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MINIREVIEWS

Evolution of Resistance to Quorum-Sensing Inhibitors

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Abstract The major cause of mortality and morbidity in human beings is bacterial infection. Bacteria have developed resistance to most of the antibiotics primarily due to large-scale and "indiscriminate" usage. The need is to develop novel mechanisms to treat bacterial infections. The expression of pathogenicity during bacterial infections is mediated by a cell density-dependent phenomenon known as quorum sensing (QS). A wide array of QS systems (QSS) is operative in expressing the virulent behavior of bacterial pathogens. Each QSS may be mediated largely by a few major signals along with others produced in minuscule quantities. Efforts to target signal molecules and their receptors have proved effective in alleviating the virulent behavior of such pathogenic bacteria. These QS inhibitors (QSIs) have been reported to be effective in influencing the pathogenicity without affecting bacterial growth. However, evidence is accumulating that bacteria may develop resistance to QSIs. The big question is whether QSIs will meet the same fate as antibiotics.

Introduction

Microbial infections are a major concern for health departments around the globe. The discovery of antibiotics almost a century ago brought hope and relief to many patients [1]. Antibiotics have continued to be one of the most effectual medications for treating bacterial infections since the 1950s [2, 3]. However, with the evolution of multiple drug-resistant strains and evasive behavior of bacteria towards antibiotics, pharmaceutical companies are finding investments in developing novel antibiotics to be counterproductive [4]. A search for novel treatment strategies has obliged researchers to deliver a re-look on the mechanisms of bacterial infection. It has been also realized that around 80 % of the infectious diseases are caused by bacteria which form biofilms [5]. Biofilms are composed of exopolysaccharides, which protect its structure and enable bacteria to resist toxic compounds and high doses of antibiotics [6]. The most important fact has been the finding that majority of the genes involved in bacterial pathogenicity are mediated through quorum sensing (QS). Bacteria are single-celled organisms, which under certain conditions behave like "multicellular" organisms. This generally happens only after bacterial population density crosses a threshold level. This unique bacterial behavior has been termed as QS. QS operates through a wide range of signals such as (1) oligopeptides, (2) acylhomoserine lactones (AHLs), (3) furanosyl borate (autinducer-2), and (4) fatty acids [7, 8]. QS in Gram-negative bacteria is mediated largely by AHLs, whereas in Gram-positive bacteria, the signals are peptidic in nature [9, 10]. The quantum of QS-mediated genes can constitute as much as around 10 % of the total bacterial genome. Thus, pathogenic bacteria with this unique characteristic are able to evade the host's defense while being at low cell densities [11–13].

Quorum Sensing Inhibition

Bacteria exist as free-living forms in mixed communities or in association with plants and animals either as pathogens or as symbionts. Although interdependent, these partners are still able to keep their identities. Bioactive molecules are produced

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by almost all living organisms; the exact significance and the scope of their uses are yet to be elucidated [3]. Plants grow and release secondary metabolites into the rhizosphere and animals develop antibodies as defense mechanisms against bacterial invasions [14, 15]. Bacteria display different survival strategies to counter the eukaryotic attacks, including secretion of hydrolytic enzymes and antibiotics [16–24]. These protective phenomena provide clues that in nature, bacterial species must also be releasing their arsenal of QS-dependent virulent factors to conform to these situations [24]. In fact, many prokaryotes and eukaryotes have been shown to produce bioactive molecules to disrupt the QS process at different stages: (i) inhibiting or reducing the activity of the QS signal-producing gene, (ii) disrupting the structure of the signal molecule, (iii) modulating the binding of the signal to the receptor sites, (iv) blocking the receptor site with antagonist-signal analogues [25–27]. This phenomenon of inhibiting bacterial QS, especially the expression of virulence genes through the production of bioactive molecules, is termed as quorum quenching [28–30].

