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Review Paper

Cell-to-Cell signal system in *Escherichia coli* Drug Resistance- a review

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Abstract

The term drug resistance refers to the ability of microorganisms to resist a drug that once stalled or killed them. Drug resistance in Escherichia coli may occur via production and elaboration of beta-lactamases, impermeability by simple closure of porin channels or lipopolysaccharide expression and removal of the anti-microbial compounds from the bacterial cell through specific and/or general efflux pumps. Drug resistance may be innate or adaptive. Cell-to-cell signal system (quorum sensing, QS) is an adaptive type of drug resistance, which depends on secreted signal molecules, to initiate response synchronized across bacterial population. The signaling molecules is similar to hormones present in higher animals. Mechanisms involved in QS systems include signals production, signals accumulation, and signals detection. In quorum sensing mechanisms, E. coli secretes chemical signal molecules during its exponential growth phase. The molecule known as autoinducers (Al-2) or pheromones is mediated by luxS gene. When a certain concentration of autoinducers is obtained, known as the threshold concentration, its presence is identified and lead to the initiation of the signal cascade. The consequence of this signal cascade may include changes of target gene expression, such as drug resistance. Factors affecting cell-to-cell signal systems are temperature, salinity, pressure, and pH. Bacteria may also be more resistant to antibiotics when they work together as a group via QS mechanism. Interfering with quorum sensing is a strategy that may be used to control bacterial virulence and antibiotic resistance. Control of QS in E. coli drug resistance include the use of AI-2 synthase inhibitors, modification of AI-2, the use AI-2 analogs, antagonism for LuxR-family receptor, signal synthesis inhibition, production of degradation enzymes and signal trapping.

Keywords: *Escherichia coli*, quorum sensing, autoinducers (Al-2), luxS gene, gene expression, drug resistance.

Introduction

Drug resistance in microorganisms is their ability to withstand a drug that once stalled them or killed them[1]. Microorganisms have found a host of mechanisms to bypass several antibiotics[2,3]. World Health Organization, WHO[4] explained that microorganisms that have the ability to resist antimicrobial agents are sometimes referred to as "superbugs".

Drug resistant microbes are found in people, animals, food, and the environment. Because of the resistance, the drug becomes in effective and diseases may persist in the body, which eventually increases their spread to others. Drug resistance can spread between animals and people, and from one individual to another. Drug resistance may occur naturally, usually through changes in genetic makeup[4].

Generally, the mechanism of antibiotic resistance can be grouped as either innate/natural or acquired/adaptive resistance[5-8]. Genes that may encode resistance to antimicrobial agents are found in bacteria and this genes may result in the production of antibiotics innate resistance. The extension of antibiotic resistance may occur because of extracted genes from bacteria. This may lead to a widespread of exposure of the resistance genes to other bacteria[9-10].

Microorganisms may also resist to some antibiotics by acquiring the resistant ability from other organisms in the environment. Adaptive resistance may occur by the following i. horizontal gene transfer[11], ii. mutation[12-13] and iii. antibiotic inactivation via enzymatic modification or destruction of the antibiotic[14-16].

Adaptive resistance may involve environmentally influenced genetic alterations that may include biofilm development and consistent development, inactivation of antibiotics, alterations in cell permeability, as well as efflux pump regulation[11]

The mechanism of drug resistance in *E. coli* include i. the production and elaboration of Extended-Spectrum Beta-Lactamases (ESBLs) which are broad spectrum enzymes capable of inactivating many broad-spectrum beta-lactam drugs[17], ii. impermeability by simply closure of porin channels and lipopolysaccharide expression in their cell wall[18] and iii. removal of the antibiotics from the bacteria through specific or general efflux pumps[19].

This review discusses cell-to-cell signal system in *E. coli* and their significance in antibiotic resistance. The majority of bacteria under long exposure to sub-inhibitory antibiotic concentrations in the presence of an external agent or, live in biofilms[20-22]. Biofilm formation (Figure-1) occurs *via* a sequence of events controlled by quorum sensing also known as cell-cell communications.

