

Review- Biological and Applied Sciences

Quorum Sensing Interfering Strategies and Their Implications in the Management of Biofilm-Associated Bacterial Infections

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HIGHLIGHTS

- Quorum sensing (QS) regulates the development of biofilm formation and its dispersion.
- Targeting QS is a potential functional alternative to antibiotics
- The QS inhibitors can be utilized to impede the virulence in bacterial pathogens
- QS inhibitors could be very useful for the development of anti-biofilm pharmaceuticals.

Abstract: The bacterial species employ various types of molecular communication systems recognized as quorum sensing for the synchronization of differential gene expression to regulate virulence traits and biofilm formation. A variety of quorum sensing inhibitors; molecules that interfere with quorum sensing among bacteria have been examined which can block the action of autoinducers. Moreover, the studies have scrutinized various enzymes for their quorum quenching activity resulting in the degradation of signaling molecules or blocking of gene expression. So far, the studies have found that these approaches are not only capable to reduce the pathogenicity and biofilm formation but also resulted in increased bacterial susceptibility to antibiotics and bacteriophages. The effectiveness of these strategies has been validated in different animal models and it seems that these practices will be transformed in near future to develop the medical devices including catheters, implants, and dressings for the prevention of bacterial infections. Although many of these approaches are still in the research stage, the increasing library of quorum quenching

molecules and enzymes will open innovative perspectives for the development of antibacterial approaches which will extend the therapeutic arsenal against the pathogenic bacterial species.

Keywords: quorum sensing; interference; autoinducers; receptors; signaling.

INTRODUCTION

Quorum sensing is recognized as a mechanism of communication between bacterial communities and to collectively acclimate their behavior under the cellular density and their immediate environment. These mechanisms are advantageous for the entire community particularly at a higher cell density for example synthesis of virulence factors, the formation of biofilms, production of siderophores, and proteases [1]. The phenomenon of quorum sensing comprises the production of small molecules i.e. autoinducers (AIs) which are released in proportion to cell density [2]. These autoinducing peptides were extensively studied and found to induce quorum sensing among Gram-positive bacteria and are species and strain-specific and have been reported particularly in Staphylococci, Enterococci, and Clostridium species. The acyl-homoserine lactones (AHLs) are another class of autoinducers that are widely reported in many Gram-negative bacteria particularly in Pseudomonas, Burkholderia, and Acinetobacter species. Many other diverse types of signaling molecules including fatty acids, ketones, epinephrine, norepinephrine, AI-2, AI-3, or quinolones have been identified in bacterial species [1].

The phenomenon of quorum quenching is to interfere with quorum sensing which is majorly driven by the enzymes that are capable to degrade the AHL signals resulting in the disruption of the signals involved in quorum sensing. This interruption of communication between the bacterial communities can be accomplished by various processes. Firstly, the small molecules known as quorum sensing inhibitors can interfere with the production of autoinducers. Secondly, the quorum quenching macromolecules for example cyclodextrins and antibodies can scavenge the autoinducers. Moreover, the autoinducers can be hydrolyzed extracellularly by using enzymes [1]. Quite a few antagonist compounds have been identified among natural products or designed to quench for example polyphenols, ajoene, eugenol, 5-fluorouracil (5-FU), and azithromycin. The mechanisms of action of some of these quorum sensing inhibitors are unknown. Few of these for example azithromycin are mainly considered as antibiotics because of their ability to inhibit the growth of bacteria above a particular concentration [3].

The increasing antibiotic resistance has compelled the researchers to decipher newer therapeutic approaches [4-7]. The approaches to interfere with the quorum sensing can potentially inhibit the bacterial virulence and formation of biofilm that plays a key role in the emergence of antibiotic resistance. The quorum sensing triggers the biofilm formation which is composed of bacterial cells and an extracellular matrix consisting of proteins, polysaccharides, and DNA that may avert the penetration of antibiotics, therefore, inducing antibiotic tolerance. Moreover, the biofilm environments provide high cell density and increased selection pressure which ultimately increases the rate of development of resistant cells through genetic mutations or lateral gene transfer [1]. The elimination of biofilms, especially in the health care environment and devices, is a rising challenge, therefore, it is quite essential to develop alternative treatments in addition to conventional antimicrobial products. In this regard, the strategies which interfere with quorum sensing are increasingly being studied.

In this review, we have highlighted the latest findings related to the quorum sensing interfering methodologies and have summarized the potential of the quorum quenching molecules by the provision of data related to their efficacy in animal models as well as clinical trials for their successive medical applications.