It has been discovered that bacteria present within the QS-mediated biofilm are up to 1,000 times more resistant to antibiotics than the planktonic forms [31]. This change in bacterial behavior severely complicates the treatment process. The aim is thus to either prevent biofilm formation process or to disrupt it. Efforts have been made to search QS inhibitors (QSIs) for each of these stages or targets. A set of criteria has been laid down for identifying QSI molecules: (i) a low molecular mass, (ii) highly specific, (iii) stable and resistant to hydrolytic enzymes of the host, (iv) no adverse effect on the host, (v) longer side chain than the native AHLs [32–34]. QSIs have been shown to be produced by plants, animals, bacteria, and other microbes [25, 26, 28, 35, 36]. The apprehension is that constant and indiscriminate usage of QSIs may put bacteria under pressure to develop means to evade this treatment procedure. The goal of this review is to provide an overview of the potential threats posed by bacteria becoming resistant to QSIs and finally what kind of QSIs should be developed to avoid this.

The Myth

Antibiotics inhibit the growth of the microbes and may eventually kill it. The microbes under this strong selective pressure tend to develop resistance to antibiotics through natural selection or genetic mutations [37]. Bacterial pathogenicity evolves to counter environmental stress and selective pressure, especially those caused by antibacterial agents [38]. It is primarily to ensure survival that bacteria evade host defences through an attack and defence mechanism [20, 39]. Bacterial genomic plasticity and mobility of genetic material are important facets in their evolution [40]. It has been reported that in a given population there are persisters which evade the lethal effect of antibiotics [41]. These persisters thus manage to develop tolerance to antibiotics [42]. The overall mechanism of resistance to antibiotics is conferred by (i) genes responsible for the degradation of antibiotics (*bla*, *pbp*) and (ii) efflux pumps (*cmc*T) [4, 37, 43]. It was thus thought that the role of "antipathogenic" compounds will be more desirable than antibacterial compounds since they would neither kill bacteria nor completely inhibit their growth. Since these bioactive molecules are perceived to manipulate the infection process without affecting bacterial growth, bacteria may not be compelled to develop resistance against them [7, 11, 24, 32, 44–48]. However, whether bacteria can become resistant to QSIs is now being questioned (Fig. 1) [38, 49, 50].

The first demonstration that bacteria do indeed develop resistance to QSI was shown using the growth of *Pseudomonas aeruginosa* on adenosine as the sole carbon source, which requires active QS [50]. When the QSI compound was added that masks the QS pathways (a brominated furanone known as C-30), growth on adenosine was impaired, and within four sequential dilutions after transposon mutagenesis, cells arose that were resistant to the QSI [50]. The gain of function mutations was in repressors of an efflux pump, and the QSI-resistant strains became resistant by having greater efflux of the QSI compound, a result that had not been anticipated in regard to QSI compounds. This result was predicted using QS mimics in the absence of a QSI compound [51]. Moreover, clinical isolates from cystic fibrosis patients that had been treated with antibiotics were found to carry the same efflux-enhancing mutations and were resistant to the same QSI compound [50]; hence, QSI resistance arises even before the use of the QSI compound. Additional results identifying clinical strains resistant to the QSI C-30 were obtained using isolates from urine, blood, and catheter tips [52]. Therefore, strains in both the laboratory and in the clinic have been shown to evolve resistance to QSIs.

Multiplicity of Quorum Sensing Systems (QSS) and QS Signals: A Latent Weapon to Counter QSIs?

The field of QSS has made rapid progress since its discovery in *Vibrio fischeri*. These works have revealed that QS circuits are much more complex than envisaged initially [53]. In majority of the persistent pathogens, virulence genes are under QS control [54]. The most prevalent bacterial QSS is mediated by the signal molecules (AHLs) produced by the synthase gene (*luxI* homologs). It binds to the receptor and activates the transcription regulator (*luxR* homologs). This complex leads to the transcription of a plurality of genes involved in pathogenicity [55]. In general, most bacteria possess a QSS having a single set of signal synthase gene and transcription regulator gene (I/R). A few other bacteria such as *Pseudomonas*,