This signal system is facilitated by excreted small signaling molecules found in Gram-negative bacteria, referred to as autoinducers[23-24].

Once the signal is detected, a cascade is initiated[13,25] and results in metabolic changes, up-regulation of virulence, adhesion, production of a protective glycocalyx, and decreased antibiotic susceptibility[13,26]. Each quorum-sensing system is unique to a particular bacterium. Signals production, accumulation, and detection is common in all quorum sensing systems[27].

Quorum Sensing

Quorum sensing mechanism coordinates cellular actions as a result of the bacterial density (Figure-2). In QS mechanisms, autoinducers (AI) are secreted by individual bacterium. As the density of bacteria increases, the AI concentration in the environment also increases. Thus, resulting in interaction of the AI with cell signal receptors in the environment[29-30]. There are three types of autoinducers: the acylated form of homoserine lactone auto inducers coordinates the communication among Gram-negative bacteria synthesized by lux-I family proteins, autoinducer peptide proteins utilised by the Gram positive bacteria and AI-2 synthesized by lux-S family[31-32].

Gonza´lez and Keshavan[33] argue that low density of bacteria in the environment dilutes the concentration of the autoinducer signals, but high population densities increase the bacterial population and leads to the accumulation of autoinducers. When this occurs, the response is activated and quorum sensing cascade is initiated (Figure-3).

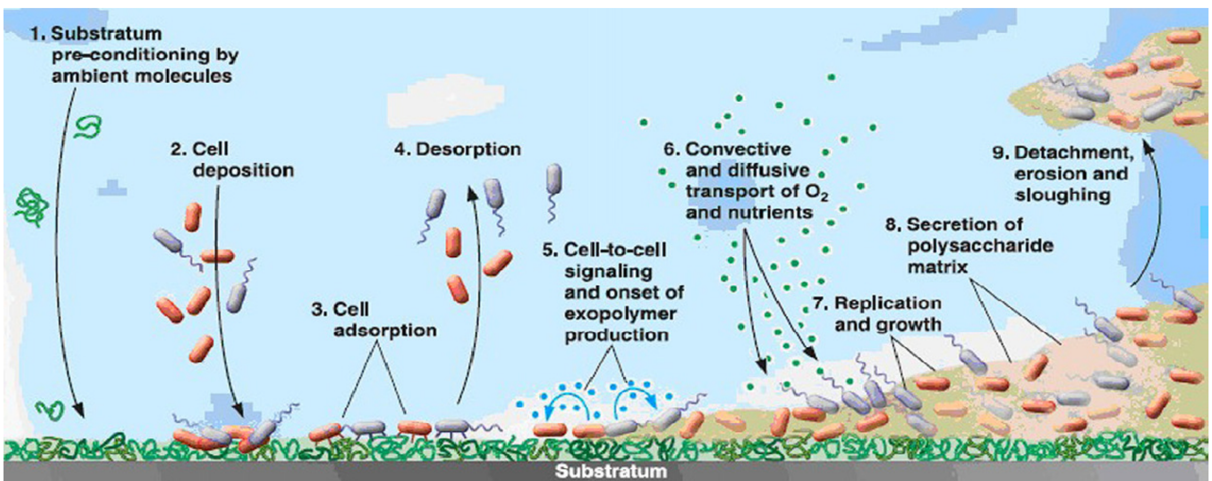

Figure-1: Biofilm formation[28].

