (AHLs), especially those induced with LuxI type proteins, are the key auto-inducers by Gram-negative bacteria. LuxR, the AHL response regulator, and LuxI, the AHL synthase of N-acyl-L-homoserine lactone transcriptionally activate target QS genes [23].

In response to the presence of particular chemical secretions, most notably AHL generated by the LuxI

Table 1 Quorum Sensing Receptor Types [20].

Receptor Type	Signal Molecule	Communication Type	Example Receptors	Representative Bacteria
LuxR-type (typical)	Acyl-homoserine lactones (AHLs)	Intraspecies	LuxR	<i>Vibrio fischeri</i>
LuxR-solo type	AHLs or alternative signals	Intraspecies/Interspecies	SdiA	<i>Escherichia coli</i>
Two-component (Gram-negative)	HAI-1	Intraspecies	LuxN	<i>Vibrio harveyi</i>
Two-component (Gram-positive)	Autoinducing peptides (AIPs)	Intraspecies	ArgC	<i>Staphylococcus aureus</i>
RRNPP family (Gram-positive)	AIPs	Intraspecies	Rap, Rgg, NprR, PrgX, PlcR	<i>Bacillus subtilis</i> , <i>Streptococcus thermophilus</i> , etc.
AI-2 receptor	Autoinducer-2 (AI-2)	Interspecies	LuxP, LsrB	<i>Vibrio harveyi</i> , <i>Salmonella typhimurium</i>
AI-3 receptor	AI-3, epinephrine, norepinephrine	Interspecies	QseC	<i>Enterohemorrhagic Escherichia coli</i>

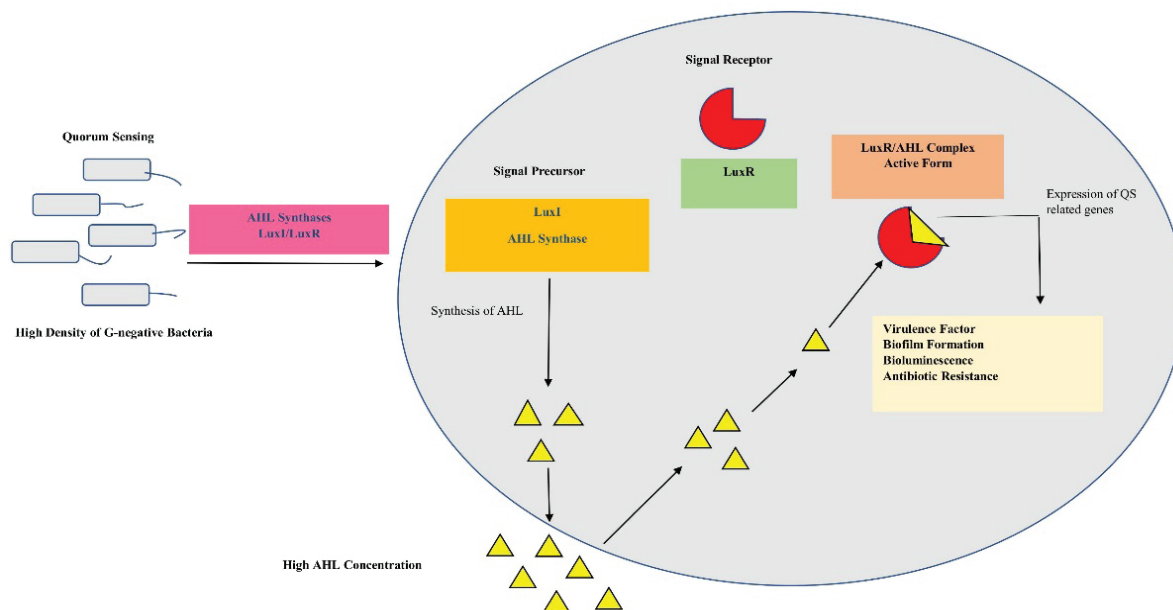


Figure 5 Mechanism of AHL through LuxR receptor [23].

(AHL Synthase) type protein, a class of common Gram-negative bacteria use LuxR-type quorum-sensing (QS) receptors to coordinate gene transcription (Fig.5). The associative mechanism of the most well-studied LuxR-type receptors allows dimerization and DNA binding to occur only when the ligand is bound.

On the other hand, a second, less studied family of proteins known as dissociatives bind to DNA when no ligand is present and unbind when a ligand is present. When compared to associative LuxR-type controllers, dissociative LuxR-type proteins are typically more operationally stable in vitro [24].

2. LuxR-solo type

The incomplete LuxR system of proteins without a corresponding synthase, commonly known as orphans or LuxR solos, is becoming more and more well-known in the field of QS. These single regulators may be distinguished by the type of origin of the ligand—endogenous or exogenous, AHL or non-AHL or neither. SdiA is part of the LuxR-type protein family which has its origin in the response regulator that controls bioluminescence in *Vibrio fischeri* [22]. There are two types of quorum sensing signals produced by other bacterial species and which are detected by SdiA: Exogenous Acyl-homoserine lactones (AHLs) and autoinducer-2 (AI-2) [25].

SdiA was initially identified as a transcriptional regu-

lator of the *ftsQAZ* operon, which codes for proteins essential for cellular division. This operon's inducer-dependent activation speeds up cell septation while also inhibiting the actions of numerous endogenous cell-division blocking factors. It was also demonstrated that AHL exposure appears to increase the regulatory action of SdiA on the *ftsQAZ* operon and that the AHL mediated quorum sensing may play a role in regulation of this particular operon [26].

3. Two-component (Gram-negative)

The primary autoinducer in *V. harveyi* is the HAI-1 molecule, which is an AHL type [27]. The three membrane-bound two-component QS receptors in *V. harveyi*, include LuxN, LuxPQ, and CqsS. These receptors recognize and bind related signaling molecules HAI-1, autoinducer-2 (AI-2) and CAI-1. These molecules are the by-products of the LuxM, LuxS, and CqsA synthases, respectively [20].

4. Two-component (Gram-positive)

The formation of *S. aureus* biofilms has been linked to a number of regulators. These are led by the accessory gene regulator (*agr*), a hybrid staphylococcal quorum-sensing system that trans-up-regulates the extracellular cysteine proteases SspB and ScpA to regulate filamentous growth. The impact of *agr*-mediated biofilm formation on the expression of several virulence factors suggests that it plays a crucial role in staphylococcal pathogenesis. By attaching itself to

the AgrC transmembrane protein and phosphorylating it, the acrocyclic peptide AIP, which contains seven to nine amino acids, controls the Agra [28].