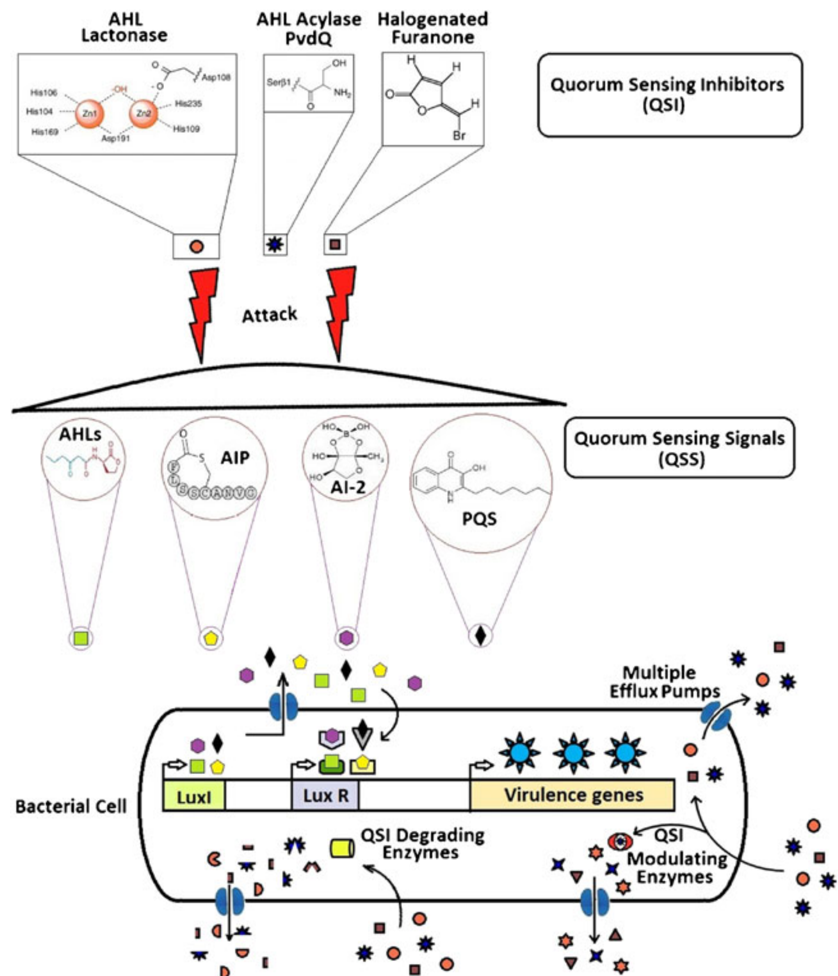

Fig. 1 Potential mechanisms of resistance to quorum-sensing inhibitors

Sinorhizobium, and *Vibrio* species have multiple (I/R) systems (Table 1) [56–61, 64, 65, 68, 69, 71]. The complexity of these systems is reflected in the diversity of the signals produced by certain bacteria (Table 1) [62–64, 66, 67, 72–75]. The multiplicity of QSS is complicated by an overlapping regulation [70]. In multiple QSS, there are chances that transcriptional regulator from different QSS may form heterodimers [76]. The binding of these heterodimers to a promiscuous promoter might lead to different gene expression profiles, allowing bacteria to sense a wide range of environmental stresses which may include QSI [77]. The questions are, as follows: Does this diversity of QSS and QS signal molecules allow bacteria to escape QSI? Is this a hidden trait, which bacteria can exploit for developing resistance to QSI? The multiplicity of QSS and their signals can prove beneficial to the bacteria to either conserve valuable resources or allow them to modulate the activity of the receptors [78]. The presence of 2–5 LuxR signal receptor homologs in *Burkholderia mallei* and the variability in the specificity of AHL synthases in *Erwinia carotovora* strains SCC3193 and SSC1 support the likelihood of their developing resistance to QSI molecules [79, 80]. It can be implied that QSIs designed to block only the *las* QSS might result in the rapid appearance of the resistant strains. It may thus be necessary to block both the *las* and *rhl* QSS to efficiently reduce production of virulence factors by *P. aeruginosa* [70].