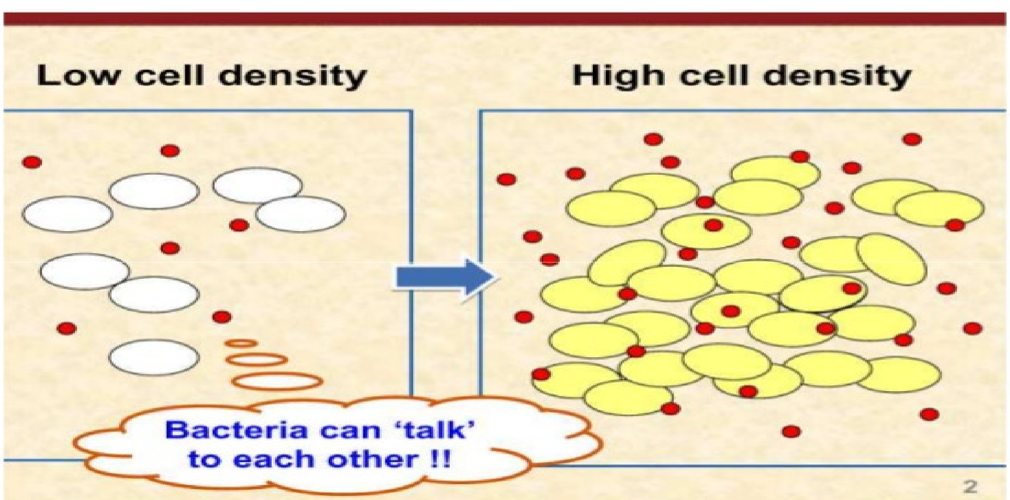

Figure-2: Bacteria communicate *via* quorum sensing mechanism[34].

According to Kendall and Sperandio[36], the primary reason why a bacterium undergoes cell-to-cell signal system is to regulate excess energy expenditure, and hence bacteria depend on varieties of mechanism to regulate the expression of genes in response to environmental changes. *E. coli* is capable of producing a signal molecule known as autoinducer 2 (AI-2)[37] (Figure-4). Bassler *et al.*[38] originally identified AI-2 as an AIs controlling the production of light by *Vibrio harveyi*. By using a *V. harveyi* strain that does not produce AI-2 but has the ability to respond to the signal, Surette and Bassler[37] discovered strains of *E. coli* that a gene, identified as luxS, which may activate AI-2 sensor in *V. harveyi*[38]. Some bacteria have the ability to produce and consume AI-2. They release it in log growth phase of growth, and import it when moving into stationary growth phase.

S-adenosylmethionine (SAM) is a key methyl donor in several microbial metabolic processes. It is an RNA cofactor and also a cofactor for protein and DNA synthesis. It is a major methyl donor in metabolic processes. Consumption of SAM leads to the production of S-adenosylhomocysteine (SAH), which is then hydrolyzed by nucleosidase(Pfs) to yield S-ribosylhomocysteine (SRH) and accumulates extracellularly with cell density. Then LuxS cleaved SRH to 4,5 dihydroxy-2,3-pentanedione (DPD), after which the DPD is further rearranged to yield AI-2 (Figure 4).

Lsr (Lux S regulated) operon imports AI-2. The Lsr operon consists of LsrA, lsrB, lsrC, lsrD, lsrF, lsrG and lsrR. The internalized Al-2 is phosphorylated *via* LsrK kinase (Figure-5).

