Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

REVIEW ARTICLE

The Role of Chemical and Herbal Antipathogenic Compounds in the Prevention of Quorum Sensing-Dependent Pathogenicity of *Pseudomonas aeruginosa* - A Review

Hira Hameed and Saiyed I Ahmed*

Institute of Microbiology, University of Agriculture, Faisalabad-38040, Pakistan
*Corresponding author: ahmedsiai@yahoo.com

ARTICLE HISTORY (14-237)

Received: May 16, 2014
Revised: June 28, 2014
Accepted: August 10, 2014

Key words:
Antipathogenic compounds
Pseudomonas aeruginosa
Quorum sensing inhibition

A B S T R A C T

Pseudomonas aeruginosa is an opportunistic pathogen of humans, animals as well as of plants and the most common Gram-negative bacterium found in nosocomial infections. *Pseudomonas* associated biofilms are highly tolerant to lethal doses of antibiotics. Recent discoveries of quorum quenching mechanisms use quorum sensing as a potential antimicrobial target. In this review we discuss the efficacy of different antipathogenic compounds against gram-negative bacteria in general and against selected *P. aeruginosa* strains in particular. Several strategies for quorum quenching have recently been deployed and recommended. The first approach is the use of quorum quenching enzymes that target a broad range of AHLs. Quorum quenching enzymes such as lactonase and acylase are able to inactivate a wide range of AHLs. The second approach involves the use of different herbal extracts as quorum quenching compounds against bacterial virulence. The third approach combines the use of quorum quenching along with other supplemental treatments, such as antibiotics, to obtain a synergistic effect in which the quorum quenching compounds deliver the primary offensive capability to reduce bacterial capacity for pathogenicity and increase the susceptibility of bacteria to antibiotic treatment. The convergent approaches are discussed here to elaborate on how new as well as previously available natural compounds such as active antipathogenic compounds from garlic and other naturally occurring herbal sources can be utilized in novel ways with the help of combinatorial chemistry to meet the challenges of increasing threats from newly emergent and potent drug resistant microbes to treat compromised individuals particularly with low immunity thresholds.

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To Cite This Article: Hira Hameed and Saiyed I Ahmed, 2014. The role of chemical and herbal antipathogenic compounds in the prevention of quorum sensing-dependent pathogenicity of *Pseudomonas aeruginosa* - A review. Pak Vet J, 34(4): 426-431.

Quorum sensing: Bacterial growth, virulence or pathogenic ability primarily depends on the ability of bacteria for cell-to-cell communication through a variety of signal molecules. This type of communication among or between bacteria is referred to as Quorum sensing (QS) (Rumbaugh *et al.*, 2012). The molecules (auto inducers) used in signaling process in Gram positive and Gram-negative bacteria are different and distinct (Galloway *et al*., 2011). Biofilm formation and sporulation in Gram-positive organisms e.g. *Bacillus subtilis* has recently been discussed thoroughly in a recent article by Liaqat *et al.* (2013). Gram-negative bacteria utilize N-acylated-L-homoserine lactones (AHLs) which are produced by LuxI-type synthase enzymes and bind to cytoplasmic LuxR receptors to exert a regulatory output and Gram positive bacteria use cyclic peptides as an auto inducer. The peptide signals are recognized by either membrane associated histidine kinase or cytoplasmic receptors (Thoendel *et al*., 2011). Another type of auto inducer AI-2 has also been identified, their chemical nature is still unknown but they initiate QS both in Gram-negative and Gram-positive bacteria (Xavier and Bassler, 2003; Galloway *et al*., 2011). Some of the auto inducers are shared between prokaryotes and eukaryotes. It is hypothesized that cross kingdom communication also exists and is more prevalent than had been previously assumed (Iyer *et al*., 2004). For example, we know that QS is functional between prokaryotes but it can also exist between prokaryotes and eukaryotes. In eukaryotes, most of the enzymes, which help in communication, are homologous to such structures, which help in quorum sensing in prokaryotes. These enzymes are present in

prokaryotes but absent in eukaryotes including plants, so prokaryotes share these enzymes (e.g. Phenylethanolamine N-methyltransferase, glutamate decarboxylase, histidine decarboxylase, etc) with eukaryotes and help them in quorum sensing (Iyer *et al*., 2004).