5. RRNPP family (Gram-positive)

The RRNPP protein family (Rgg, Rap, NprR, PlcR and PrgX), which are covalently bound to intracellular signaling peptides associated with QS in gram-positive bacteria, includes intracellular receptors associated with intracellular signaling peptides [21]. The perfect examples of RRNPP the signal transducers are PrgX regulator in *Enterococcus faecalis*, the NprR regulators of *Bacillus cereus* group, the PlcR proteins of *B. cereus* group, the Rap phosphatases of *Bacillus subtilis* and the Rgg proteins of the *Streptococcus* species. All the most critical cellular functions are regulated in the family of RRNPP which are sporulation, competence, virulence, biofilm development, necrotrophic life style, conjugative plasmid transmissions, and antibiotics indifferences [29].

6. AI-2 receptor

The receptors LuxP AI-2 were first found in *Vibrio* species and LsrB are widespread throughout enteric bacteria and microorganisms of the Rhizobiaceae, Bacillaceae, and Clostridiaceae families [30].

The LuxS enzyme is widely expressed in several bacteria and participates in the production of the AI-2 signal. As a result, AI-2 is produced as an interspecies communication signal rather than being specific to a single bacterial strain. Three validated receptors of AI-2 are LuxP protein in the *Vibrio* species, LsrB protein of *Salmonella Typhimurium* and *Escherichia coli* and the RbsB protein of *Aggregatibacter actinomycetemcomitans*. As a receptor, LsrB positively promotes internalization of AI-2 but LuxP participates in cascades of signal transduction and thus determines downstream gene expression. Following internalization, AI-2 becomes phosphorylated. This phosphorylated AI-2 then binds to the LsrR protein, which in turn causes the Lsr system to be expressed and accelerates the conversion of AI-2 to its final form [31].

7. AI-3 receptor

Like hormones, AI-3 can be considered as an extracellular signal transduced via the binary system QseBC, a histidine kinasephosphatase (QseC) and response regulator pair (QseB). In a small number of Gram-negative species, including the enteropathogenic *E. coli*, the periplasmic QseC domain is retained, and AI-3 resembles the eukaryotic hormones in its effects since QseC is a bacterial adrenergic receptor to the

eukaryotic host hormones noradrenaline and epinephrine. The other consequence of this structural resemblance is that adrenergic receptor antagonists block AI-3. Furthermore, epinephrine/norepinephrine possesses the ability to activate the QseC/QseB cascade and become a QS signal that is then transferred to the quorum of the gut microbiota.

The human hormones noradrenaline and epinephrine are used by enterohemorrhagic *E. coli* O157:H7 (EHEC) to activate virulence genes that may be linked to the stress hormone cascade and irritable bowel syndrome brought on by extended stress [32].

Using histidine sensor kinase QseC, the bacteria can detect and respond to hormone-like host-produced factors like autoinducer-3 (AI-3), epinephrine (Epi), and norepinephrine (NE). The QseBC two-component system (TCS) is formed when the transmembrane C-terminus of QseC binds to the cytoplasmic membrane. However, it then undergoes autophosphorylation and the phosphate is transferred to an intracellular component known as QseB. After activation, QseB normally binds the specific sequence of DNA on the bacterial cells regulating the growth and motility, biofilm formation, and expression of virulence genes in bacteria. It acts as a virulence global regulator in enterohemorrhagic *Escherichia coli*, and the quorum sensing (QS) system QseBC was demonstrated to promote intracellular colonization and systemic infection [33].

3. Quorum sensing role in pathogenicity

3.1 Expression of Virulence Factor

Pathogenesis is a multilayered, interdependent process that involves multiple components interacting with one another as a pathogenic disease infects a host. Most strains follow a common path, even though the specific biology of infection can vary from organism to organism. The initial step that traps the virus by attaching itself to the host's microbiota and causing dysbiosis is adhesion. In order to alter the native cells' metabolism and evade immune responses, the invader releases a pattern of molecules, proteins, enzymes, and siderophores into the host cells after anchoring [34].

3.2 Formation of Biofilm

Biofilms are populations of microbial cells that are primarily adhered to a substrate and physically adherent to one another by a matrix of polymers, many of which are released by the microorganisms. In ad-

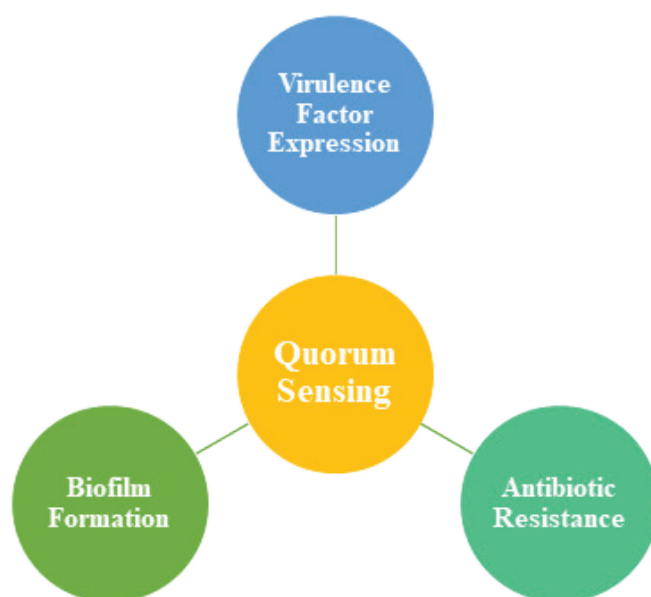


Figure 6 Quorum Sensing role in pathogenicity

dition to creating additional channels and microcolonies, the matrix provides the cells immersed inside it with physical protection as well as a microenvironmental gradient of oxygen and nourishment. These gradients are thought to produce phenotypic and genotypic plasticity and heterogeneity in populations linked to biofilms. Therefore, the created microenvironments encourage interactions between microorganisms since they are being driven inwards in the matrix and diffusion is being reduced [35].