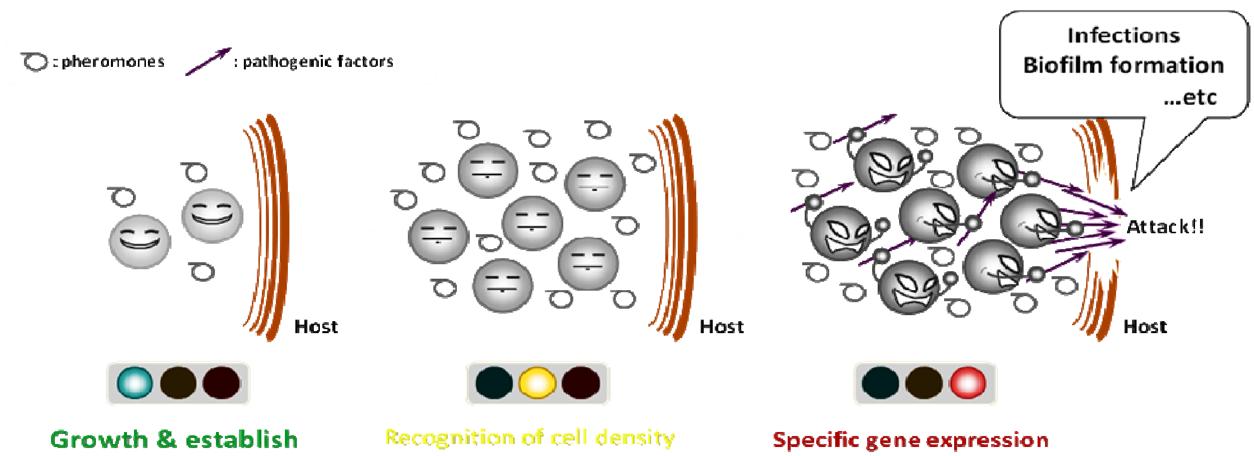

Figure-3: Bacterial quorum sensing at high bacterial density[33-35].

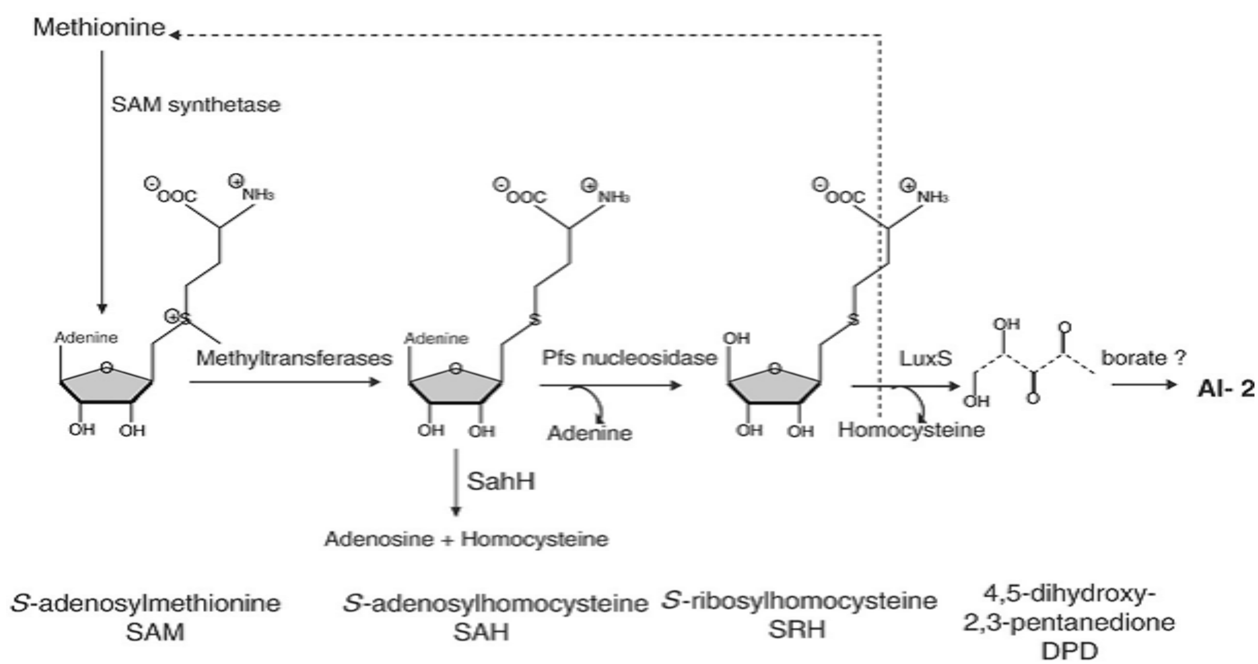

Figure-4: Production of Al-2[40].