Pseudomonas, an opportunistic pathogen, expresses a wide range of genes, which helps it in surviving under harsh conditions prevailing on the surface and within the host organism [20]. These are also effective in challenging the host immune system and cause infectious diseases. *P. aeruginosa* causes diseases such as cystic fibrosis and microbial keratitis largely through AHL-dependent QSS, which activates genes responsible for biofilm formation (chronic infections) and represses genes involved in the expression of Type III secretion system (TTSS) [81]. More recent works have shown that TTSS can also be expressed in biofilms [82]. It was opined that AHL-dependent QS partially represses TTSS expression and that other QS signals of *P. aeruginosa* may be instrumental in modulating the expression of TTSS within biofilms [83]. Enterohemorrhagic *Escherichia coli* activates the transcription of their virulence genes through three types of signals: (i) aromatic autoinducer (AI-3), (ii) hormones—epinephrine and nonepinephrine [84]. The QseC membrane-bound sensor

Table 1 Diversity of quorum sensing systems and signal molecules

Organism	Quorum sensing		Reference
	Systems[a]	Signal molecules	
Pseudomonas aeruginosa	LasI(Synthase)/R (Receptor) RhlI/R	3OC12HSL C4HSL	[56–62]
	QscR (Quorum sensing control repressor)	3OC12HSL (LasI regulates QscR)	
	PqsABCD	2-heptyl-4-quinolone (HHQ)	[62]
Pseudomonas fluorescens, and *P. alcaligenes*	LuxR	Diketopiperazines (DKPs): Cyclo(L-Phe-L-Pro)	[63]
Sinorhizobium meliloti	SinI/R TraM/R and Mel	C14HSL to C18HSL C4HSL	[64, 65]
Rhizobium leguminosorum	RhiI/R, CinI/R	OHC6HSL, 3OC6HSL, C7HSL, 3OHC14HSL	[66, 67]
Vibrio harveyi	HAI-1 (Species specific)	OHC4HSL	[68]
	CAI-1 (Intergeneric communication)	(S)-3-hydroxytridecan-4-one	[69]
	LuxP (Interspecies communication)	AutoInducer-2	[68]
Burkholderia cepacia	CepI/R	C8HSL	[67]
Burkholderia pseudomallei	BpmI2/R2 BpmI3/R3	C8HSL, C10HSL, OHC8HSL, OHC10HSL, 3OC14HSL	[67]
	PmlI1/R1	C8HSL, C10HSL, 3OHC8HSL, 3OHC10HSL	
Erwinia carotovora	ExpI/R, CarI/R	3OC6HSL	[67]
Bacillus cereus	PlcR	Peptide PapR	[70]
Staphylococcus aureus	AgrABCD	Autoinducing peptide	[62]

HSL homoserine lactone, *C4HSL N*-butanoyl HSL, *C7HSL N*-heptanoyl HSL, *C8HSL N*-octanoyl HSL, *C14HSL N*-tetradecanoyl-HSL, *C18HSL N*-octadecanoyl-HSL, *OHC4HSL N*-hydroxybutyrylHSL, *OHC6HSL* 3-hydroxy-*N*-hexanoyl-HSL, *OHC8HSL* 3-hydroxy-*N*-octanoyl-HSL, *OHC10HSL* 3-hydroxy-*N*-decanoyl-HSL, *3OHC14HSL* 3-hydroxy-7-cis-tetradecanoyl-HSL, *3OC6HSL* 3-oxo-*N*-hexanoyl-HSL, *3OC8HSL* 3-oxo-*N*-octanoyl-HSL, *3OC12HSL* 3-oxo-*N*-dodecanoyl-HSL

[a] I/R system: Synthase/Receptor

kinase can be triggered by any one of these signals resulting in transcription of virulence genes [85, 86].