Quorum sensing inhibition (QSI)/quorum quenching: The cure or treatment of conventional infectious diseases is based primarily on the use of antibiotics that aim to kill or inhibit bacterial growth (Sharon *et al.,* 2009). The major problem with this approach is the frequent development of antibiotic resistant strains of microbes under attack. For example, one prime strategy of resistance that *P. aeruginosa* utilizes is the development of biofilm mode of growth. Through the use of this strategy *Pseudomonas* effectively evades the action of antibiotics since biofilms prevent effective concentrations of antibiotics to penetrate to arrest and prevent the further development of such opportunistic pathogens (Rasmussen and Michael, 2006). The emergence of antibiotic resistant strains of *P. aeruginosa* demands new approaches for combating infections (Kaufmann *et al.,* 2008). Accordingly, the use of selected signal molecules or cell-to-cell communication disrupting drugs (that will arrest the ability of invading microbes to launch their virulence mechanisms through activation of responsible genes) can potentially prove to be one of the most effective strategies. Although, we will focus our attention here primarily on *P. aeruginosa,* a Gram-negative bacterium, it behooves to remind the reader that this strategy can be equally effective against a variety of Gram-positive bacteria. Compounds capable of such a type of interference have been termed antipathogenic drugs and this phenomenon is also called quorum sensing inhibition (QSI) or quorum quenching (Rasmussen and Michael, 2006; Kalia and Purohit, 2011). Actually there are three main strategies for accomplishing quorum quenching either by targeting signal generation inhibition or by inhibition of signal dissemination or signal receptor inhibition (Dong *et al.,* 2007).

***Pseudomonas aeruginosa* and its pathogenicity:** *P. aeruginosa* is an opportunistic pathogen of humans, animals as well as of plants and the most common gram-negative bacterium found in nosocomial infections of urinary tract, bloodstream, pneumonia, and burn wound. It is also known for its connection with chronic infections of the respiratory tract diseases including cystic fibrosis, diffuse panbronchiolitis, and bronchiectasia (Bjarnsholt and Givskov, 2007). Its survival and pathogenicity depends on the production of lytic enzymes like protease, elastase, pyocynanin pigment, exopolysaccharide (EPS), and swarming motility (Adonizio *et al*., 2008; Vu *et al*., 2009).

Quorum sensing in *Pseudomonas aeruginosa*: It has been found that *P. aeruginosa* uses at least two QS systems for the formation of biofilm and other virulence expression factors (Duan and Surcttc, 2007). The first QS system of *P. aeruginosa* uses the LasI/R and the concerned genes are LuxI/R homologues. In the LasI/R system, LasI synthase directs the synthesis of N-(3-oxodoecanoyl)-L-homserine lactone (3O-C_{12}- HSL), which can bind to receptor Las R. This complex controls the transcription of genes for several virulence factors, including alkaline protease, toxin A and elastases (Schuster *et al.,* 2003; Duan and Surcttc, 2007). The second type of QS system is named rhlI/R, where RhlI synthase directs the production of N-butyryl-L-homoserine lactone (C4-HSL) binding to receptor Rhl R (Bottomley *et al*., 2007). The complex so formed i.e. C4-HSL-RhIR activates transcription of several genes including rhl AB operon that encodes for the enzyme responsible for rhamnolipid synthesis which are intricately involved in biofilm formation.

P. aeruginosa also uses a third signal, *Pseudomonas* quinolone signal (PQS) and the PqsR receptor protein (Dubern and Diggle, 2008). This QS signal is responsible for the expression of many virulence factors in *P. aeruginosa* (Schuster *et al.,* 2003; Bottomley *et al*., 2007; Bredenbruch *et al*., 2006).