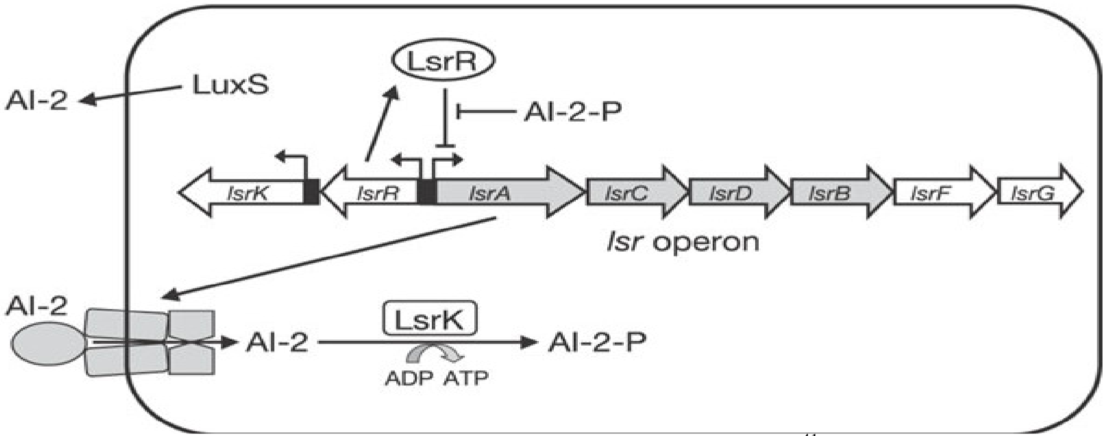

Figure-5: The *E. coli* Lsr transporter imports AI-2[41].

Factors affecting Cell-To-Cell signal system in *E. Coli*

There are many factors that influence cell-to-cell signal system in bacteria, and in particular *E. coli.* This chapter reviews some of the important factors such as salinity, pH, temperature and pressure. This chapter also elucidates the implications of autoinducer signaling system in *E. coli* on health care system and the control measures of cell-to-cell signal system in drug resistance.

Temperature: Quorum sensing may occur in moderate temperature environment [42]and in cold environment[43]. The production of precursors for acylatedhomoserine lactone during *Thermus* sp. cold shock was associated with the production of biofilm[43]. A particular gene in *Thermotoga maritima* that codes for short chain amino acids are considerably used to produce and elaborate AHLs precursors in higher rate at high temperatures in increased cell density and it was thought to be a sensing molecule[43].

Psychrophiles however, have an ecological importance, which its quorum sensing has been related to the evolution of bio-informatics[44-45]. Psychrophiles such as *Pseudoalter-monashaloplanktis* has been known to produce the AHL precursor putative Al-2 signals although they show no identification of Luxs production[44]. The putative Al-2 signal is elaborated when there is high temperature that can cause damage to the cells.

The survival of bacteria is highly dependent on their adaptation to changes on the temperature of a particular environment[46]. The effect of temperature shifts on *E. coli* [for example, inside versus outside the human body] has resulted to the production and elaboration of AHL's and its precursors[47]. Indole and hydroxyl indoles have been used to determine the effects of temperature on both non-pathogenic and Enterohemorrhagic *E. coli* (0157:H7)[48] and this effect has been observed to influence biofilm formation. The AHLs production and elaboration has shown a good response at 30^0C, and a weak one at 37^0C and hence, at the temperature 30^0C, *E. coli* colonies tends to form a cell-to cell sensing for its resistance[49] by producing AI-2 proteins with the SdiA regulator generally in the presence of indole.

Salinity: Salinity and alkalinity are generally interwoven[49]. Gram-negative bacteria generally tend to produce and elaborate autoinducers at a salinity level of 2.5 M [49-50]. This response has been observed to form biofilms and exopolysaccharide in *E. coli* and the EPS protects *E. coli* from dryness and enhance the cell-to-cell communication occurs *via* specific channels formation[51].