The most advantageous of the many survival benefits that microorganisms receive from their biofilm style of life is the spread of antibiotic resistance. It may surprise you to learn that bacteria in biofilms are far more resistant to drugs than their free-living planktonic counterparts. The microorganisms in biofilms prefer to live a sessile lifestyle and constantly adapt to environmental changes. When conditions that support rapid development become available, these cells can alter their composition and return to a planktonic lifestyle [36].

The infamous biofilm-forming bacteria *Pseudomonas aeruginosa* successfully adheres to a variety of surfaces, resulting in persistent infections that are challenging to treat. The disease's opportunistic human pathogen, *P. aeruginosa*, causes severe and potentially fatal symptoms and quickly infects people with cystic fibrosis, resulting in significant morbidity and fatality rates. Because of its capacity to build biofilms, *P. aeruginosa* possesses the trait of being resistant to several medications.

Elements of *P. aeruginosa* that controls the biofilm

The extracellular polymeric substance (EPS), which contains proteins, polysaccharides, eDNA, and lipids, makes up the biofilm matrix. In addition to acting as a selective sieve that allows a small number of nutrients to enter and prevents antimicrobial probes from penetrating the matrix, the EPS helps the bacteria adhere to the surfaces. When the concentration of autoinducers in the host system exceeds a threshold, *P. aeruginosa* produces extracellular polymeric substances (EPS) [37].

3.3 Resistance to Antibiotics

Even though Antonie Van Leeuwenhoek examined biofilms with a crude microscope as early as 1674, Bill Costerton didn't come up with the word "biofilm" until 1978. The relationship between antimicrobial resistance and biofilm production varies depending on the kind of bacteria. The growth of biofilm and strains that produce beta-lactamases was responsible for the expansion and dissemination of biofilm and multidrug-resistant Gram-negative bacilli [38].

4. Therapeutic Approach of Quorum Quenching

Alternative methods of combating such an infection are required due to the misuse and overuse of antibiotics in medicine, the development of antimicrobial resistance, the failure of antibiotics to control microbial infections, and the detrimental effects on the environment's sustainability requirements. One such option is quorum quenching (QQ), which is the mechanistic suppression, retardation, or interruption

of bacterial social behavior or communication [39]. The term quorum quenching (QQ) was coined in 2000 when the pathogenicity of the plant pathogen *Erwinia carotovora* was demonstrated to be highly attenuated by an enzyme AiiA lactonase in the strain *Bacillus* sp. 240B1. Quorum quenching disrupts bacterial communication by stopping the process of synthesizing signaling molecules, by blocking or crafting signaling molecules or by blocking receptors, one of the three critical elements that make up the QS system [40].

4.1 Strategies of Quorum Quenching

The three types of AHL (G-Negative Bacteria)-inactivating enzymes—known as lactonases, amidases, and oxidoreductases—can be categorized according to their mechanistic characteristics. In a reversible mechanism, lactonases in the AHL family hydrolyse the homoserine lactone ring to produce acyl homoserine. AHL acylases hydrolyse the AHL on the amide bonds, resulting in an irreversible reaction that produces fatty acid chains and homoserine lactone. AHL oxidoreductases, which can either oxidize or decrease the AHLs, have hardly ever been examined (refer to Fig. 7) [41].

Degradation of Signal Molecule

The three types of AHL-inactivating enzymes—known as lactonases, amidases, and oxidoreductases—can be categorized according to their mechanistic char-

acteristics. In a reversible mechanism, lactonases in the AHL family hydrolyze the homoserine lactone ring to produce acyl homoserine. AHL acylases hydrolyze the AHL on the amide bonds, resulting in an irreversible reaction that produces fatty acid chains and homoserine lactone. AHL oxidoreductases, which have the ability to either oxidize or decrease the AHLs, have hardly ever been examined [42].

Biosynthesis of Signal Inhibitor

In addition, the acyl carrier protein (ACP), the acyl component of the AHL signal, and S-adenosylmethionine (SAM), the amino donor of the synthesis of the homoserine lactone ring moiety, might be used to prevent the formation of AHL. It was discovered that the other SAM analogues, such as sinefungin, S-adenosylhomocysteine, and S-adenosylcysteine, were also efficient AHL synthesis inhibitors [43].

Other inhibitors that are utilized to prevent the synthesis of AHL have been found. For example, triclosan prevents the synthesis of AHL by preventing the enoyl-ACP reductase from producing the precursor [44].

Signal Receptor Antagonist

The formed receptor-AI complex controls the transcription of genes that control virulence, biofilm formation, conjugation, and sporulation in addition to bioluminescence and competence once the autoinducers have ultimately attached to their respective receptors. Furthermore, QS facilitates communica-

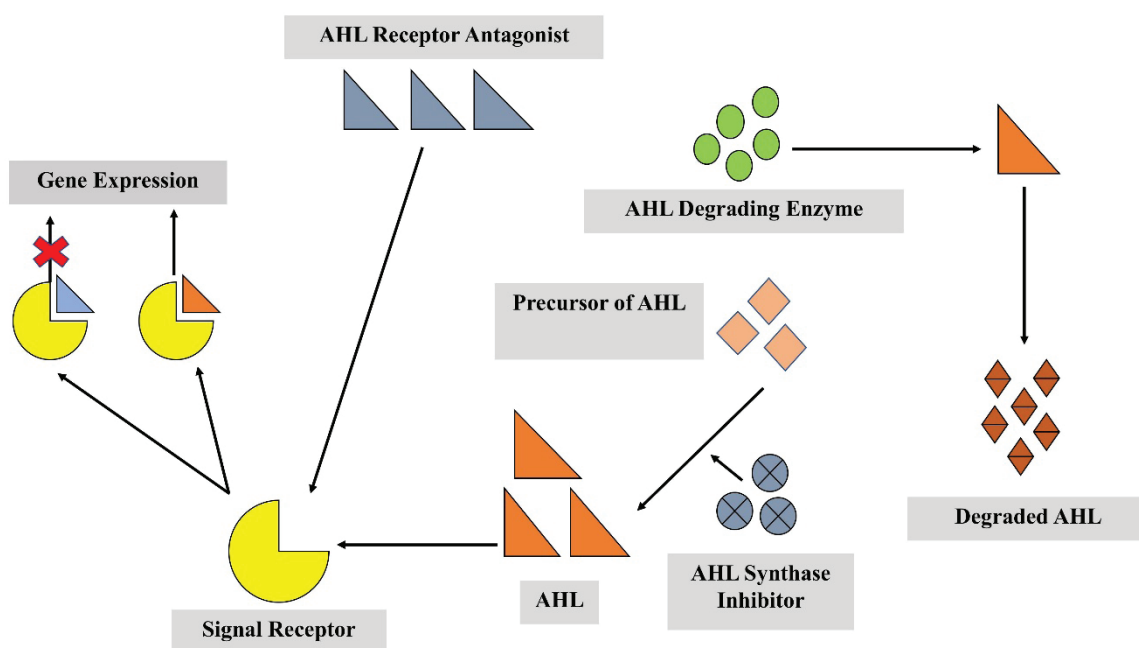


Figure 7 AHL inhibition strategies [5].