Quorum sensing: a bacterial crosstalk

The different bacteria usually coexist forming multi-species communities within the host, for example, the oral cavity or the gastrointestinal tract. It is quite interesting that both gram-negative and gram-positive bacteria are capable of interacting with each other through the recognition and processing of signaling molecules among various species [8]. For instance, the biofilm is formed within the lungs of the infected patients suffering from cystic fibrosis mainly involving *Burkholderia cepacia* and *Pseudomonas aeruginosa*. It was reported that these two species utilized the acylated homoserine lactones (AHLs) autoinducer molecules for communication in a murine infection model. Here *B. cepacia* might recognize signal molecules processed by *P. aeruginosa* and not vice versa [9].

Further, it was indicated that eukaryotic cells can cross-talk with infectious bacteria and vice versa by using the signals. The Enterohemorrhagic *Escherichia coli* (EHEC); the causative agents of bloody diarrhea

utilize the quorum sensing for cellular interaction. It was indicated that *E. coli* strains can interact with the host cell signaling molecules [10]. A knock-out mutant of EHEC that was not capable of synthesizing the autoinducer, was observed to react with the signaling molecules synthesized from the host which might activate the expression of the numerous virulence genes. Under other conditions, this might be possible that the signaling molecules processed via host may suppress quorum sensing systems of bacteria. Moreover, it is reported that the human respiratory epithelium can inhibit the cellular communication of *P. aeruginosa*. Furthermore, it is proposed that the mammalian respiratory system use this defense mechanism against the pathogenic organisms [11].

Potential of quorum sensing as an antibacterial target

Quorum sensing has also been evaluated as a promising antibacterial target as the strains having mutations within the essential quorum sensing genes were found to have reduced pathogenicity as observed in animal models. Quorum sensing systems in pathogenic microorganisms like *P. aeruginosa* remain active during human infections as seen in clinical trials where the signal molecules have been found in tissues or body fluids of infected patients [12]. As quorum sensing generally relies upon the interactions of various small-molecule ligands and receptors, it provides an explicit pharmacological pathway towards the inhibitor development. Whereas the biosynthetic pathway of signal molecules comprised a target for the pharmacologic inhibition, therefore, quorum sensing could also be inhibited through the prevention of pathogenic groups by the antibody-based sequestering or through the enzymatic inactivation of the signal molecules of quorum sensing [13].

At present, our knowledge about the capability of quorum sensing as the antibacterial target indicates that it is of great value among a few pathogenic organisms where it is obvious that disrupting the quorum sensing possesses a major effect on virulence [14]. In this case, clinically effective quorum sensing inhibitors are limited to the bacterial pathogens like *P. aeruginosa* and *S. aureus* [15]. The regulatory network of quorum sensing is more thoroughly studied in *P. aeruginosa* regulating biofilm maturation and various virulence determinants. Several *P. aeruginosa* quorum-sensing inhibitors have been characterized. Remarkably, several antibiotics comprising of ciprofloxacin, ceftazidime, and azithromycin have reported to hinder the quorum sensing at the sub-inhibitory concentrations [16]. The biofilm formation and quorum sensing interference to inhibit the surface colonization is shown in Figure 2.

Quorum sensing and bacteriophages susceptibility

Currently, the interests have risen significantly for the use of bacteriophages to treat the infections caused by multiple drug-resistant bacterial pathogens [17]. These phages are extremely abundant predators of bacteria on the earth and are increasingly being common for the treatment of bacterial infections. Though the bacteriophages exhibit an attractive way to evade the antibiotic resistance, bacteria also have acquired the mechanisms to respond to the phage attack. Foremost, the phage entry could be reduced by the production of the extracellular matrix or through the modification of the phage receptor expression or structure. Once within the cell, the phage DNA could be identified and destroyed by the adaptive inducible CRISPR-Cas system or the restriction enzymes [18].

The relationship among bacteriophage and quorum sensing was initially examined in *P. aeruginosa* however, in *E. coli* the capability of quorum sensing for regulating the defense mechanisms of phage was determined years later [19]. It was reported that N acyl-homoserine lactones caused a decrease in the adsorption rate by decreasing the number of receptors present on the surface of the cell. In *V. cholerae*, the lack of autoinducer synthase genes, and consequently in quorum sensing induction, reduces the resistance of phage that might be reestablished by adding the exogenous autoinducers, AI-2, CAI-1. The increase in phage resistance after the activation of quorum sensing was described through the downregulation of synthesis of O-antigen that reduced the adsorption of bacteriophages and increased the production of hemagglutinin protease that was shown to inactivate the bacteriophages [20].

Taking into consideration, the use of quorum quenching compounds is an extremely promising technique to develop newer therapeutic applications. The use of these compounds in combination with phage therapy can enhance the sensitivity of bacteria towards phages through synergistic effects. Moreover, disrupting the quorum-sensing system of one species has shown to cause a decrease in the total biomass in multi-microbial cultures during the phage attack which leads to the fact that quorum quenching in conjunction with the phage therapy might be effective against the infections caused by multiple bacterial species [21].