Brominated furanone produced by *Delisea pulchra* could inhibit the QS-regulated swarming motility of *Serratia liquefaciens*. Nevertheless, the inhibitory effect of QSI was reversed by higher concentrations of exogenously supplied *N*-butanoyl HSL (C4HSL) [87]. In a mouse model, the inhibition of LuxR-controlled PluxI-gfp (ASV) fusion by QSI-Furanone C-30 was negated by increasing the dose of 3-hydroxy-*N*-hexanoyl-HSL from 400 to 1,200 μM. Here, the effect of a single C-30 injection dose was found to last for a short period of 6 h [11]. An interesting observation on the effect of C-30 was its ability to repress the expression of a few QS-mediated virulence genes such as *lasA*, *lasB*, *hcnAB*, *rhlAB*, *chiC*, *phnAB*, and *phzABCDEFG*. It however, allowed bacteria to exploit their multiple QSS to continue with an uninterrupted expression of *lasI/R* and *rhlI/R* gene clusters [11]. Since a basal concentration is sufficient to activate QS, 3OC12HSL may not be a limiting factor [11, 54, 74]. A QSI targeting 3OC12HSL alone may not affect QS and the operation of QSS in parallel may mimic a scenario where bacteria have become resistant to QSI [88]. Thus, mechanisms seem to be already in place in *P. aeruginosa* to evade the effect of QSI by having multiple QSS and their signals [11, 38].

Mutations in QS Circuitry

Another feature which helps bacteria to withstand antimicrobial agents is their innate ability to undergo mutations or acquire genes from closely or widely related organisms, through horizontal gene transfer [4, 40, 89, 90]. Genetic mutations in *raiI* and *raiR* genes of *Rhizobium elti* enabled the elucidation of seven different AIs involved in the nitrogen fixation process in *Phaseolus vulgaris*. It revealed that expression of *raiI* in *R. elti* mutants could be activated by one or more AIs [74]. Similarly, *Agrobacterium tumefaciens*, *P. aeruginosa*, and *Rhizobium* species can withstand mutations in their QS circuitry because of their abilities to get activated by multiple AHL signals, which vary from three to seven [72, 91, 92].

P. aeruginosa mutant PAO-R1, defective in *lasR* gene, is unable to produce most of the QS-dependent virulence factors [93, 94]. Although, it was proposed to be a potential target to control *P. aeruginosa* infections; however, it was envisaged that a reverse mutation may lead to restoration of *lasR* functioning even if *las* QSS is absent [70]. Mutations in the LuxR receptor lead to their failure to recognize synthetic antagonist *N*-(propylsulfanylacetyl)-L-HSL [95]; however, resistance to a QSI was not tested, and these authors argued that such

resistance was unlikely to evolve. Unfortunately, this item has not been recognized by several authors who have claimed that resistance to QSI was seen in this report and that "QSI resistance can easily be obtained" in this manner [38] although there is no proof to date that such mutations render cells resistant to QSI compounds. Spontaneous mutations arising in the QSS of *Vibrio cholerae* strains make them either non-functional or constitutive in nature [96]. *Serratia marcescens* has a unique genetic makeup, the location of *spnIR* QSS on a transposon (Tn*TIR*), which may position it to take advantage to overcome any hindrance in their QSS [97]. In fact, *P. aeruginosa lasI* mutant PAO1-JP1, which was not able to produce 3OC12HSL could still form strong biofilms, under hydrodynamic conditions of high shear flow [98]. This observation has been underpinned by the effect of QSI from *P. aeruginosa* extracts, which affected virulence factors controlled by the *las* and *rhl* system to different extents. However, the extract did not affect the biofilm formation progress [99]. *P. aeruginosa* mutant PAO-R1, defective in one of the 2 QSS (LasR-PAI-1), showed up-regulation of its second QSS (RhlR-PAI-2), under carbon (C) and nitrogen (N) stress conditions [70, 88]. Enhanced growth under certain physiological conditions was also recorded in *lasR*-deficient isolates from cystic fibrosis patients and *P. aeruginosa* strain PA14 [100]. It raised doubts that targeting the biofilm formation process may not be the best option to moderate bacterial infections [98, 99].

