Indian J Microbiol (Apr–June 2015) 55(2):121–130
DOI 10.1007/s12088-015-0520-1

REVIEW ARTICLE

Meddling *Vibrio cholerae* Murmurs: A Neoteric Advancement in Cholera Research

M. Hema · Srikkanth Balasubramanian · S. Adline Princy

Received: 8 December 2014 / Accepted: 17 February 2015 / Published online: 24 February 2015

Abstract Cholera, a known diarrheal disease is associated with various risk factors like hypovolemic shock, rice watery stools, and death in developing countries. The overuse of antibiotics to treat cholera imposed a selective pressure for the emergence and spread of multi-drug resistant *Vibrio cholerae* strains. The failure of conventional antimicrobial therapy urged the researchers to find an alternative therapy that could meddle the cholera murmurs (Quorum Sensing). It seems to effectively overcome the conventional cholera therapies in parallel to decrease the morbidity and mortality rate in the developing countries. The paramount objective of this review essentially focuses on the different Quorum Sensing (QS) regulatory switches governing virulence and pathogenicity of *Vibrio cholerae*. This review also provides an insight into the plausible QS targets that could be exploited to bring about a breakthrough to the prevailing cholera therapy.

Keywords *Vibrio cholerae* · Quorum sensing · Multi drug resistance · Cholera

Cholera, the Diarrheal Disease

Diarrheal outbreaks have hampered the normal lives of people in developing and under -developed countries. Poor sanitation and hygiene practices have been the root causes behind such outbreaks. Cholera, the diarrheal disease is an outcome of infestation of the human intestine and its milieu with *Vibrio cholerae*. Two serotypes of the organism viz., the O1 and the O139 have been responsible for pandemic outbreaks throughout. Cholera has been a subject of research from many centuries and has influenced people throughout in their perspective views regarding the disease, ranging from the Miasmatic theory of 19th century which made people believe that this disease was spread through miasma (bad air) [1] to the current bacterial communication (Quorum Sensing) mediated disease progression [2].

World Health Organization (WHO) reports suggest that about 1.4–4.3 million cholera cases are incident every year in the globe of which nearly 28,000–1,42,000 lives are swiped out. Fifteen discrete outbreaks of cholera were documented during 1971–2010 [3]. The mortality rates due to cholera in 2011 increased by 57.96 and 3.62 % when compared to 2009 and 2010 [4, 5]. Reports suggest that numbers of cholera cases in India are more than the ones that are submitted to WHO Weekly Epidemiological Record. Epidemic outbreaks in India were largely due to the regular pilgrims to the Ganges river [6]. According to one of the reports, about 37,783 cholera cases (84 deaths) were incidents in the country between 1997 and 2006 but only very less numbers were given for WHO Records [7]. Among Asian region, the Indian subcontinent continues to be a hub for cholera cases with an occurrence of 78 %. In the year 2013, an aggregate of 47 nations from all mainland's reported 1,29,064 incidences of cholera to WHO, of which 43 % was accounted for from Africa and 47 % from the America where a huge episode that began in Haiti at the end of October 2010 additionally influenced the Dominican Republic.

The cholera tainted patient ought to be basically supplemented with liquids and electrolytes along with antibiotics to counteract lack of hydration formally. This was done through intravenous using sterile, pyrogen free

M. Hema · S. Balasubramanian · S. A. Princy (✉)
Quorum Sensing Laboratory, Centre for Research on Infectious Diseases (CRID), School of Chemical and Biotechnology, SASTRA University, Thanjavur 613 402, Tamil Nadu, India
e-mail: adlineprinzy@biotech.sastra.edu