Antibiotics as QS inhibitors

Since quorum sensing implies an overall change in the cell physiology and gene expression of bacteria, so the association between antibiotic tolerance and quorum sensing is multifactorial. For example, adding AHLs to the growing culture of *P. aeruginosa* has shown to enhance the number of bacterial persister cells after treating them with ciprofloxacin and carbenicillin [22]. Moreover, the transcriptional analysis in *P. aeruginosa* with the quorum sensing transcription regulator i.e., MvfR (PqsR) have shown that quorum sensing stimulates the expression of the peroxidases that protects the bacteria from the reactive oxygen species like hydrogen peroxide and beta-lactam antibiotics [23]. Further, the study involving the *P. aeruginosa* strain PAO1 and a regulator i.e. VqsM which is essential for quorum sensing induction was found to facilitate the antibiotic resistance by stimulating the expression of *nfxB* gene; an antibiotic tolerant regulator conferring increased resistance to the tetracycline, kanamycin, and quinolones by regulating the expression of operon *mexC-mexD-oprJ* [24].

Even though several biochemical factors may be involved in the quorum-sensing mediated resistance to antibiotics, numerous studies have focused on the impact of biofilm formation in the antibiotic resistance among bacteria leading to treatment failure. These consequences have been examined within *P. aeruginosa* in animal models along with some other species like *Staphylococcus aureus* and *Klebsiella pneumoniae* [25]. Certain conditions in which the biofilm formation facilitates bacteria include defense mechanisms like alteration of gene expression, cellular physiological conditions, and physical barriers [26]. The *las* and *rhl* quorum-sensing systems are necessary for the formation of biofilm and its disruption is associated with the high sensitivity to antimicrobial compounds as well as the host immune system among *P. aeruginosa* strains [11]. Furthermore, in *P. aeruginosa* an additional quorum sensing system called pqs system has shown mediating the programmed cell death causing the release of extracellular DNA that promotes antibiotic resistance and biofilm formation favoring the remaining cell population. In *Acinetobacter baumannii*, the antibiotics specifically the meropenem or levofloxacin has shown to stimulate the expression of an efflux pump that promotes the release of an AHL and therefore increases the quorum sensing mediated biofilm formation [27].

Considering the significance of quorum sensing in antibiotic resistance and biofilm formation, combination therapies using quorum quenching compounds were studied. In *P. aeruginosa*, the use of pharmacological compound i.e. benzamide benzimidazole and the inhibition of quorum sensing regulator MvfR restored the antibiotic susceptibility and reduced the biofilm formation [16]. Hamamelitannin and baicalin hydrate, a peptide-based quorum sensing inhibitor and the AHL targeting quorum sensing inhibitor increases the biofilm disruption in gram-positive bacteria e.g. *S. aureus* as well as in gram-negative bacteria like *Burkholderia cepacia* and *P. aeruginosa* and exhibited the synergistic effects in co-treatment with vancomycin, clindamycin, and tobramycin during *in vivo* and *in vitro* experiments [28]. The effectiveness of a large number of antimicrobial agents including the aminoglycosides, polypeptides, quinolones, glycopeptides, and cephalosporins, has been increased by adding the quorum sensing inhibitors [29, 30].

Combining all these findings indicates that the quorum-sensing inhibitors are promising agents to enhance antibiotic sensitivity and consequently reducing the massive doses of antibiotics. Moreover, related efficiency and trend have been examined with the antibiotic ciprofloxacin and lactonase enzyme for quorum quenching in mice model of infection [31]. Taken together, the quorum quenching, and antimicrobial agents possess encouraging effects, emphasizing that quorum quenching is an effective way of reducing the use of antibiotics and combat against the rising problem of antibiotic resistance.

Antibodies as QS signal blockers:

The control of quorum sensing using monoclonal antibodies seems to be an attractive approach due to its increased specificity and less cytotoxicity as seen in different studies. The antibody (RS2-1G9) was found to inhibit the 3-oxo-C12-HSL, therefore, decreasing the inflammation in the host during the *Pseudomonas aeruginosa* infection. Another antibody (XYD-11G2) catalyzed the hydrolysis of 3-oxo-C12-HSL signaling that resulted in decreased pyocyanin production among Gram-negative bacteria. The antibody (AP4-24H11) significantly attenuated the tissue necrosis *in vivo* using the infection model. Though the monoclonal antibodies have successfully blocked the QS signaling among pathogenic bacterial species, their therapeutic applications for various bacterial infections are yet to be confirmed in the clinical trials [32].

Natural QS inhibitors

The natural compound libraries can also serve as quorum quenchers or inhibitors. Particularly, plants are well known for producing highly complex compounds with a variety of therapeutic or medicinal properties.