The inherent ability of *Pseudomonas* allows it to exercise a stringent control over the infection process. QscR regulator of *P. aeruginosa* modulates the expression of the virulence gene responsible for the production of factors such as hydrogen cyanide, pyocyanin, and elastase [59, 76]. At low cell densities, QscR inhibits the expression of these genes by forming inactive heterodimers with LasR and/or RhlR. This complex consequently inhibits the QS-mediated gene expression [76]. On the other hand, it may also be interpreted that suppressing QscR may lead to uninterrupted supply of QS signals, leading to "constitutive" virulent behavior. Thus, while QSI will be reducing the concentration of AHLs, a mutant of *P. aeruginosa*, lacking *qscR* will counter its attack [53].

Another mechanism which retards the production of QS signal is through RsaL, the global regulator of QSS in *P. aeruginosa* [101]. It can repress transcription of the signal synthase gene *lasI*. RsaL is a QS repressor, which acts by binding to LasR and *rsaL-lasI* two-way promoter. It is a homeostatic mechanism which limits the production of the QS signal (3O12CHSL) [102, 103]. However, a mutation in the *rsaL* gene, allows uninterrupted production of 3OC12HSL throughout the growth process [104]. It can help bacteria to continue with its functions of QS-mediated virulence and pathogenicity. This regulatory protein controls more than 130 genes in *P. aeruginosa* physiology and can be a potential mechanism to develop resistance to QSI by counterbalancing 3O12CHSL-dependent gene activation [104].

The Cheats

QS is a social system where a large population of bacteria work together and release goods (signal molecules) into the extracellular space. The system also leads to the production of enzymes necessary for their survival. These bioactive molecules are "freely" available for all individuals in the population to sense and take advantage. This social cooperation is susceptible to exploitation by cheaters, who can derive benefit from it without spending their energy and are likely to destabilize the QSS [42, 79, 88, 105–108]. Studies have been conducted to elucidate the role of cheaters. QS cheats can be categorized as (i) signal negative, the *lasI* mutants, which can avoid the energy needed for producing the QS signals and related activities and (ii) signal blind, the *lasR* mutants, which have the ability to produce QS signals but do not use them for production of virulent factors [109].

The growth of wild-type and *lasI* and *lasR* mutants of *P. aeruginosa* is influenced by the nutrients in the surrounding medium [106]. In nature, the proportion of the signal-blind strain is more common in mixed populations [106, 110], the social cheaters grow initially but they are not able to sustain as there are fewer co-operators available for exploitation. However, this scenario is anticipated to reverse with the evolution of QSI-resistant bacterial strains [106, 109, 111]. The growth and behavior pattern of these social cheaters was gathered by allowing a small proportion of QS-deficient mutants to grow along with QS-proficient wild-type strains of *P. aeruginosa* [51]. In a population composed of QSI-resistant (wild-type) and QSI-sensitive strains (signal-blind *lasR rhlR* double mutants) of *P. aeruginosa,* a selective pressure was created for distinguishing the two population types by providing two different carbon sources: (i) bovine serum albumin (BSA) and (ii) adenosine. The utilization of both the C sources is QS-mediated. BSA is metabolized by extracellularly produced proteases and thus benefits the entire population (public goods), and adenosine is degraded in the periplasmic space by nucleoside hydrolase (Nuh), useful only to the producer cell (private goods) [51]. In a co-culturing of QSI-sensitive mimics with varying proportions of wild-type *P. aeruginosa* (QSI-resistant mimics), growth characteristics varied with C source. A significant delay in the growth on BSA (public goods) was observed in the cases of co-culture having a larger proportion of QSI-sensitive mimic population. This growth retardation was not observed in the case of adenosine (private goods). This behavior of QSI-sensitive and QSI-resistant populations of *P. aeruginosa* implies that social cheating will enhance the chances of developing resistance to QSI in mixed populations, in privately acquired nutrition [51].