intravenous solution or by using ORS which contains salts like NaCl (3.5 gm), KCl (1.5 gm), $NaHCO_3$(2.5 gm) and glucose (New formula of all rehydration salt, which reduce osmolarity [8]. Conventional therapy employs the administration of antibiotics as a means of relieving the worsened diarrheal conditions. As defined by CDC (Center for Disease Control) doxycycline/tetracycline are given as a first line treatment in adults whereas azithromycin/erythromycin are given for children and pregnant women. Furazolidone, erythromycin, trimethoprim-Sulphamethoxazole, chloramphenicol, azithromycin, ciprofloxacin are preferred for adult administration [9–12]. The significant detriment of the antibiotic is that it will abbreviate the time of infection instead of counter-acting the disease. Currently used vaccines are WC-rBS and BivWC, where the WC-rBS (marketed as Dukoral) is a monovalent vaccine consist of attenuated *V. cholerae* O1 along with recombinant cholera toxin B subunit. BivWC (marketed as Shancol and mORCVAX), a bivalent vaccine consist of inactivated *V. cholerae* O139. *Vibrio cholerae* has a unique property so that they can exist in an autochthonous state in river, brackish water estuaries and coastal waters and also in dwarfish forms under nutrient deprived conditions as a viable but non cultivable form.

The foremost limitation with the conventional antibiotic therapy is the development of MDR strains of *Vibrio cholerae* make this therapy abortive [9, 13]. Clinical isolates of *V. cholerae* strain causative executor for various outbreaks have become impervious to numerous antibiotics including tetracycline, ampicillin, nalidixic acid, streptomycin, sulphonamides, trimethoprim, gentamicin, ciprofloxacin etc. [14, 15]. Reports say that people infected with these MDR strains exhibits features indicating more severe illness than patients affected with wild strains. The infection cause by the MDR strains are paramount not just on the grounds that they are harder to treat, additionally, on the grounds that they may prompt higher morbidity and mortality rate [16]. Therefore, it is an era to overlook the limitations of anti-bacterial approach with anti-virulent therapies on meddling the quorum sensing (*Vibrio cholerae* murmurs) to counteract cholera [17]. This can be achieved either by interrupting the synthesis of QS signaling molecules or by blocking the receptor molecules thus disturbing the interaction between signaling molecules and receptor [18, 19].

Quorum Sensing: The Bacterial Samvada

The discrete behavioral pattern of bacteria to the diverse milieu they face is an outcome of the cell to cell communication between them called Quorum Sensing (QS) [20]. Chemicals known as autoinducers serve as dialect for the bacterial cells [21]. These autoinducers promotes group behavior in bacteria to establish their virulence traits as they are being sensed by their own populations/different populations [22, 23]. Bacteria define a high degree of signal specificity owing to the differences in the structures of QS signals and its binding domains of the receptor protein [24]. It is also known that bacteria use three classes of highly species specific and non-species specific autoinducers to elicit QS response. The most widely used species specific signal are Acyl-homoserine lactone (AHL) in the Gram negative bacteria, whereas oligopeptide in the Gram-positive bacteria [25]. Most of the other class of autoinducer (AI-2), is known to be a derivative of 4,5-dihydroxy-2,3-pentanedione (DPD), was found to be non-species specific that mediates crosstalk between inter-specious [26]. Just like people of different regions have different dialects; prokaryotes also have cell signaling variants e.g., *Streptococcus pyogenes* that resides in the pharyngeal tract has peptides as the cell signaling molecule whereas, Uropathogenic *E.coli* (UPEC), a resident of urinary tract has Acyl Homo Serine Lactones (AHSL) as the cell signaling moiety [27, 28]. The autoinducer concentration is directly proportional to the cell density and its concentration reaches a threshold level at HCD is being sensed by surface receptors and elicits a response at the target gene level expression via activation or repression [29]. This adaptive aggressive behavior is most predominantly observed in bacteria like *Pseudomonas aeruginosa*, Uropathogenic *Escherichia coli* etc., paradoxically, the converse happens in case of *Vibrio cholerae* i.e., under conditions of LCD (Low Cell Density) state virulence factors are expressed and at HCD (High Cell Density) state, virulence is repressed. Additionally, at HCD activation of HapA (Haemagglutinin Protease) occurs which facilitates the dissemination of the bacterial cells from the human intestinal cells.