Though, the synthesis of various molecules is difficult, because of their complex chemical scaffolds; yet, the direct extraction method would be the best option to get these products. Enough compounds extracted from plants have already been found to have quorum inhibitor activity. Recently, it has been reported that the phenolic compounds extracted from plants can inhibit the biofilm formation in the PAO1 strain of *P. aeruginosa* when used in high concentrations [33]. However, these compounds have the opposite effect on the subinhibitory concentrations and supported biofilm growth. A few of these compounds include gallic acid, vanillin, and 4-hydroxybenzoic acid, which do not resemble the prototype of AHLs. Furthermore, derivatives of ellagic acid from the *Terminalia chebula*, also the aqueous extracts from *Callistemon viminalis*, *Bucida buceras*, and *Conocarpus erectus* have also been reported for the inhibition of QS of *P. aeruginosa* [34]. The derivatives of ellagic acid disrupt Rh1R and LasR, while simultaneously decreasing the production of virulence factors and increasing the sensitivity of the PAO1 strain of *P. aeruginosa* biofilm towards the tobramycin treatment. Another study was carried out among the *Caenorhabditis elegans* and found that the aqueous extracts have reduced the gut infection in 60% of nematodes and decreased the mortality rate by 50%. Most importantly, all the extracts were useful with no toxic effects to the host and could be useful as therapeutic agents [34].

Apart from the extracted compounds from plant sources, various other natural compounds have also been derived from marine life and insects as well [35]. Considering the vast diversity of the natural world, a lot of beneficial compounds are still to be revealed, and researches into the uncharacterized and novel extracts would extend the list of useful compounds libraries.

Inhibition of QS in animal models

Caenorhabditis elegans:

The soil nematode worm *C. elegans* along with its associated bacterial species i.e. *P. aeruginosa* has been used as a model organism to figure out the efficiency of several quorum sensing inhibitors. Moreover, it is reported that the strains of *P. aeruginosa* that are capable of causing the disease in mice and humans are also able to kill the *C. elegans* worm [36]. Additionally, the pathogenesis of the *P. aeruginosa* strains in *C. elegans* is controlled with a similar cell density-dependent quorum sensing system as seen in human infections. Further, the sequencing of the *C. elegans* and *P. aeruginosa* genomes have made this association ideal for studying the disease biology. The ease of growth of *C. elegans* in the research laboratory is an additional advantage for its wide usage as a model organism to study the host defenses and pathogenesis in humans as well as animals. The nematode generally survives by feeding on the nontoxic bacteria; however, they are killed when feeding on the lawn of *P. aeruginosa*. The quorum sensing was found to regulate the hydrogen cyanide and phenazines production which has been associated with the killing of these worms. The wild-type *P. aeruginosa* has killed 100% worms while the quorum sensing knockout mutants killed only 10% of them which indicates the significance of quorum sensing in the virulence phenotype of the pathogen. The quorum-sensing inhibitors such as 4-NPO or garlic extract with the wild-type strains of *P. aeruginosa* were found to decrease the mortality rate of these worms to almost 40% and 5% [37]. The addition of the PvdQ acylase into the worms which were infected with the PAO1 strain of *P. aeruginosa* resulted in an enhanced lifespan and reduced the pathogenicity of the worms [38].

Mice model:

The mice were challenged with the *P. aeruginosa* embedded in the alginate beads that resulted in a chronic lung infection because of the bacteria caught in beads which seems like that of cystic fibrosis. Further, the bacterial AHL monitor having the quorum sensing regulated *gfp* gene implanted into beads resulted in the expression of the quorum-sensing controlled genes in the lungs. The quorum sensing inhibitor compounds inoculated with bacteria might be screened due to their capability to block or reduce the expression of *gfp* as the green fluorescent bacteria may be identified within lung tissues [39]. The extracts from Garlic were indicated to decrease the *P. aeruginosa* pathogenicity in mice when the quorum sensing defective *P. aeruginosa* mutant LasR was used in the burn mice model [40].

Other models:

Various models such as the rabbit osteomyelitis model, rabbit endophthalmitis model, and mouse septic arthritis model have been used to observe the role of accessory gene regulator in the pathogenesis of *S. aureus* [41]. The *Drosophila melanogaster* was used to study the quorum sensing and quorum sensing inhibitors and to study the dynamics of *P. aeruginosa* infection and the association between the pathogenicity and formation of biofilm in this bacterial species. The natural products were found to stimulate the organo-

sulfur compounds such as S-phenyl-L-cysteine sulfoxide which has been indicated as the quorum sensing inhibitor which inhibited the *P. aeruginosa* infection, biofilm formation as well as quorum sensing [42].

QS inhibitors in clinical trials

The previously certified and commercialized quorum sensing inhibitors were implicated in the clinical trials, even though their basic uses and specialized biological activity shows no association with bacterial quorum sensing, although they were cytotoxic and possess antimicrobial activities and were bactericidal [43, 44]. The azithromycin has been widely used for the treatment of pulmonary transplanted and cystic fibrosis patients in clinical trials. This antibiotic enhanced the quality of life of the affected patients but was unable to reduce the bacterial load among infected patients [45]. Simultaneously, the capability of azithromycin to disrupt the bacterial signaling at non-bactericidal concentrations for *P. aeruginosa* strains was exhibited *in vitro* [46]. Further, the effect of azithromycin on the *P. aeruginosa* quorum-sensing among the patients with ventilator-associated pneumonia was determined [44]. These studies explained the anti-virulence impact of the azithromycin among the high-risk group patients, but the results obtained were not significant.