Pressure: *Photobacterium profundum* is a piezophile that is known to be related to the *A. fischeri* and *V. harveyi* in which were the first organisms to be quorum sensed[52]. *P. profundum* AHL elaboration at a high pressure was observed to facilitate metabolism and thus quorum sensing is essential in high pressure environment and comparing this to *E. coli* that is a moderate piezophile, if they are exposed to high pressure, they signal their cells to produce and elaborate the LuxR encoding genes and this provides the basis for their few numbers in deep ocean or sea bodies[53].

pH: *E. coli* inhibits the guts of humans and ruminant animals and the pH surrounding such environment is nearly neutral and hence the changes in acidity or alkalinity of such environment tend to affects the existence of *E. coli*[54]. Acidophiles such as *Ferroplasma acidarmanus* is an acidophilic archeon that is isolated from mountain mile precursors and can form a cell-to-cell signaling or sensing to a little extent with other microbes that are typically not acidophiles [such as *E. coli*] for the formation of biofilms and motility characteristics[54].

Furthermore, the acidophile bacterium *Acidithiobacillus-ferroxidans* contains the axeI and axeR that have been linked to produce the LuxI-LuxR proteins that are co-relatedly enrolled with *E. coli* for bioleaching in an environment with pH 1-2[55]. It has been observed that the two divergent gene [lttI and lttR] isolated from *Leprospirillum ferroxidans* are of high quorum sensing and has drawn similarities to *E. coli* genes elaborate cell growth, production of biofilm, chemotaxis and flagella formation[56].

The acidic, oxic and anoxic conditions of the human body, can certainly be considered an "extreme" location, and offers some challenges for microbes[45]. *E. coli* infection generally results in enteric fever, which is endemic in many regions of the world. *E. coli* elaborates shigatoxins which is totally dangerous. *E. coli* is characterized by a highly developed quorum sensing system and this accounts for its virulence and hence allows it to survive in the human host[57]. *E. coli* has a large number of genes that produces Shiga polysaccharides and this result in biofilm production. This ability gives *E. coli* the capability to respond to environmental alterations by modifying their biofilm to suit their survival[45].

The highly acidic nature [pH<1] of the human stomach environment is generally a challenge for the establishment of bacteria. Entrance of *E. coli* to the stomach results in the production of excess Shiga polysaccharides that elaborates thick biofilm. The production of excess shiga toxins is enhanced by the absence of Hap which is a quorum sensing regulator that regulates the expression of the shiga polysaccharide operon[59].

Implications of Cell-To-Cell signaling in *E. coli* on health care system

The two major implications of cell-to-cell signaling of microorganisms generally *E. coli* in several health care system has been the expression of virulence factors on the affected hosts system and the concordant resistance of antibiotic therapy on these *E. coli* strains[11].

Promotion of virulence

Virulence refers to the ability of bacteria to infiltrate, colonize and cause disease in a compromised system[60]. Virulence expressions are regulated by specific genes that encodes for virulence factors in *E. coli* and this genetic regulation is inter-wired and connected[61]. In the presence of changes of the environmental factors of a host system, cell to cell signaling results in the manifestation of these virulence Shiga toxins[62].

E. coli strain Shiga toxin producing (STEC) may be present as a mild gastroenteritis, diarrhea, grossly bloody diarrhea and Hemolytic Urenic Syndrome [HUS][63]. Transmission of STEC to susceptible humans occurs through contact with affected person, animals or contaminated environment[64]. The AB5 shiga toxin targets the host cells responsible for the expression of the glycolipid globotriaosylceramide (Gb3), subsequently disrupting the host protein synthesis and causing cell death[65].