tion between bacteria of various species, and while some species lack the ability to synthesize their own AIs, they do have receptors for other species' AIs. It's interesting to note that bacterial infections' pathogenicity is much decreased by disrupting their QS systems and blocking the appropriate QS receptors [45]. By either competing with the bacteria at the receptor level or deactivating the receptor in the QS signaling, one can decrease the virulence and infection caused by the bacteria. Flavonoids and furanones have been identified as the two classes of QS inhibitors that bind to the receptors of different pathogenic bacteria. Furanones, whose halogenated derivatives are generated by the marine alga *Delisea pulchra*, were the first identified QS inhibitors [46].

The most prevalent AI receptor protein found in Gram-negative bacteria is LuxR-AHL. The addition of an active methylene group to the AHL also reduces the receptor's protein-signal binding by 50%, making the AHL alterations a very powerful tool for controlling processes with QS signals. Gram-positive bacteria control two systems of the QS: an active transcriptional regulator and a membrane-bound histidine kinase receptor. Currently, it is possible to block these receptors in Gram-positive bacteria against their pathogenicity by utilizing certain AIP antagonists to inhibit the receptors. Specifically, the four variant thiolactone peptides (AIP I-IV) of *S. aureus*, which employs the AIP-mediated QS system *agr*, have an impact on its bacterial activity [5].

Plant derived or Natural inhibitors of QS

Some of the secondary metabolites formed by medicinal plants target the QS system: phenols and phenolic acids, polyacetylenes, flavonoids, terpenoids, tannins, saponins, quinones, coumarins, alkaloids [47].

5. Applications and Advances in Anti-QS Therapy

The goal of anti-QS tactics is to interfere with the QS signaling pathways that allow bacteria to interact and coordinate their actions [48].

Anti-virulence drugs development

On the one hand, a new method of therapy known as precision antimicrobials was developed in order to overcome the increasing antibiotic resistance rate amongst microorganisms. Because precision antimicrobial drugs tend to target the pathogen-specific virulence determinants without affecting the resident microbiome, they are expected to establish restricted selective forces that significantly lower the risk of

acquired resistance. More significantly, these antivirulence medicines target pathogen-specific virulence factors, ensuring that their activity is selective against only the disease-causing strain, rather than merely killing pathogens on a broad spectrum [49].

Nanoparticles targeting QS

In terms of therapeutic approaches, nanotechnology has been receiving a lot of interest. It was also demonstrated that nanomolecules, nanocomposites, and generally nano- and microcomposites, including composites based on Ag or ZnO, could successfully quench the quorum because they inhibited the microcolony, which decreased the creation of biofilms and changed their structure [47].

When it comes to bacterial biofilm development, metal and metallic nanoparticles like silver, selenium (SeNPs), tellurium (TeNPs), and gold (GNPs) have shown remarkable promise in the counteracting of bacterial resistance through nanotechnology. Projections reveal that nanotechnology could play a significant role in combating bacterial infections and managing them, specifically in fighting multidrug-resistant bacteria and bacterial biofilms [50].

Synergistic use with antibiotics

The QSIs do not kill or eliminate the bacteria; instead, they stop a biofilm from growing. Therefore, it is necessary to look for a synergistic interaction between QSIs and antibiotics [51]. Antibiotic resistance may be decreased by a combination therapy that uses synergistic medicines with distinct targets and mechanisms of action. Drugs like trimethoprim/sulfamethoxazole (Bactrim), tazobactam/piperacillin (Zosyn), and amoxicillin/clavulanate (Augmentin) are examples of combinations that have already received approval [52].

6. Challenges and Limitations in QS inhibitors

The organisms need to have a built-in defense system against an attack that could endanger their existence. A continuous state of AHL signals, albeit at a slower rate, can even be seen at low cell densities because quorum-sensing inhibitors (QSIs) are designed to essentially only engage QS without stimulating bacterial growth. Therefore, bacteria may be released to express their virulence once the concentration of QSI falls below the threshold. For bacteria to continue to be resistant to QSIs, they do not even need to undergo any genetic change. By keeping its QS under control until the concentrations of QSI exceed those of signal molecules, bacteria can evade QSI. It is stat-

ed that the goal going forward should be to build QSI with a lower chance of resistance. Every living thing will eventually develop defense systems because survival is their primary goal [53].

In the past few years there are a number of factors that have contributed towards the complex issue of multiple drug resistance (MDR). With periodic exposure to a particular antibiotic, multidrug and widespread drug resistance have occurred [5].

Bacteria have also evolved various forms of antibiotic resistance. First, chemical modification inhibits antibiotic effect by the secretion of enzymes which alter the chemical structure of the antibiotic either by disabling the drug molecule or by derivatizing its chemical functional groups, thus preventing the antibiotic to react with its target. Second, drug efflux pumps are an important mechanism for developing microbial resistance against antibiotics, wherein bacteria develop efflux pump proteins in the cell membrane, thus actively pumping out the antibiotic from the cell before it can reach the concentration needed for its effect. Third, bacteria can develop resistance by altering drug-target genes, either by shielding the target site against the binding action of an antibiotic, or by changing the target site itself to allow it to be less specific to the antibiotic molecule [54].