The extracts from Garlic are widely known for its quorum quenching activities and have been evaluated in a clinical trial for the treatment of patients suffering from cystic fibrosis, however, the results were unable to prove the therapeutic benefits of garlic in these patients [47]. The anti-cancer drug, 5-Fluorouracil which is a pyrimidine analog was shown to impede the quorum-sensing regulated virulence factors in *P. aeruginosa* as seen *in-vitro* experiments, therefore, its use as a coating for catheters was suggested in the clinical trials [43].

Eventually, quite a few numbers of quorum quenching molecules have reached up to the clinical trials however they tend to exhibit some valuable effects of quorum sensing inhibitors. Even though many pieces of evidence of the theory were implemented in the animal models, further work needs to be devoted to authenticating these methods in the clinical stages to verify its therapeutic application.

QS inhibitors in medical devices

Most of the hospital-acquired infections are linked with the use of various medical devices. Biofilm forming and multi-drug resistant bacteria are mostly responsible for hospital-acquired infections that cause serious medical complications, also a high risk of mortality and morbidity. Considering the capability of quorum quenching to inhibit the bacterial virulence, developing innovative medical devices by using quorum quenching is of utmost interest [48]. New generations of dressings, catheters, contact lenses, aerosols, trauma, and orthopedic devices or implantable devices are recently being developed [49-51]. The studies using quorum sensing inhibition for various medical devices are summarized in table 1.

Initially, a quorum-sensing inhibitor was studied for the functionalization of the catheters. The furanones have been covalently attached which are shown to reduce the formation of biofilm by *Staphylococcus epidermidis* reference strain and to control the infection (up to 65 days) within a sheep model [52]. For the coating of a central venous catheter, the 5-fluorouracil was used and was found effective and analogous to the traditionally used chlorhexidine and silver sulfadiazine coated catheters during a study comprising of 960 patients admitted in intensive care units in the USA [43]. Though the association with quorum sensing was not much appraised in the studies, the 5-fluorouracil coated catheters exhibited a decreased level of contamination especially the gram-negative bacteria, compared with the conventional coatings that suggest the interference of 5-fluorouracil with the AHL dependent quorum sensing.

The coatings using polyethylene glycol comprising of quorum sensing inhibitor DHP(5-methylene-1-(prop-2-enoyl)-4-(2-fluorophenyl) dihydropyrol-2-one) was indicated to decrease the colonization of *P. aeruginosa* strain MH602 and an *S. aureus* strain. The combinations of DHP derivatives and furanone and remained covalently attached on the surface of the glass which considerably decreased the adhesion of *P. aeruginosa* strain PAO1 and an *S. aureus* strain [53]. The delivery systems based on the varnishes, emitting quorum sensing inhibitor TZD-8 (thiazolidinedione-8) were used for the catheters and were found to be active against the biofilms produced by *Candida albicans* [54]. In a recent study, polyphenols from honey have been used into the scaffold of selenium nano-vectors for the quenching of *P. aeruginosa* strain PAO1 for *in vivo* and *in vitro* evaluation [55].

The inhibition of agr-based quorum sensing in *S. aureus* was accomplished using inhibiting peptides for various biomaterials. In a study, the macrocyclic peptides were inserted into the non-woven polymer nanofibers which were confirmed to maintain their biological activity against the *S. aureus* for three weeks [56]. The surface coating using click chemistry has been considered for coating of pro and anti-quorum sensing peptides i.e. AIP-I and TrAIP-II which were found significantly efficient for the inhibition of *S. aureus* biofilms [57]. The interaction of antibiotics with the quorum sensing inhibiting peptides FS3 was also

determined using daptomycin and found quite effective in combination with the FS3-coated prosthesis against the *S. aureus* infections [58]. Likewise, the RNAIII-inhibiting peptide was found very effective inside a Dacron tube graft for decreasing infections associated with *S. epidermidis* strains [59].