Resistance to antibiotics therapy

E. coli tends to showcase or express biofilm during environmental stresses. This occurs due to series of cell-to-cell communication as regulated by released autoinducers[24]. Signaling cascades in bacteria initiates decrease in the antibiotic susceptibility[23] and therefore the antibiotic strategy that involves the disruption of the several structural make up of *E. coli* is been altered due to the formation and expression of biofilm[66-67].

Most antibiotic resistance of *E. coli* through the formation of a biofilm results from underlying heterogeneous bacterial sub-populations[67-68]. This sub-population varies in the mechanism by which they achieve their state or resistance in different *E. coli* strains [i.e. some strains produce enzymes that degrades antibiotic compounds and other strains do have an up-regulated efflux pump][24]. The *E. coli* biofilm community enforces innermost oxidative stress on infected individuals that subsequently forms a hyper-mutation state[69].

Control measures of Cell-To-Cell signal system in *E. Coli* drug resistance

Pathogenic bacteria are may also be more resistance to antibiotics when they work together as a group *via* QS mechanism. Blocking the interactions among these bacteria would force them to live as individual cells and thereby making them more susceptible[27]. Therefore, disrupting the cell signaling becomes a strategy for the control of virulence and antibiotic resistance in pathogens. This is useful in antimicrobial therapy to overwhelmed bacterial diseases. Methods applicableto interfere with quorum sensing in bacteria comprises of the following:

Antagonism of Lux Rreceptor: The first phase in cell-to-cell signaling is the binding of specific autoinducer signals to LuxR protein. Therefore, interfering with this system will result in inhibiting quorum sensing[70]. Example of this cell-to-cell signaling inhibition has been detected in *Vibrio fischeri*, *Agrobacterium tumefaciens* and *Pseudomonas aeruginosa*[71-74]. Halogenated acyl-furanones is a natural compound that acts as naturalcell-to-cell signaling antagonist[70,75,76].

Signal synthesis inhibition

Preventing the synthesis of AHL is another approach for the inhibition of QS. For example, SAM, has been reported to inhibit the reaction of the LuxI[77]. Chung *et al.*[78] also reported that C_8-HSL inhibits the enzymatic activity when it binds to AHL synthase and Gutierrez *et al.* [79] reported some compounds that targets the activity of 5′-methylthioadenosine nucleosidase involved in recycling SAM.

Signal trapping

Cell-to-cell signaling certainly will not occur when concentration of AHL is below the critical limit discussed earlier. Therefore, trapping the AHL signals to maintain the signal below the threshold results in quenching the QS signals. Cyclodextrin is used as a method of cell-to-cell signaling trapping as reported by Vance and Peake[80].

The use of AI-2 synthase inhibitors, modification and application of AI-2 Analogs

Inhibiting enzymes elaborated in the production of 5'-methylthioadenosine/*S*-adenosylhomo-cysteine nucleosidase (MTAN) using for example brominated furanones, will inhibit the concentration of the AI-2[1]. AI-2 can also be inhibited by alteration of AI-2 by the use of the kinase ATP and LsrK, once modified, the phosphorylated form of AI-2 will be unable to cross the bacteria cell thereby the AI-2 signaling will be quenched[81]. Another method is compounds that chelate AI-2 using polymeric material that contains boron[82] and the use of Analogs of AI-2 as QS Inhibitors e.g. the use isobutyl DPD (an analogue of DPD) is also capable of inhibiting AI-2 synthesis[1,83].

Conclusion

Cell-to-cell system in bacteria is an important mechanism for drug resistance. Bacteria may also be more resistant to antibiotics when they work together as a group *via* QS mechanism. Blocking the interactions among these bacteria would force them to live as individuals and thereby making them more susceptible. Therefore, meddling with cell-to-cell system is a promising strategy towards controlling bacterial virulence and antibiotic resistance. Further study may be carried out to explore additional mechanisms of interfering with quorum sensing system in *E. coli* and other bacteria for the control of drug resistance.

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