In mixed populations of *P. aeruginosa*, wild-type strains are not able to compete with the cheats—*lasR* mutants, which cannot release iron-scavenging siderophores into the extracellular milieu [112]. In situations, where the growth of cheaters

may prove detrimental for the survival of the population as a whole, a compensatory mutation was anticipated to take place. The protease-producing ability of *lasR* mutants (*las*R5) was restored by the *rhl* system, which thus showed an enhanced C4HSL production [70, 88].

The impact of anti-virulence interventions on the evolution of QSS was demonstrated in a placebo-controlled clinical trial. The response of antibiotic, azithromycin, which acts as QSI [113, 114] was followed by evaluating the behavior of using *lasR* mutants in intubated patients colonized by *P. aeruginosa* [115]. Mutation in *lasR* resulted in reduced expression of elastase and the mutant had a reduced growth compared to wild-type *P. aeruginosa*. Azithromycin treatment, however, prevented the selection for *lasR* mutants and consequently increased the proportion of wild type. It was therefore concluded that such anti-virulence intervention may increase the prevalence of highly virulent QS wild-type isolates [115].

Efflux Pumps

Bacteria develop resistance to antibiotics and biocides by various mechanisms, the most instrumental being efflux systems [37]. An active and efficient efflux system enables bacteria to evolve resistance against toxic metals, drugs, and even structurally unrelated antibiotics [116–118]. *P. aeruginosa* has numerous efflux pumps which regulate membrane permeability and the uptake of compounds [119]. The expression of multidrug efflux systems has been found to be a consequence of mutations in regulatory genes. Antibiotics such as azithromycin (AZM), ceftazidime, and ciprofloxacin affect QS possibly by altering membrane permeability and affecting the 3OC12HSL efflux, which can contribute to the consequences of selective pressure and development of QS resistance [120, 121]. Certain antimicrobial drugs at sub-inhibitory antimicrobial concentrations can induce or interfere with QSS and even promote biofilm formation [122]. Vanadium-resistant mutants in *ncr* (non-coding region), *mexI*, and *opmD* showed drastic decrease in production of the QS-mediated phenazine pigment pyocyanin [123, 124]. In fact, *nfxC*-type mutants over-expressing MexEF-OprN efflux pump had reduced transcription of the AI synthase gene *rhlI* and also exhibited 20 times less pyocyanin production [118, 125]. The reduced transcription of *rhlI* was also assigned to efflux of tryptophan, the precursor of PQS and leading to its reduced intracellular levels, which consequently affected QS functions [126, 127]. Hence, AHL homeostasis may be a potential mechanism to circumvent QSI, i.e., by reducing AHL production [118, 127]. *P. aeruginosa* mutants for *mexR* and *nalC* show increased resistance to a well-studied QSI-brominated furanone C-30. *P. aeruginosa mexR* mutant in the presence of C-30 could infect and cause disease in *Caenorhabditis elegans* [50]. These mechanisms are supported by additional genes responsible for the inactivation or modification of the drug [4, 37, 128, 129].

Environmental Impact

Bacteria may produce diverse QSS and also the corresponding signals, however, their stability may be affected by certain environmental conditions. Within biofilms, pH varies from 6–11 during the diurnal cycle [130, 131]. During daylight, pH values >9 results in hydrolysis of AHLs [132]. This physiological hydrolysis due to alkaline pH does not affect AHLs with acyl chain longer than C12. In this scenario, shorter chain AHLs might be produced during night cycle and long chain AHLs would remain largely intact during photosynthetic activity period [77]. The QS-mediated exopolysaccharide matrix of the biofilm regulates the mobility of a molecule through the nanopores between polymers [133, 134]. These responses to changes in environmental conditions can help bacteria to manipulate the production and release of QS signals and withstand adverse conditions by engineering the flow of signals and ions [6, 135].