The quorum sensing inhibiting materials have been obtained following the immobilization of peptides or QSIs, quorum quenching enzymes have also been extensively scrutinized as these acts on the secreted autoinducers and do not require direct contact with the microbial surface to disrupt the communication. *Aspergillus melleus* Acylase has been incorporated successfully within silicon catheters and polyurethane coatings decreasing the biofilm formation of *P. aeruginosa* ATCC10145 and PAO1 strain [60, 61]. The acylase in combination with α -amylase obtained from the *Bacillus amyloliquefaciens* was found to delay the biofilm formation of *E. coli* and *P. aeruginosa* reference strains for almost 7 days in a rabbit model of infection [62]. The acylase from the porcine kidney immobilized onto the carboxylated polyaniline nanofibers (cPANFS) to produce Nano biocatalysts reduced the formation of biofilm by *P. aeruginosa* PAO1. The topical application using lactonases obtained from *Bacillus* species has also been examined in a mice model of burn infection using the *P. aeruginosa* reference strain PAO1. The gel containing lactonase stopped the systemic spread of bacteria, reduced mortality, and offered synergistic activity with ciprofloxacin in burn infections [31].

Since the stability of the enzyme is the main obstacle in developing the bio-based materials, the various enzymes from the extremophiles were also considered. Specifically, the PLL SSoPox enzyme from the *Sulfolobus solfataricus* has found to possess an exquisite mode of quenching bacterial virulence [49]. This highly stable enzyme was initially immobilized on the Nanoporous alumina membranes while maintaining the strong effect of decreasing the secretion of virulence factors, elastase, and pyocyanin of *P. aeruginosa* PAO1 [63, 64]. SsoPox-W2631, a variant enzyme, has been exhibited to considerably decrease the virulence of *P. aeruginosa* of the 51 clinical isolates from the diabetic foot ulcerations by retaining its efficacy towards the bacterial strains after immobilization in the polyurethane coating through glutaraldehyde cross-linking [65]. These enzymes have also used for intratracheal administration which significantly increased the survival rate in rats as observed in a pneumonia infection model for a reference strain of *P. aeruginosa* [51]. Apart from the studies using the acyl-homoserine lactones-based quorum sensing quenchers, the latest study has explained the use of AI-2 synthesizing kinase LsrK. The enzyme was combined with the biological polymers i.e. alginate and chitosan and ATP were added which ultimately decreased the AI-2 mediated quorum sensing [66].

A recent study has measured the QS inhibition potential of various alkyl gallates used in food preservation including octyl gallate (OG) and propyl gallate (PG) and hexyl gallate (HG) against *P. aeruginosa*. The QS gene expression experiment suggested that that the HG inhibited RhlR, whereas OG inhibited PqsR. The study highlighted that alkyl gallates great potential as a QS inhibitor against *P. aeruginosa* [67]. Quorum quenching based devices have got special attention as that they could potentially prevent hospital-acquired infections through effective control of biofilm formation and bacterial virulence. Still, more efforts are required to verify the concepts *in vitro* as well as in animal or clinical studies. The efficiency of these devices must be determined not only in bacterial strains model but also on phenotypically and genetically diverse clinical strains. Even though the development of medical devices looks less difficult compared to the drugs, the regulatory issues need to be addressed for authenticating the capability of these systems for their therapeutic applications. The wide range of quorum quenching enzymes and quorum sensing inhibitors and their medical applications will ultimately lead to the development of innovative medical devices.

Quorum quenching; in silico approaches:

The detection and discovery of enzyme inhibitors in medicinal chemistry are also supported by the computer-based methods for the exploration of anti-quorum sensing substances which helps to save the cost and time in performing experiments by preselection of the potential candidates through in silico screening.

A study conducted for the virtual screening to find the RhlR and LasR quorum sensing inhibitors within *P. aeruginosa*. Among the 1,920 compounds, docking detected five candidate binders for RhlR and LasR receptors. Their capability to inhibit the expression of the virulence factors i.e. elastase, hemolysin, and protease was further validated through *in vitro* experiments [68]. In another study, a library was screened comprising of 3,040 natural compounds for finding the new quorum-sensing inhibitors of the receptor LasP from *P. aeruginosa*. Based upon the molecular docking results, the *in vitro* experimental processes were limited to just 22 auspicious candidate inhibitors. These compounds were further investigated to downregulate various virulence factors [69]. The structural compound library namely SuperDrug and SuperNatural which comprise of natural compounds were screened to find the inhibitors for the LasR receptor

from *P. aeruginosa*. By employing molecular docking and virtual screening, few compounds were found capable to inhibit the quorum sensing signaling cascade [70].

A variant Boolean network modeling as an *in silico* multistep modeling approach to study the time-dependent characteristics of the *Pqs*, *Las*, and *Rh1* signaling systems in *P. aeruginosa* [71]. Their goal was to examine the metabolic and regulatory interaction between quorum sensing inhibitors, signaling molecules, receptor antagonism, and expression of various virulence factors e.g. rhamnolipids, pyocyanin, and elastase. During the simulation, they revealed that the signaling molecules PQS and HHQ are reduced when *PqsBCD* expression is suppressed by suitable inhibitors. This network approach also allowed them to anticipate the quantitative impact of PqsR antagonists and Pqs inhibitors. In *S. aureus* a crystal structure of AgrA LytTr domain was determined which was necessary to bind the DNA. Afterward, the virtual screening using a small library comprising of almost 500 compounds revealed three inhibitors that disrupt the AgrA binding to DNA [72].