7. Future Prospects

Approaches based on CRISPER

The bacteria acquired the CRISPR-Cas system (Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR)-associated proteins) as a natural evolutionary immunity to bacteria viruses (bacteriophages). It was first found in *E. coli* in the 1980s, but its ability to actually form an adaptive immune system in bacteria, which is thought to be an analog of the mammalian system, was not realized until the 2000s. The invention of the CRISPR-Cas9 technique of gene editing in 2012 has turned the world of biotechnology upside down, enabling the editing of the genome. Scientists have recently concentrated on employing CRISPR-Cas, a novel antimicrobial intervention, to fight harmful bacteria and infections, especially those that are resistant to many drugs [55]. The CRISPR system is the most cutting-edge technology that promises to address the present issues with genome editing. It so happens that the CRISPR system is a natural immune response that protects against bacterial phage invasion. These main Cas protein and other nomenclature attributes have pro-

vided the classification of CRISPRCas systems into two classes, six types, and 19 subtypes [56]. Since its discovery, the "CRISPR-Cas9" system—a form of acquired immune system that shields several bacteria and archaea—has attracted a lot of attention [57].

By interfering with or removing the genes involved in autoinducer synthesis, detection, and downstream signaling, CCS has shown useful in controlling quorum sensing circuits [58]. Bacterial defense against viral infections depends on three key elements of the CRISPR-Cas system: "adaptation (spacer acquisition), crRNA synthesis (expression), and target interference". The Cas protein, sometimes referred to as the nuclease protein, is produced by the Cas gene and cleaves and destroys the foreign viral DNA [59].

Discovery of QS pathway by OMICS technologies

Through omics science, the host-pathogen relationship is consistently thoroughly and precisely studied, and the pathogen's unique proteins can be identified. Researchers are currently leveraging multi-omics data to update and reshape the microbial risk assessment role model.

Transcriptomics methodology employed in recent biofilm studies also enables the full detection of a certain set of genes that participate in antimicrobial resistance and biofilm development.

Although alterations in the whole protein profile can be detected using proteomics, nothing is known about the biological changes at the transcriptome level. The term "proteomics" describes the full expression of an organism's proteins under specific conditions.

Metabolomics is the detection, identification, and evaluation of a biological system's metabolome. Metabolite profiling can provide insights into the biochemistry, pathophysiology, and physiology of cells. Metabolomics is a useful technique for tracking the end points since, in contrast to genes and proteins, metabolite profiling is directly linked to the defined phenotype [60].

Through the deciphering of the molecular complexity of microbial life and the application of cutting-edge future technologies to these advancements, multi-omics technologies will address significant issues like food security and antibiotic resistance (ABR) [61]. Bacteria can coordinate gene expression and related metabolism through intercellular signaling, which can result in metabolic diversity in the products of bacterial metabolism. Metabolomics is the qualita-

tive and quantitative analysis of all of the low-weight molecular mass metabolites of a given organism or a given cell in a state of normal physiology [62].

By comparing samples taken under settings with and without QS induction, the transcriptome sequencing technique is fairly effective at identifying the genes linked to QS. Finding the QS-mediated expression profiles of various plant and human pathogens has been the most popular use of the technology [63].

Use of quorum sensing in the industry and agriculture beyond medicine

Quorum-sensing signaling systems are intimately linked to the network between the host plant and the associated microbial population, which is crucial for the holobiont's establishment. The balance of healthy or disease-causing bacteria and their host plants, which impart immunity and growth characteristics, is disrupted by interkingdom signaling. Although further research is needed to completely understand the precise chemical process by which bacterial AHL signals impact plant performance, it is said that they do. Depending on the chemical structure of the QS signal, there is evidence that AHLs change the balance of phytohormones, mediate morphological changes of roots (elongation of the primary root, stimulation of root growth), increase salt tolerance, etc. [50].

CONCLUSION

Inhibition of quorum sensing (QS) is also a potent non-lethal approach to microbial antimicrobials because it interferes with communication networks regulating virulence and biofilm formation in microorganisms without killing microbes. Using QS-based medicines as a sustainable replacement of traditional antibiotics is an attractive prospect and emphasizes pathogenicity, biofilm formation and resistance mechanisms. The cutting-edge area in the potential application of this strategy pertains to the recent advances in the translational research efforts, including quorum quenching enzymes, natural, and synthetic enzyme inhibitors, nanotechnology-based drug and enzyme delivery vehicles, and combinatorial therapy using medications.

But multidisciplinary analysis connecting the domains of microbiology, chemistry, bioinformatics, nanotechnology and clinical sciences is important to achieve the full potent of attainment of its benefits. Future directions such as new pathway discovery via

omics and fine-tuning of QS genes via CRISPR editing can accelerate clinical use. The introduction of QS-specific strategies into the process of antimicrobial therapy may completely change the landscape of the control of infections, allowing to limit the development of resistance and protecting the microbiome balance.