Quorum quenching against AHL mediated QS

1) Quorum quenching enzymes: QSI or quorum quenching in *P. aeruginosa* or gram-negative bacteria in general can be interfered with in several ways. Since we know that in *P. aeruginosa* or in Gram-negative bacteria quorum sensing is AHL based, so it can be blocked by inhibition of AHL biosynthesis involved enzymes of acyl chain (acyl-acyl carrier protein) (ACP) and S-adenosylmethionine synthase (Parveen and Cornell, 2011), as well as LuxI homolog proteins. For example, various analogs of SAM (S-adenosyle methionine), such as *S*-adenosylhomocysteine, *S*-adenosylcysteine, and sinefungin, have been demonstrated to be potent inhibitors of AHL synthesis catalyzed by the *P. aeruginosa* RhlI protein (Pechere, 2001).

Second way in which quorum quenching can be exercised is through destruction of the QS signaling molecules. In this way signal is not accumulated in the environment and is unable to initiate and express QS phenomena. Two enzymes that play a significant part in degradation of AHL have been discovered: i.e., AHL lactonase and AHL acylase (Dong *et al.,* 2007).For example *Bacillus* spp. which is a Gram-positive bacterium, inhibits quorum sensing in Gram-negative or AHL autoinducer producing bacteria by lactonase enzymes (A ii A) which cleave acyl moiety from lactone rings of AHL and utimately inactivate AHL. However, in this mode it only acts as quorum quencher for AHL auto inducer producing bacteria and its own communication threshold remains uninterrupted (Dong *et al.*, 2000; 2001). Similarly, human paraoxonase (PON2) enzymes are lactonase in nature so they also inhibit QS by targeting the signal transmission (Draganov *et al*., 2005; Ozer *et al.,* 2005). From other bacteria as diverse as *Agrobacterium tumefaciens*, *Erwiniacarotovora*, and *Xanthomonas* spp. there are reports of autoinducer degradation activities (Haudecoeur *et al.,* 2009). Similarly, many other bacteria produce AHL acylase and act as a quorum quencher by targeting AHL molecules. They degrade the amide bonds resulting in the yield of homoserine lactone and fatty acid. By this mechanism the AHL ring becomes open, which can serve as a ready source of energy and nitrogen compounds. As a further example *Variovorax paradoxus*

and *Ralstonia* specie XJ12Bendeavor toopen AHL ringsin order to use products of AHL as carbon and nitrogen sources (Leadbetter and Greenberg, 2000; Lin *et al.,* 2003;Nishino and Spain, 2006). In conclusion, lactonases and acylases are quite efficient quorum-quenching enzymes which can be utilized as effective antivirulence or antipathogenic tools against *P. aeruginosa* or other Gram negative bacteria because of their ability to inactivate signaling molecules without interfering with the enzymatic mechanisms inside the bacterial cells, thereby reducing the selective pressure exerted by the use of antibiotics.

Finally, the third mode of quorum quenching in bacteria can be achieved by the inhibition of LuxR homolog proteins (Qs receptors) (Koch *et al*., 2005; Chen *et al.,* 2011). For example, synthetic halogenated furanones from *Delisea pulchra* inhibit AHL-dependent quorum sensing by displacing the AHL signal from its reporter protein (Manefield *et al*., 1999). Furanones from marine algae *Delisea pulchra* do not act as quorum quenching compounds against *P. aeruginosa* (de Nys *et al.,* 1993**)** but synthetic furanones have demonstrated good efficacy (Rasmussen and Givskov, 2006). On the other hand a chemical compound, Iberin from horseradish inhibits QS in *P. aeruginosa* by acting on RhIR receptors. These receptors are used in the second type of QS system deployed by *P. aeruginosa* (Jakobsen *et al.,* 2012).