Quorum Sensing in *Vibrio cholerae*

There are three QS systems in *Vibrio cholerae* which converge to regulate virulence identified till date. System I is the Cholera Autoinducer-1 (CAI-1)/CqsS system responsible for inter-vibrio and intra-species communication [2, 30, 31] System II—Autoinducer-2 (AI-2)/LuxP/Q responsible for inter species communication [2, 30, 31] and the System III – Unknown signals/VarS which is yet to be explored [32]. At LCD, both the enzymes CqsA and LuxS that synthesize CAI-1 & AI-2 [30, 33] produce them in a lower concentration. CqsS is the transmembrane receptor for CAI-1 and LuxP/Q is the periplasmic/transmembrane receptor for AI-2. These receptors are bifunctional i.e. at LCD they behave as kinases transferring the phosphate group to the downstream proteins and HCD they behave as phosphatases removing the phosphate groups from the

phosphorylated proteins. Lower concentration of these signals leads to an ineffective binding to their cognate receptors. Consequently, the receptors serve as kinases to mediate the phosphate flow a protein called LuxU and this, further transfers it to the regulator protein LuxO (Fig. 1). LuxO is the point of convergence of both the system I and II [2, 31, 34, 35]. System III converges with System I and System II at LuxO protein through activation of VarS/A pathway but the exact mechanism of the series of steps that occur to activate LuxO is unknown [2, 32]. Phosphorylated LuxO along with σ^{54} is in turn, a transcriptional activator of the Quorum regulatory RNAs (Qrr1-4). Qrr1-4 bound to the RNA chaperone Hfq, binds to mRNA transcript of HapR (the global virulence repressor protein in *Vibrio cholerae*) and renders the translation of HapR impossible [36].

HapR is a transcriptional repressor of VpsT, the latter is a transcriptional activator of genes required for biofilm formation [30, 37]. HapR also represses the production of AphA, a protein needed for activation of Cholera Toxin (CT) and Toxin Co-regulated Pilus (the main virulence factors of *Vibrio cholerae*). HapR represses haemolysin, the major virulence factor of *Vibrio cholerae* Biotype Eltor both transcriptionally and post-translationally [38]. HapR is also an activator of HapA protease and RNA polymerase sigma factor (rpoS σ^{54}). HapA protease serves as a "detachase" in detaching the *Vibrio cholerae* cells from the human cells, via digestion of GM-1 receptor of human intestinal cells, which serves as a bridge between bacterial and human cells [39]. σ^{54} increases the stress response of the bacterial cells to extreme nutrition and oxidative conditions [40]. Thus, under LCD conditions, virulence factors like Cholera toxin, Toxin co-regulated pilus, Haemolysin, Biofilm are expressed but factors like HapA protease, σ^{54} are repressed because of the absence of the regulatory protein of HapR. Hence, it could be inferred that "virulence and autoinducer concentration are inversely proportional" in case of *Vibrio cholerae*.

At HCD, the binding of the signals, CAI-1 and AI-2 to their respective cognate receptors CqsS and LuxP/Q will be achieved. This binding switches these receptors from functioning as kinase to phosphatase enzymes. Ultimately, dephosphorylation of LuxO protein, takes place which brings about repression in qrr1-4 production. Qrr1-4 repression subsequently leads to a successful translation of HapR mRNA transcript. As a result, virulence factors like haemolysin, Biofilm, Cholera Toxin, Toxin co-regulated pilus are repressed and HapA protease and σ54 are expressed at HCD conditions (Fig. 2). Thus, virulence is repressed at