REFERENCES

1. Biondo C. New insights into bacterial pathogenesis. *Pathogens*. 2022 Dec 26;12(1):38. <https://doi.org/10.3390/pathogens12010038>
2. Soni J, Sinha S, Pandey R. Understanding bacterial pathogenicity: a closer look at the journey of harmful microbes. *Frontiers in Microbiology*. 2024 Feb 20;15:1370818. <https://doi.org/10.3389/fmicb.2024.1370818>
3. Castillo-Juárez I, Maeda T, Mandujano-Tinoco EA, Tomás M, Pérez-Eretza B, García-Contreras SJ, Wood TK, García-Contreras R. Role of quorum sensing in bacterial infections. *World Journal of Clinical Cases: WJCC*. 2015 Jul 16;3(7):575. <https://doi.org/10.12998/wjcc.v3.i7.575>
4. Rajkhowa S, Hussain SZ, Agarwal M, Zaheen A, Al-Hussain SA, Zaki ME. Advancing Antibiotic-Resistant Microbe Combat: Nanocarrier-Based Systems in Combination Therapy Targeting Quorum Sensing. *Pharmaceutics*. 2024 Sep 3;16(9):1160. <https://doi.org/10.3390/pharmaceutics16091160>
5. Naga NG, El-Badan DE, Ghanem KM, Shaaban MI. It is the time for quorum sensing inhibition as alternative strategy of antimicrobial therapy. *Cell Communication and Signaling*. 2023 Dec;21(1):1-4. <https://doi.org/10.1186/s12964-023-01154-9>
6. Singh S, Bhatia S. Quorum sensing inhibitors: curbing pathogenic infections through inhibition of bacterial communication. *Iranian journal of pharmaceutical research: IJPR*. 2021;20(2):486. doi: 10.22037/ijpr.2020.113470.14318
7. Muras A, Mallo N, Otero-Casal P, Pose-Rodríguez JM, Otero A. Quorum sensing systems as a new target to prevent biofilm-related oral diseases. *Oral Diseases*. 2022 Mar;28(2):307-13. <https://doi.org/10.1111/odi.13689>
8. Bouyahya A, Chamkhi I, Balahbib A, Rebezov M, Shariati MA, Wilairatana P, Mubarak MS, Benali T, El Omari N. Mechanisms, anti-quorum-sensing actions, and clinical trials of medicinal plant bioactive compounds against bacteria: a comprehensive review. *Molecules*. 2022 Feb 22;27(5):1484. <https://doi.org/10.3390/molecules27051484>
9. Su Y, Ding T. Targeting microbial quorum sensing: the next frontier to hinder bacterial driven gastrointestinal infections. *Gut Microbes*. 2023 Dec 18;15(2):2252780. <https://doi.org/10.1080/19490976.2023.2252780>
10. Polizzi A, Donzella M, Nicolosi G, Santonocito S, Pesce P, Isola G. Drugs for the quorum sensing inhibition of oral biofilm: New frontiers and insights in the treatment of periodontitis. *Pharmaceutics*. 2022 Dec 7;14(12):2740. <https://doi.org/10.3390/pharmaceutics14122740>
11. Kumar L, Patel SK, Kharga K, Kumar R, Kumar P, Pandohee J, Kulshresha S, Harjai K, Chhibber S. Molecular mechanisms and applications of N-acyl homoserine lactone-mediated quorum sensing in bacteria. *Molecules*. 2022 Nov 4;27(21):7584. <https://doi.org/10.3390/molecules27217584>
12. Filik, N., & Filik, F. (2023). Bacteria-to-bacteria communication, Signaling Molecules: AHLs, AIPs and AI-2, I can't talk now matey, gone to pathogenesis!. *International Journal of Secondary Metabolite*, 10(4), 590-604. <https://doi.org/10.21448/ijism.1248987>