2) Quorum quenching pressure exerted by herbal extracts: Many natural food products, herbs and spices possess quorum sensing inhibitory properties for example, garlic, carrot, and chamomile (Fulghesu *et al.,* 2007). Among these, garlic (*Allium sativum*) is renowned for its antifungal, anticancerous, antiviral, antiprotozoal and antimicrobial activities (Block, 2010). It was demonstrated that the antimicrobial activities related to the presence of growth inhibitory compounds, such as allicin, which is the active ingredient in garlic (Ankri and Mirelman, 2001). Later on it was reported that ajoene from garlic are naturally occurring substances which act as quorum quenching compound active against *P. aeruginosa.* Recently tested mouse model studies of pulmonary infection have shown that ajoene elicited significant clearance of *P. aeruginosa* in these studies (Jakobsen *et al.,* 2012). Ajoene is a sulfur-containing compound, which is produced when garlic is crushed. Michael and his team found that ajoene from garlic acts as a quorum quencher for almost 11 virulence genes that are responsible for *P. aeruginosa* infections (Michael, 2012). Gorgonian corals are naturally occurring invertebrates, which are found in coral reefs worldwide in marineeco system (Bayer, 1961). Laura *et al.* (2012) tested different non-marine human pathogenic Gram-positive and Gram-negative bacteria as well as a variety of marine bacteria against the antimicrobial and quorum sensing inhibitory activities of gorgonian extracts. They found that among the non-marine human pathogenic bacteria, *P. aeruginosa* PAO1, *Bacillus subtilis, Vancomycin resistant enterococcus, methicillin sensitive staphylococcus* and *Escherichia coli* were sensitive to gorgonian extracts. They proceeded to examine the quorum sensing inhibitory effects of long chain AHL from *P. aeruginosa* PAO1 and short chain AHL from *Chromobacterium violaceum* CV026 and concluded that gorgonian extracts possessed significant QS inhibitory activities on long chain AHL from *P. aeruginosa* PAO1

Basavaraj *et al*. (2008) reported that natural fruit juices like grapefruit are known to inhibit biofilm formation in *Escherichia coli* O157:H7, *Staphylococcus aureus, Salmonella* and *P. aeruginosa* by acting on QS mechanisms of these bacteria**.** They concluded that particularly furocoumarins from grapefruit possess such inhibitory capabilities on a number of bacterial species. Dietary phytochemicals are secondary metabolites in plants. Vattem *et al.* (2007) have tested some common phyto chemicals from dietary fruit, herb and spice extracts in model bioassay test systems. The effect of the substances as antipathogenic drugs at sub-lethal concentrations (SLCs) were investigated and resulted in the conclusion that indeed such compounds possessed inhibitory QS related functions that were effective on different bacterial properties like swarming motility of pathogens *Escherichia coli*O157:H7and *P. aeruginosa*, violaceum production in *Chromobacterium violaceum o26* (CVo26). Almost 200,000 compounds have been screened which are potential inhibitors of LasR-dependent gene expression and the two best inhibitors among these are tetrazole and V-06-018, with phenyl ring that normally binds to Las R. Müh *et al.* (2006) observed that both of these compounds inhibited the production of two QS dependent virulence factors, elastase and pyocyanin in *P. aeruginosa.*