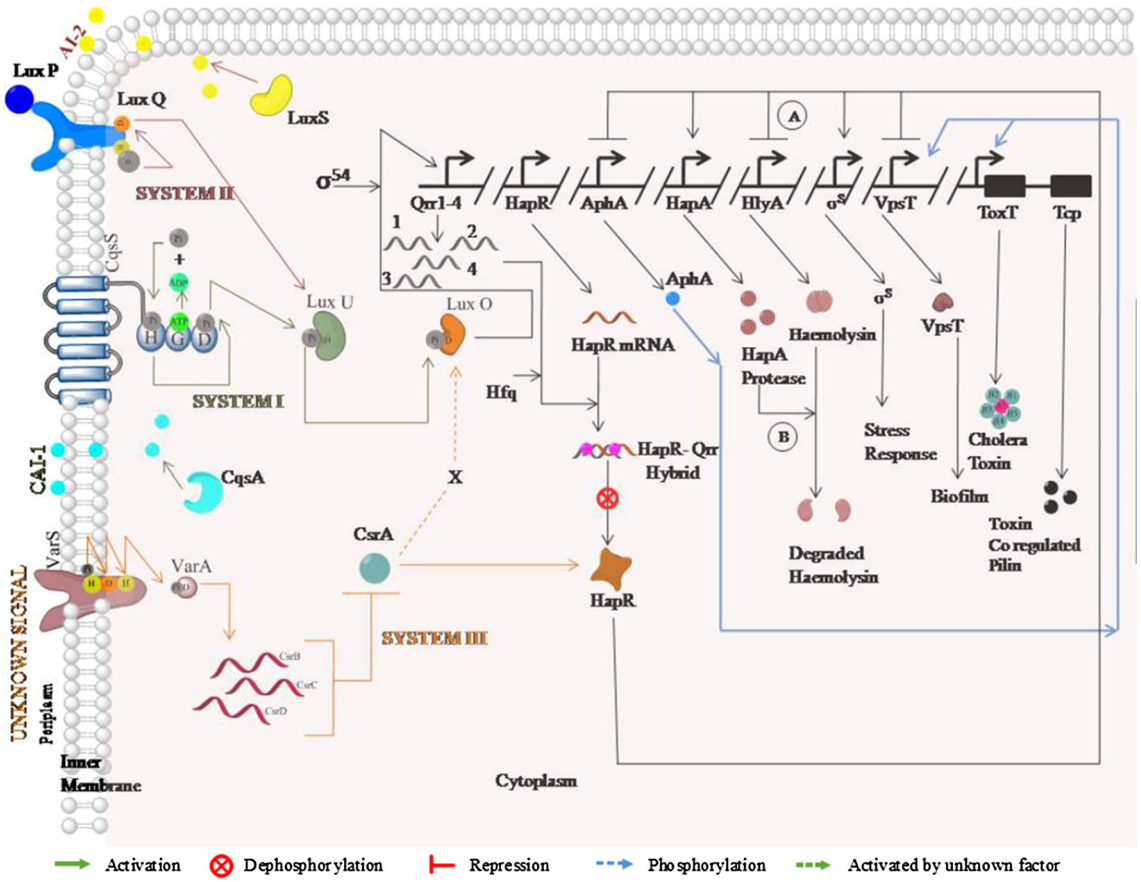


Fig. 1 *Vibrio cholerae* quorum sensing circuit at LCD state

HCD and established at LCD reflecting on the fleeting and ephemeral life style of *Vibrio cholerae* i.e. they have a short stay at intestinal environment completion of LCD gives them a positive signal to switch their life style in fecal environment and get disseminated from the intestine of host cells. Therefore, probing QS systems in *Vibrio cholerae* is an approach to arrive at novel anti-virulent drugs for an MDR scenario free Cholera therapy. So, throughout this review the plausible QS components (signals, receptors, sensors, regulators, regulatory RNAs) have been addressed that could be exploited to disrupt or attenuate the species specific and non-specific mediated quorum sensing as potential antimicrobial targets.