13. Zhang Q, Li S, Hachicha M, Boukraa M, Soulère L, Efrif ML, Queneau Y. Heterocyclic chemistry applied to the design of N-Acyl homoserine lactone analogues as bacterial quorum sensing signals mimics. *Molecules*. 2021 Aug 24;26(17):5135. <https://doi.org/10.3390/molecules26175135>
14. Martínez OF, Duque HM, Franco OL. Peptidomimetics as potential anti-virulence drugs against resistant bacterial pathogens. *Frontiers in Microbiology*. 2022 Apr 18;13:831037. <https://doi.org/10.3389/fmicb.2022.831037>
15. Nagano M, Ishida S, Suga H. Inner residues of macrothiolactone in autoinducer peptides I/IV circumvent spontaneous S-to-O acyl transfer to the upstream serine residue. *RSC Chemical Biology*. 2022;3(3):295-300. <https://doi.org/10.1039/D1CB00225B>
16. Bhatt VS. Quorum sensing mechanisms in gram positive bacteria. In *Implication of quorum sensing system in biofilm formation and virulence* 2019 Jan 29 (pp. 297-311). Singapore: Springer Singapore. https://doi.org/10.1007/978-981-13-2429-1_20
17. Haque S, Yadav DK, Bisht SC, Yadav N, Singh V, Dubey KK, Jawed A, Wahid M, Dar SA. Quorum sensing pathways in Gram-positive and-negative bacteria: potential of their interruption in abating drug resistance. *Journal of Chemotherapy*. 2019 May 19;31(4):161-87. <https://doi.org/10.1080/1120009X.2019.1599175>
18. Davares AK, Arsene MM, Viktorovna PI, Vyacheslavovna YN, Vladimirovna ZA, Aleksandrovna VE, Nikolayevich SA, Nadezhda S, Anatolievna GO, Nikolaevna SI, Sergueïevna DM. Quorum-sensing inhibitors from probiotics as a strategy to combat bacterial cell-to-cell communication involved in food spoilage and food safety. *Fermentation*. 2022 Dec 6;8(12):711. <https://doi.org/10.3390/fermentation8120711>
19. Neil B, Cheney GL, Rosenzweig JA, Sha J, Chopra AK. Antimicrobial resistance in aeromonads and new therapies targeting quorum sensing. *Applied Microbiology and Biotechnology*. 2024 Dec;108(1):205. <https://doi.org/10.1007/s00253-024-13055-z>
20. Yi L, Dong X, Grenier D, Wang K, Wang Y. Research progress of bacterial quorum sensing receptors: Classification, structure, function and characteristics. *Science of The Total Environment*. 2021 Apr 1;763:143031. <https://doi.org/10.1016/j.scitotenv.2020.143031>
21. Verdugo-Fuentes A, Gastélum G, Rocha J, de la Torre M. Multiple and overlapping functions of quorum sensing proteins for cell specialization in *Bacillus* species. *Journal of Bacteriology*. 2020 Apr 27;202(10):10-128. <https://doi.org/10.1128/jb.00721-19>
22. Schwieters A, Ahmer BM. Role of the LuxR solo, SdiA, in eavesdropping on foreign bacteria. *FEMS Microbiology Reviews*. 2025 Apr 16:fuaf015. <https://doi.org/10.1093/femsre/fuaf015>
23. Ampomah-Wireko M, Luo C, Cao Y, Wang H, Nininahazwe L, Wu C. Chemical probe of AHL modulators on quorum sensing in Gram-Negative Bacteria and as antiproliferative agents: A review. *European Journal of Medicinal Chemistry*. 2021 Dec 15;226:113864. <https://doi.org/10.1016/j.ejmech.2021.113864>
24. Stoutland I, Aguirre-Figueroa G, Blackwell H. Chemical probes to control a dissociative LuxR-type quorum sensing receptor in Gram-negative bacteria. *ChemRxiv*. 2024; doi:10.26434/chemrxiv-2024-mmfn9-v2. <https://doi.org/10.26434/chemrxiv-2024-mmfn9-v2>
25. Panchal J, Prajapati J, Dabhi M, Patel A, Patel S, Rawal R, Saraf M, Goswami D. Comprehensive computational investigation for ligand recognition and binding dynamics of SdiA: a degenerate LuxR-type receptor in *Klebsiella pneumoniae*. *Molecular Diversity*. 2024 Dec;28(6):3897-918. <https://doi.org/10.1007/s11030-023-10785-6>
26. Mayer C, Borges A, Flament-Simon SC, Simões M. Quorum sensing architecture network in *Escherichia coli* virulence and pathogenesis. *FEMS microbiology reviews*. 2023 Jul;47(4):-fuad031. <https://doi.org/10.1093/femsre/fuad031>
27. Zhou T, Wang J, Todd JD, Zhang XH, Zhang Y. Quorum Sensing Regulates the Production of Methanethiol in *Vibrio Harveyi*. *Microorganisms*. 2023 Dec 24;12(1):35. <https://doi.org/10.3390/microorganisms12010035>
28. Huang Q, Xie Y, Yang Z, Cheng D, He L, Wang H, Liu Q, Li M. The cytoplasmic loops of AgrC contribute to the quorum-sensing activity of *Staphylococcus aureus*. *Journal of Microbiology*. 2021 Jan;59(1):92-100. <https://doi.org/10.1007/s12275-021-0274-x>
29. Do H, Kumaraswami M. Structural mechanisms of peptide recognition and allosteric modulation of gene regulation by the RRNPP family of quorum-sensing regulators. *Journal of molecular biology*. 2016 Jul 17;428(14):2793-804. <https://doi.org/10.1016/j.jmb.2016.05.026>
30. Liu X, Wei Z, Yang M, Zhang X, Wang Z, Li S, Li C, Zhu L, Zhang L, Zhang X, Shen X. A global perspective on autoinducer-2-mediated cell communication in prokaryotes. *iScience*. 2025 Jun 13. <https://doi.org/10.1016/j.isci.2025.112908>
31. Liu Y, Hu H, Luo F. Roles of autoinducer-2 mediated quorum sensing in wastewater treatment. *Water Science and Technology*. 2021 Aug 15;84(4):793-809. <https://doi.org/10.2166/wst.2021.278>
32. Juszczyk-Kubiak E. Molecular aspects of the functioning of pathogenic bacteria biofilm based on quorum sensing (QS) signal-response system and innovative non-antibiotic strategies for their elimination. *International Journal of Molecular Sciences*. 2024 Feb 24;25(5):2655. <https://doi.org/10.3390/ijms25052655>
33. Qin T, Chen K, Xi B, Pan L, Xie J. QseBC regulates in vitro and in vivo virulence of *Aeromonas hydrophila* in response to norepinephrine. *Microbial Pathogenesis*. 2023 Jan 1;174:105914. <https://doi.org/10.1016/j.micpath.2022.105914>
34. Venkateswaran P, Vasudevan S, David H, Shaktivel A, Shanmugam K, Neelakantan P, Solomon AP. Revisiting ESKAPE Pathogens: virulence, resistance, and combating strategies focusing on quorum sensing. *Frontiers in cellular and infection microbiology*. 2023 Jun 29;13:1159798. <https://doi.org/10.3389/fcimb.2023.1159798>
35. Falà AK, Álvarez-Ordóñez A, Filloux A, Gahan CG, Cotter PD. Quorum sensing in human gut and food microbiomes: Significance and potential for therapeutic targeting. *Frontiers in Microbiology*. 2022 Nov 25;13:1002185. <https://doi.org/10.3389/fmicb.2022.1002185>
36. Saxena P, Joshi Y, Rawat K, Bisht R. Biofilms: architecture, resistance, quorum sensing and control mechanisms. *Indian journal of microbiology*. 2019 Mar 5;59(1):3-12. <https://doi.org/10.1007/s12088-018-0757-6>
37. Brindhadevi K, LewisOscar F, Mylonakis E, Shanmugam S, Verma TN, Pugazhendhi A. Biofilm and Quorum sensing mediated pathogenicity in *Pseudomonas aeruginosa*. *Process Biochemistry*. 2020 Sep 1;96:49-57. <https://doi.org/10.1016/j.procbio.2020.06.001>
38. Odularu AT, Afolayan AJ, Sadimenko AP, Ajibade PA, Mbese JZ. Multidrug-Resistant Biofilm, Quorum Sensing, Quorum Quenching, and Antibacterial Activities of Indole Derivatives as Potential Eradication Approaches. *BioMed Research International*. 2022;2022(1):9048245. <https://doi.org/10.1155/2022/9048245>
39. Rather MA, Saha D, Bhuyan S, Jha AN, Mandal M. Quorum quenching: a drug discovery approach against *Pseudomonas aeruginosa*. *Microbiological Research*. 2022 Nov 1;264:127173. <https://doi.org/10.1016/j.mi>