Many other plants produce compounds which have good QS inhibitory effects as an example, carrot, soya bean, water lilly, tomatoes pea seedlings, bean sprouts, vanilla and similar natural compound scan be very effective QS inhibitory agents (Rasch *et al.,* 2005; Choo *et al.,*2006; Niu *et al.,* 2006; Jaramillo *et al*., 2012). Most of these plant extracts exert a strong antagonistic effect on LuxR based QS. Due to the quorum quenching ability of these plants, they have been used in food and flavor industry as a preservative and to delay the onset of food spoilage (Rasmussen *et al.,* 2005; Blana, 2011).The commonly used cuminum (*Cuminum cyminum*) and its secondary metabolite, methyl eugenol has been used against Gram-negative bacterial pathogens such as*P. aeruginosa* and it was found that it promoted the loosening of biofilm architecture and strongly inhibited in vitro biofilm formation of this organism (Packiavathy *et al.,* 2011). Garlic and other similar spices in daily culinary use as additives such as ginger and turmeric have been reported to possess QSI properties (Vattem *et al.,* 2007). Similarly, several other commonly used substances like essential oils of cinnamon (Niu *et al.,* 2006) and clove (Khan *et al.,* 2009) can also act as potent QSI compounds against Gram-negative or AHL mediated QS. Several natural compounds have been shown to possess QSI properties, like edible fruit (grape fruit), as well as marine sponges and seaweeds (Skindersoe *et al.,* 2008; Musthafa *et al.,* 2010) and even some bacteria themselves exhibit QSI activities (Thenmozhi *et al.,* 2009; Nithya *et al.,* 2010). Extracts from edible plants were tested as QS inhibitors for examples, Melicope lunu-ankenda which is a Malay garden salad, has been found by Tan *et al.* (2012) to inhibit QS dependent virulence determinants of human pathogen *P. aeruginosa* PAO1's pyocyanin and swarming

motility. These plant extracts also inhibited other Gram-negative bacterial virulence like that of *Chromobacterium violaceum* CV026, violacein production and bioluminescence expression in *E. coli* [pSB401] by hindering response of their QS signal N-hexanoyl homoserine lactone.

3) Synergistic effect of combinatorial therapy with antimicrobial agents and herbal extracts enhances quorum quenching effects: Given the emergence and increasing occurrence of multidrug resistance among a variety of pathogenic bacteria, the development of novel therapies for the treatment of bacterial infections, such as those based on quorum quenching would be of huge clinical significance. Selective disruption of quorum sensing should attenuate pathogenicity without imposing the level of selective pressure associated with antibacterial treatment (Defoirdt *et al*., 2010; Maeda *et al*., 2012).

In most of pathogenic bacteria and especially in the case of *P. aeruginosa* biofilm formation is critical QS regulated phenomena. Biofilm production causes many problems such as surgical instrument associated infections (nosocomial infections) and catheter-related bloodstream infections (Donlan, 2001; Taylor and Webster, 2011). If QS could be controlled or disrupted then the formation of biofilms could also be avoided or minimized but sometimes with the disruption of QS in *P. aeruginosa*, biofilm formation is not entirely prevented. However the simultaneous use of QSI compounds could make the infection more susceptible to antibiotic compounds (Estrela and Abraham, 2010; Brackman *et al.*, 2011). Rhamno lipids that form as a result of QS, provide the bacteria such an environment in order to escape from the host immune defense system. The quorum quenching mechanisms render the bacteria more sensitive to PMNLs by depressing the strength of biofilm or rhamno lipid formation (Bjarnsholt *et al.*, 2005).

Christensen *et al*. (2012) reported synergistic effects of antibiotic and herbal extract against *P. aeruginosa* and found much more pronounced results. e.g. ajoene from garlic combined with the antibiotic tobramycin killed over 90% of bacteria which were involved in biofilm formation instead of tobramycin alone which killed less than 10%. Michael (2012) subsequently reported that combinatorial therapy of garlic with antibiotic decreased the dose of garlic used. The dose of garlic, which had given good results against *P. aeruginosa* in adult human, was 50 bulbs of garlic for several days but that also generated a multitude of unwanted side effects. However, when garlic was given in combination with antibiotic (tobramycin), their synergistic effect permitted the reduction in very high doses of garlic.