Plausible Targets

Signals/Autoinducers

CAI-1 and AI-2

The two autoinducers, cholera autoinducer-1 (CAI-1) and autoinducer-2 (AI-2) synergistically impose their control to their target gene expression. It is well documented that the species specific signal, CAI-1 is the stronger, whereas the LuxS is known to synthesize the weaker signal AI-2 (furanosyl borate diester (2*S*, 4*S*) -2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran borate). The catalytic process of LuxS involves the fragmentation of a byproduct of SAM metabolism, (S) -ribosyl-homocysteine (SRH), to produce homocysteine and the AI-2 precursor, 4,5-dihydroxy- 2,3-pentanedione (DPD). DPD spontaneously cyclizes and borate is chelated to yield AI-2, identified as (2S,4S)-2-methyl-2,3,4- tetrahydroxytetrahydrofuran-borate [41, 42]. The CAI-1 (Cholera Autoinducer-1), a stronger signal, was previously identified as (S)-3-hydroxytridecan-4-one known to require the enzyme CqsA for its biosynthesis [33, 43]. The sole substrate for CqsA substrates are (S)-2-aminobutyrate and decanoyl coenzyme A, and the product of the reaction is 3-aminotridecan-4-one, dubbed amino-CAI-1 is a pyridoxal phosphate-dependent acyl-CoA transferase reaction. Further, amino-CAI-1 is being converted to CAI-1 via a CqsA-independent mechanism and greater than or equal to100 times more CAI-1 release is observed than amino-CAI-1. *V. cholerae* QS response is being elicited on sensing the signals, amino-CAI-1 or CAI-

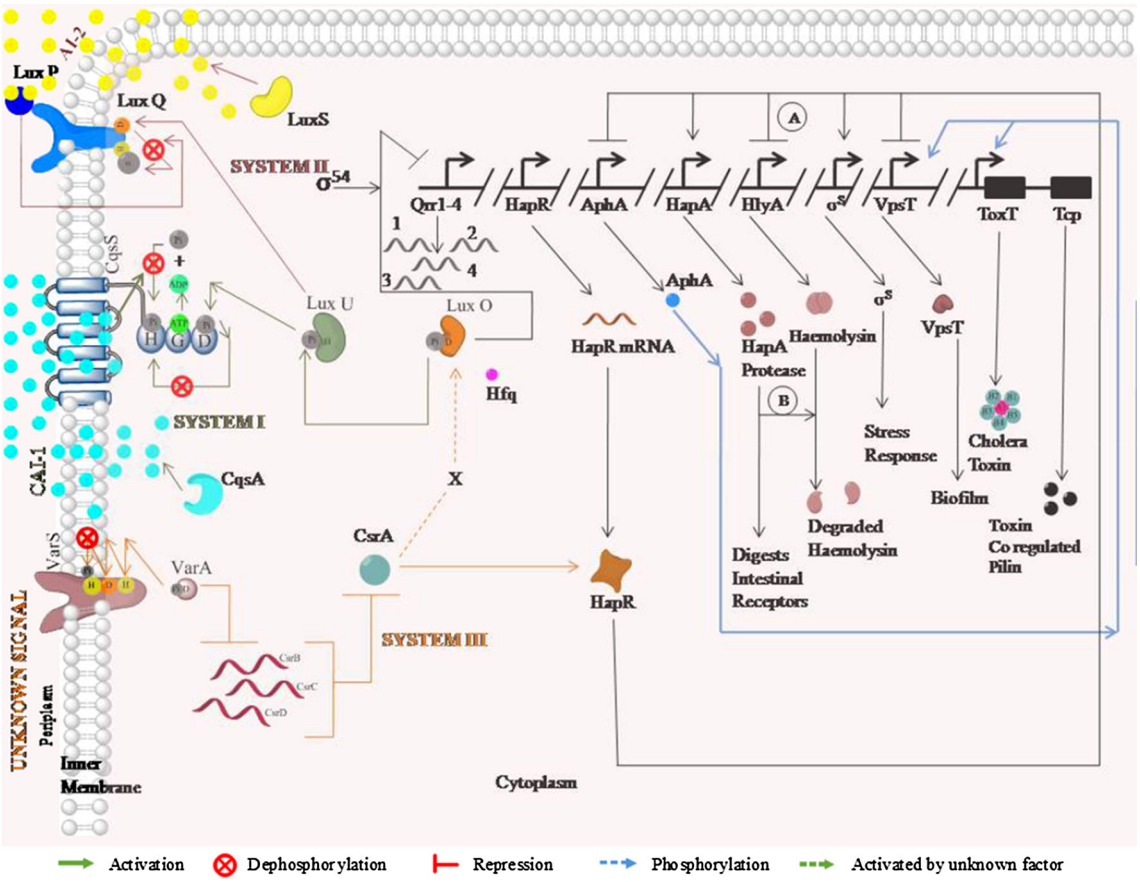
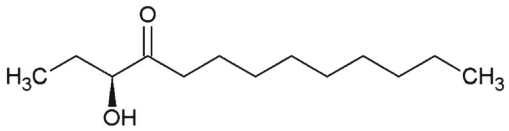