Co-evolution

Marine organism, *D. pulchra* has been widely studied for its QSI abilities [136, 137]. In natural marine environments, quite a few bacteria have been found to be associated with *D. pulchra* [138, 139]. Bacterial isolates from the distal portion of the thallus of *D. pulchra* had the ability to form biofilms. Crude extracts of *D.* pulchra strongly inhibited the attachment of the bacterial isolates from rock surfaces at significantly much lower concentrations (10 ng cm^{-2}) than those (1 μg cm^{-2}) needed for isolates from the surface of *D. pulchra* [138]. *Nautella italica* R11 and *Phaeobacter gallaeciensis* LSS9 isolated from the surface of red macro alga *D. pulchra* could cause bleaching disease on the host whereas other isolates could grow well and form biofilms but did not induce the disease [140–142]. It implies that isolates from the epiphytic bacterial community closely associated with the marine algae have evolved resistance to QSI, produced by the host. As a result, it is quite probable that the algae may in turn evolve mechanisms to further avoid biofouling.

A Never-Ending Battle

In the past, our enthusiasm with the large-scale utilization of antibiotics was tempered by the fact that bacteria evolve to become resistant to selection pressure. In the present scenario, QSIs are proving out to be the most promising alternatives

and/or supplements to antibiotics. Most of the research in this area seems to be well aligned to meet the basic criteria of a QSI but is yet to succeed to clinical stage. Another very interesting scenario, which is quite easy to visualize, is that our battle against infectious diseases may be a never-ending process [143]. The experts in the area are quite apprehensive about its success; hence, it may be judicious to be prudent lest QSI may meet the fate of antibiotics [15]. We may presume QSI resistance mechanism(s) to be similar to those which confer resistance to antibiotics, such as restricted availability, inactivation or even modification of the target [51]. The big question is why bacteria will develop resistance against QSI. We may argue that the functioning of QS is so vital for bacteria that it may "invest" up to 10 % of their total genome in it. The organisms are likely to have a natural protection mechanism in place to counter attacks which may endanger their existence. Since QSI are envisaged to involve "only" QS, without affecting bacterial growth, a constant supply of AHL signals, albeit at a slower rate, can even be imaged at low cell densities. Therefore, once QSI concentration gets below the threshold level, bacteria may be free to express its virulence. Apparently, bacteria do not even need to undergo any genetic change to withstand QSIs [77]. Bacteria can thus evade QSI by keeping its QS under control till the concentration of QSI is higher than that of the signal molecules. It is being suggested that in the future, the target should be to develop QSI with lower risk of developing resistance. In contrast to QSI causing competitive inhibition [144], it may be desirable to look for non-competitive or uncompetitive inhibitors. It is expected to exert less pressure on QS gene expression [49]. The associated drawback with the use of non-competitive biomolecules as QSIs is their narrow range of activity and may become ineffective by genetic mutations in the binding sites [49–51]. Caution should also be exercised that QSI should not affect efflux pumps [145, 146]. Taking into account the multiplicity of receptors, it is unlikely that a single molecule will be able to act as a broad-range QSI [53]. The existence of natural bacterial communities is closely linked with the environment. The physiochemical conditions thus drive the bacterial activities and regulate their survival. Since survival is the essence of life, each organism will continue to evolve its defense mechanisms. The likelihood of bacteria developing resistance to QSI is less probable than that observed with conventional antibiotics [147]. Observations on bacterial resistance to QSI oblige us to be cautious while developing drugs against QSS [83]. The composition of the bacterial community will change if they are not able to withstand environmental stress [77]. The genetic engineering of plants with AHL degrading enzyme may also be a reason to worry as it may create selective pressure for the evolution of bacterial strains, which may evolve mechanisms (i) to inhibit the activity of these enzymes and/or (ii) to become independent of AHL-based expression of virulence factors [15, 148]. We may conclude that the need is to employ innovative and novel strategies to extend the range of QSIs against multidrug-resistant organisms.

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Conflict of interest The authors declare that they have no competing interests.

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