- res.2022.127173
40. Malešević M, Jovčić B. Targeting Gram-Negative Bacterial Biofilm with Innovative Therapies: Communication Silencing Strategies. *Future Pharmacology*. 2025 Jul 3;5(3):35. <https://doi.org/10.3390/futurepharmacol5030035>
 41. Zhu X, Chen WJ, Bhatt K, Zhou Z, Huang Y, Zhang LH, Chen S, Wang J. Innovative microbial disease biocontrol strategies mediated by quorum quenching and their multifaceted applications: A review. *Frontiers in Plant Science*. 2023 Jan 12;13:1063393. <https://doi.org/10.3389/fpls.2022.1063393>
 42. Boakye A, Seidu MP, Adomako A, Laryea MK, Borquaye LS. Marine-derived furanones targeting quorum-sensing receptors in *Pseudomonas aeruginosa*: molecular insights and potential mechanisms of inhibition. *Bioinformatics and Biology Insights*. 2024 Sep;18:11779322241275843. <https://doi.org/10.1177/11779322241275843>
 43. D'Aquila P, De Rose E, Sena G, Scorza A, Cretella B, Passarino G, Bellizzi D. Quorum quenching approaches against bacterial-biofilm-induced antibiotic resistance. *Antibiotics*. 2024 Jul 3;13(7):619. <https://doi.org/10.3390/antibiotics13070619>
 44. Lima EM, Winans SC, Pinto UM. Quorum sensing interference by phenolic compounds—A matter of bacterial misunderstanding. *Heliyon*. 2023 Jul 1;9(7). <https://doi.org/10.1016/j.heliyon.2023.e17657>
 45. Cavalu S, Elbaramawi SS, Eissa AG, Radwan MF, S. Ibrahim T, Khafagy ES, Lopes BS, Ali MA, Hegazy WA, Elfaky MA. Characterization of the anti-biofilm and anti-quorum sensing activities of the β -adrenoreceptor antagonist atenolol against gram-negative bacterial pathogens. *International Journal of Molecular Sciences*. 2022 Oct 28;23(21):13088. <https://doi.org/10.3390/ijms232113088>
 46. Hetta HF, Ramadan YN, Rashed ZI, Alharbi AA, Alsharif S, Alkindy TT, Alkhamali A, Albalawi AS, Battah B, Donadu MG. Quorum sensing inhibitors: an alternative strategy to win the battle against multidrug-resistant (MDR) bacteria. *Molecules*. 2024 Jul 24;29(15):3466. <https://doi.org/10.3390/molecules29153466>
 47. Paluch E, Rewak-Soroczyńska J, Jędrusik I, Mazurkiewicz E, Jermakow KJ. Prevention of biofilm formation by quorum quenching. *Applied microbiology and biotechnology*. 2020 Mar;104(5):1871-81. <https://doi.org/10.1007/s00253-020-10349-w>
 48. Elfaky MA. Unveiling the hidden language of bacteria: anti-quorum sensing strategies for gram-negative bacteria infection control. *Archives of Microbiology*. 2024 Mar;206(3):124. <https://doi.org/10.1007/s00203-024-03900-0>
 49. Lau WY, Taylor PK, Brinkman FS, Lee AH. Pathogen-associated gene discovery workflows for novel antivirulence therapeutic development. *EBioMedicine*. 2023 Feb 1;88. <https://doi.org/10.1016/j.ebiom.2022.104429>
 50. Abbamondi GR, Tommonaro G. Research progress and hopeful strategies of application of quorum sensing in food, agriculture and nanomedicine. *Microorganisms*. 2022 Jun 10;10(6):1192. <https://doi.org/10.3390/microorganisms10061192>
 51. Knap K, Kwiecień K, Ochońska D, Reczyńska-Kolman K, Pamuła E, Brzychczy-Włoch M. Synergistic effect of antibiotics, α -linolenic acid and solvent type against *Staphylococcus aureus* biofilm formation. *Pharmacological Reports*. 2024 Dec;76(6):1456-69. <https://doi.org/10.1007/s43440-024-00669-3>
 52. Beasley JM, Dorjsuren D, Jain S, Rath M, Tieghi RS, Tropsha A, Simeonov A, Zakharov AV, Muratov E. Breaking the Phalanx: Overcoming Bacterial Drug Resistance with Quorum Sensing Inhibitors that Enhance Therapeutic Activity of Antibiotics. *bioRxiv*. 2025 Jan 22. <https://doi.org/10.1101/2025.01.17.633658>
 53. Kalia VC, Wood TK, Kumar P. Evolution of resistance to quorum-sensing inhibitors. *Microbial ecology*. 2014 Jul;68(1):13-23. <https://doi.org/10.1007/s00248-013-0316-y>
 54. Zhao X, Yu Z, Ding T. Quorum-sensing regulation of antimicrobial resistance in bacteria. *Microorganisms*. 2020 Mar 17;8(3):425. <https://doi.org/10.3390/microorganisms8030425>
 55. Jacobowski AC, Boleti AP, Cruz MV, Santos KF, de Andrade LR, Frihling BE, Migliolo L, Paiva PM, Teodoro PE, Teodoro LP, Macedo ML. Combating Antimicrobial Resistance: Innovative Strategies Using Peptides, Nanotechnology, Phages, Quorum Sensing Interference, and CRISPR-Cas Systems. *Pharmaceuticals*. 2025 Jul 27;18(8):1119. <https://doi.org/10.3390/ph18081119>
 56. Salaria M, Frazee J, Nautiyal R, Dhiman SS, Sharma J. Role of the CRISPR technique in decoding the principles of quorum sensing. In: Schuster M, Greenberg EP, editors. *Quorum sensing: microbial rules of life*. Washington (DC): American Chemical Society; 2020. p. 49–63. DOI: 10.1021/bk-2020-1374.ch004
 57. Yang B, Fang D, Lv Q, Wang Z, Liu Y. Targeted therapeutic strategies in the battle against pathogenic bacteria. *Frontiers in pharmacology*. 2021 May 12;12:673239. <https://doi.org/10.3389/fphar.2021.673239>
 58. Nag A. CRISPR-Cas System and its Role in Quorum-Sensing Processes of Bacteria and Fungi. In *Gene Editing in Plants: CRISPR-Cas and Its Applications 2024* Mar 19 (pp. 817-838). Singapore: Springer Nature Singapore. https://doi.org/10.1007/978-981-99-8529-6_29
 59. Junaid M, Thirapanmethee K, Khuntayaporn P, Chomnawang MT. CRISPR-based gene editing in *Acinetobacter baumannii* to combat antimicrobial resistance. *Pharmaceuticals*. 2023 Jun 23;16(7):920. <https://doi.org/10.3390/ph16070920>
 60. Dutta B, Chatterjee D, Sarkar N, Lahiri D, Nag M, Ray RR. Multi-omics technology in detection of multispecies biofilm. *The Microbe*. 2024 Sep 1;4:100128. <https://doi.org/10.1016/j.microb.2024.100128>
 61. Taj Z, Keishing S, Chattopadhyay I. Fundamentals and Applications of Omics in Microbiology. In *Omics Approaches in Biofilm Research: Perspectives and Applications 2025* Jul 8 (pp. 63-90). Cham: Springer Nature Switzerland. https://doi.org/10.1007/978-3-031-91863-6_3
 62. Li Y, He J, Wei G, Shi Y, Tao W, Huang A. Metabolomics-based insights into the mechanism of quorum sensing-enhanced conjugated linoleic acid production in *Limosilactobacillus fermentum* L1. *Lwt*. 2025 Feb 15;218:117477. <https://doi.org/10.1016/j.lwt.2025.117477>
 63. Zeng YH, Cheng KK, Cai ZH, Zhu JM, Du XP, Wang Y, Zhou J. Transcriptome analysis expands the potential roles of quorum sensing in biodegradation and physiological responses to microcystin. *Science of the Total Environment*. 2021 Jun 1;771:145437. <https://doi.org/10.1016/j.scitotenv.2021.145437>