Rasmussen *et al.* (2005) studied the QSI properties of fungi and identified two compounds from fungi, which have QSI activity on *P. aeruginosa* biofilm. These compounds not only attenuate *P. aeruginosa* but make *P. aeruginosa* biofilms highly susceptible to treatment with conventional antibiotics like selective *P. aeruginosa* antibiotic tobramycin. Most of the secondary metabolites isolated from filamentous fungi, which are already used clinically as antimicrobial drugs, also act as QSI at sublethal doses. Patulin and penicillic acid have been identified as being biologically active QSI compounds. Patulin was found to enhance biofilm susceptibility to tobramycin treatment and patulin or penicillic acid also rendered the *P. aeruginosa* susceptible to polymorphonuclear (PMLN) cells effect.

Other benefits of quorum quenching: Bacterial enzymes, which have been reported to be regulated by QS, for example different microbial extracellular enzymes such as Pectate lyase, pectin lyase, polygalactouronase, cellulase, lipases, chitinase, nuclease and protease have been known to cause food spoilage. As these enzymes play a part in food spoilage, by inhibiting the regulatory system of these enzymes by using various preservatives with duel QSI properties, food spoilage could be delayed or entirely prevented (Pirhonen *et al.*, 1993; Rasmussen *et al.*, 2005; Van *et al.*, 2006; Van *et al.*, 2007).

Anti pathogenic drugs whose mode of action is primarily QSI based mainly target the QS activities of bacteria and hence prevent the expression of bacterial virulence genes or stress survival genes but do not kill the pathogen itself. Accordingly, by using this mode of action of anti pathogenic drugs, prevalence of the development of antibiotic resistant strains can be avoided or minimized (Tan *et al.*, 2012).

Food borne pathogens cause great economic losses to the extent of about 5-6 billion euros each year in Europe alone and the pathogens that are primarily involved as causative agents are *Listeria monocytogenes, Salmonella, Escherichia coli, Pseudomonas* spp. and *Entero-bacteriaceae* (Koutsoumanis 2009; Scallan *et al.*, 2011).

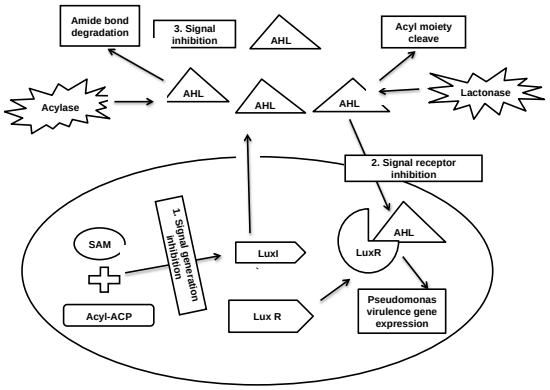

Fig. 1: Three pathways of QSI in *Pseudomonas aeruginosa* AHL mediated QS

Conclusion: As frequent or indiscriminate use of antibiotics against bacterial infections leads to increasing development of resistant strains which is becoming a global threat to public health. Accordingly the use of novel therapeutic agents are becoming of ever-greater importance. Towards this end the target of the scientist is to attenuate virulence without causing selection pressure. QS is a key regulatory system that is responsible for the expression of virulence determinants, thus by under-standing the potential of quorum sensing as an effective target against bacteria, new forms of anti pathogenic drugs can be developed and promoted. This effort can lead to the development of novel therapeutics that can prevent and control the spread of microbial infections.

QSI compounds are naturally found in many of our daily consumed foods (plants, herbs, vegetables, spices and these selective studies of these products and their active ingredients which result in specific beneficial effects including control of selected pathogens would render the dawn of a new age in pharmacotherapy. We are currently embarked on studies of active ingredients from Moringa (*Moringa oleifera*), Neem (*Azadirachta indica*), ginger (*Zingiber officinale*), onion (*Allium cepa*) and a variety of other herbs and spices as sources of QSI compounds for testing against the pathogenicity of *P. aeruginosa*. Our goal is to shed additional light and attempt to elucidate intricate mechanisms by which QSI compounds from such sources exercise their beneficial effects on human pathophysiology.

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