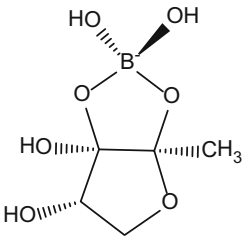
Fig. 2 *Vibrio cholerae* quorum sensing circuit at HCD state

1, but forseen to show any significant response to the CAI-1 variants [44]. In coherence to this, a commensal *E. coli* manipulated to express the cqsA gene that produced a variant CAI-1 inhibits pathogenesis of *V. cholerae* in an infant mouse model [45] provides an insight that targeting the stronger signal with its structural and functional mimic would probably lead to the development of an anticholera treatmnt.



CAI-1

AI-2



Receptor

CqsS

Bacteria are ubiquitous with Two-component systems (TCS) systems that are used to adapt to environmental changes [46]. CqsS, a unique TCS system in *Vibrio cholerae* (CqsSvc) known to be highly conserved (70 %) in the transmembrane ligand sensing domains among all Vibrio species as it detects CAI-1 molecules with the extended side chains either with 10-carbon or 8-carbon [47]. The residues Cys^{170}/Phe^{175} was found to be crucial in the CqsSvc as it signifies the ligand chain length [44]. The CqsSvc Sensor histidine kinases has a *N*-terminal transmembrane sensing domains, dimerization histidine phosphotransfer (DHp) domains and C-terminal catalytic ATP-binding (CA) domains [48]. It has been theoretically defined to have two-state model for histidine kinases with a "kinase on" and a "kinase off" mechanism [49]. In response to the CAI-1 (HCD), the binding switches the CqsS to an "kinase off" state (phosphotase) and results in the dephosphorylation of phosphorylated response regulators (LuxU ~ P; LuxO ~ P). Significantly at LCD condition, the switch is reversed to "kinase off" state, where the His194 is critical for the phosphate flow to the DHp domain as it is evidenced that the H194Q do affect the phosphate flow. Also mutation in the highly conserved glycine residue CqsG379A/G381A of the CA domain was also found to be incapable of autophosphorylation are known to be critical for ATP binding. In accordance to this, researchers have also evidenced to hinder the QS system on targeting those key residues of the CqsS receptor that affect the phosphate flow [50].

LuxPQ

LuxP, a factor belonging to the large protein family PBPs (Periplasmic Binding Protein) sense the weaker non species specific autoinducer, AI-2 to elicit its QS response. [51]. Notably, it interacts with AI-2 molecules in conjugation with a two-component sensor kinase, LuxQ with a hybrid of a periplasmic sensor domain and cytoplasmic histidine domains [52]. Irrespective of LuxP interaction with AI-2 or not, the unliganded apoform hold open to have specific interaction through the PAS domain with the LuxQ (LuxQp) [53]. The PAS domain is quite similar to that of PBPs protein and the LuxP will bind to the tandem PAS folds present in the LuxQ. These PAS folds have prosthetic qroups that help them to bind to ligand molecules [54]. Therefore, the ligand AI-2 interaction regulates LuxQ activity on bringing structural changes in the constitutively associated LuxP: LuxQ complex. Notably, at LCD state, the intrinsic kinase activity of LuxQ would cross phosphorylate the histidine residues within the histidine kinase domain [55] and at HCD state, a retrograde flow of phosphate group. It is also known, that the LuxQ intrinsic activity of kinase is being reduced/converted to phosphatase with AI-2 interaction via integrating the signal to the regulator, LuxR [56].