Quorum sensing interference using animal infection models; bottlenecks

So far, several animal infection models have validated that the mutants which are deficient in quorum sensing are far less infectious than their parental strains; therefore, quorum sensing interference stimulates the potential of microbes by increasing the survival rate of host and resulted in a decrease in the bacterial counts and bacterial associated damage [73]. Few of the commonly used infection models include the nematodes like *Caenorhabditis elegans*, arthropods such as *Galleria mellonella*, zebrafish, and fruit fly which are informative and valued yet lacks the accurate human physiological features. Furthermore, the mice and immunocompetent individuals were also used as a model; however, it is understood that the *P. aeruginosa* is an opportunistic pathogen, therefore it was not clear that the immune response of the immunocompromised patients will be able to clear the bacterial infections following the therapeutic use of quorum sensing interference therapies. Besides affecting the immunocompromised individuals, the *P. aeruginosa* strains are the primary cause of the death in cystic fibrosis patients; therefore, various mice models exist that combine particular mutations in the membrane protein termed as cystic fibrosis transmembrane conductance regulator (CFTR) that could mimic several properties of the infection in humans. Nevertheless, cystic fibrosis is a quite complicated disease the number of reported mutations within *cftr* gene is much more (more than thousand) which are associated with this disease; thus, it seems to be challenging to develop a murine model which closely resemble the human disease [74].

Many of the modern models are appropriate for studying acute infections but very few are suitable for chronic infections i.e. cystic fibrosis. This particular model was used to conclude that quorum sensing interference by using azithromycin can impede with the alginate production by the mucoid strain NH57388A, therefore, reducing the damage to the host, still, was unable to considerably enhance the survival of mouse [75]. The thermally induced injury model is an alternative kind of model which is similar in a situation as observed in humans as it involves the production of second or third-degree burns on the dorsal side of the mouse using hot water followed by inoculation of burn. It was observed that quorum sensing deficient mutants e.g. *lasI*, *lasR*, *pqsA*, *rh1I*, and *lasI rh1I* were less virulent as compared to the parental strains [76]. Furthermore, it has also been employed to examine the inhibitory effect of anthranilic acid analogs including 4CABA, 6CABA, and 6FABA for the blockage of quinolone quorum-sensing signals. These compounds significantly decreased the mortality rate in the mouse at the appropriate doses. However, these compounds were assessed on a single dose, so it is not much clear whether these can exert dose-response effects [76].

CONCLUSION AND FUTURE PROSPECTS

Many studies have confirmed that the quorum quenching based approaches have significant potential as antibacterial agents over the past few years. Several studies signify the use of these methods for the functionalization of medical devices. Thus far, there is limited knowledge about the possible mechanisms of resistance that bacteria could develop in response to the quorum quenching based therapies. The phenomenon of resistance resulting from the evolution in the milieu of selection pressure favoring the progress of resistant strains as have already observed in the case of antibiotics. Although, few quorum sensing inhibitors for instance azithromycin have found to inhibit the growth, many others possess a moderate or quite little effect on growth rate. The quorum quenching enzymes may serve as some ideal contenders with minimal side effects and effective inhibitory activity against the biofilms. However, the quorum-sensing inhibition is still a prospective approach to increase the therapeutic arsenal for the management of bacterial infections in addition to the conventional antimicrobial agents. The comprehensive effect of these strategies on the bacterial physiological properties has shown that quorum interference-based approaches would be

appropriate for reducing bacterial virulence and restoring the antibiotic resistance through decreasing the biofilm formation and decreasing bacteriophage resistance, therefore, opening the way for imminent combinational therapies. Further studies are desirable to establish the effects induced these strategies for single bacterial species as well as for bacterial communities. The future explorations will direct the extent of the action of quorum sensing interfering molecules and their implications as therapeutic agents, as coating agents for medical implants and as combinational therapeutics.

Table 1. Quorum sensing interference molecules for medical devices

Devices	Quorum quenching molecules	Strategies	References
Catheters	Furanones 5 fluorouracil Acylase (<i>Aspergillus melleus</i>) Acylase (<i>Aspergillus melleus</i>) combined with α -amylase (<i>Bacillus amyloliquefaciens</i>)	Quorum sensing inhibitor Quorum quenching enzymes	[43] [60, 61]
Urinary catheters	Thiazolidinedione-8	Quorum sensing inhibitor	[54]
Coatings	5-methylene-1-(prop-2-enoyl)-4-(2-fluorophenyl)-dihydropyrrol-2-one PLL SsoPox from <i>Sulfolobus solfataricus</i>	Quorum sensing inhibitor Quorum quenching enzymes	[53] [51, 65]
Capsules	Autoinducer-2 processing kinase LsrK	Quorum quenching enzymes	[66]
Colonization-resistant materials	Truncated autoinducer peptide-II	Peptides	[57]
Implanted medical devices	DHP derivatives and furanones	Quorum sensing inhibitor	[77]
Dacron graft	RNA-III inhibiting peptide	Peptides	[59]
Nanofibers	Acylase from porcine kidney Macrocyclic peptides	Quorum quenching enzymes Peptides	[78] [56]
Topical therapies	Lactonase (<i>Bacillus</i> sp.ZA12)	Quorum quenching enzymes	[31]
Nano-vectors	Polyphenols of honey	Natural compounds	[55]
Prosthesis	FS3	Peptides	[58]

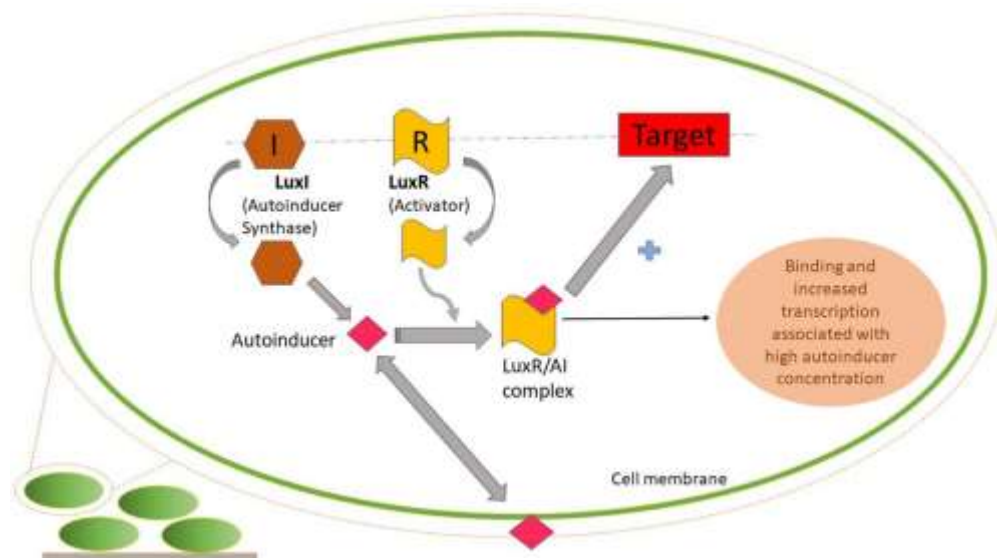


Figure 1. The LuxI, LuxR type quorum sensing. The autoinducer synthase (LuxI-like) catalyzes the synthesis of acyl-homoserine lactone (AHL) which can diffuse through the bacterial cell membrane. The transcriptional regulator (LuxR) binds with the diffusing AHL which can upregulate the transcription of the target genes.

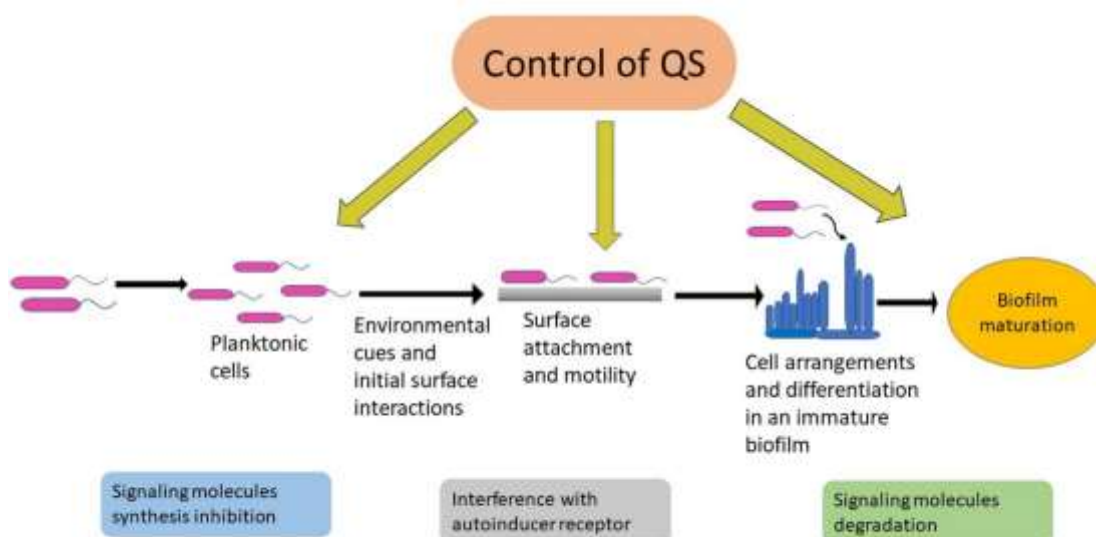


Figure 2. Step involved in biofilm formation and control of quorum sensing for the inhibition of surface colonization.

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