Regulators

HapR

The master regulator, HapR belong to the family members, TetR and QacR with nine α helices [57]. The C-terminal dimerization interface mediates its contact with its target DNA binding site, aphA promoter. The HapR DNA-binding domain at the N-terminal region is composed of three α

helices, α 1, α 2 and α 3 and a dominant electropositive surface was noticed in the HTH motif (helices α 2 and α 3). The dimerization interface promote the HTH motif to be competent to interact to its DNA binding domain without making any significant conformational change in the kink in the α 7 helices as it results with an extended α 5 helix. The residues surrounding helix α 3, (Ser50, Val51, Ala52, Thr53, Phe55, and Asn56) were found to show either polar or hydrophobic base-specific interactions in the major groove of DNA. Also, these residues Arg10, Arg12, Arg18, Lys19, Arg33, Arg37, His40, and Arg61 was found to show significant interaction with the phosphate backbone to provide additional stability to the regulator binding to DNA. The mutational studies (F55A- defective; T53A–weak) have revealed to show higher DNA binding affinity (base-specific interaction) lies in the α 3 (Thr 53 and Phe55) to efficiently bind to aphA promoter to repress biofilm phenotype [58]. Also, HapR molecules has tunnel (C-Terminal) to help the movement of the solvent molecules that contacts an binding pocket (amphipathic cavity) for an unidentified ligand similarly present in the TetR protein molecules. The cAMP- receptor protein (CRP) was found to lower the *Hap*R expression level via integrated with the environmental signals to enhance the growth of organisms in both the human host and the environment [59].

LuxU

LuxU a Phosphorelay protein has two independent functional domains to receive sensory signals (phosphate receiver domain) from a sensory kinase (phosphate donor domain) and integrate the signals to LuxO [60]. At LCD state, kinase switch on and phosphate flow from the sensory kinase, is being transferred to response regulatory protein, named LuxO and it is reversed at HCD state [61]. LuxU with an aminoacid sequence of length 133a.a show greater sequence similarity to other phosphorelay proteins, (HPt), BvgS, ArcB, and Ypd. Its phosphorelay function is highly defined by a conserved His residue (His 58) and it is consistent with the surrounded positively charged residues (Lys54 and Lys61) provides an ultimate binding site for the negatively charged phosphate [62]. We also speculate LuxU, a hub for signal integration as *V. cholerae* QS relies on two parallel sensory pathways, CAI-1/CqsS and AI-2/LuxPQ where the output converges at LuxU [2]. Similar sensory mechanism has been revealed in the related bacterium *V. harveyi*, where the information flow from three parallel sensors, CqsS, LuxPQ and LuxN converges at LuxU [63]. The *Vibrio* QS pathway ensures to have a central domain architecture (LuxU) that means to perform the enzymatic activities required for phosphotransfer to and dephosphorylation of its target proteins. Also LuxU mediated phospho-flow in both directions is well documented as a feed-back regulation [64].

LuxO

LuxO, a NtrC family regulator act as a genetic switch to influence the biofilm formation, toxin production, phosphorylation and bioluminescence in Vibrio species [31]. The LuxO has two separate N-terminal regulatory and C-terminal DNA binding domain where the oligomerization or AAA^+ ATPase domain is located between the receiver domain and a helix turn helix (HTH) motif [31, 65, 66]. The HTH motif are known to recognize enhancer like sequence between 100 and 150 base pairs present in upstream of the promoter of the target genes. ATPase domain with its walker A and B motif and Arginine finger motif at active state will recruit the core RNA polymerase with the sigma factor (σ) to promote the transition from closed to open complex. The Walker A motif has consensus sequence GXXXGXGX (X means any amino acid) shows higher affinity of interaction with the target DNA binding site and the magnesium ion present in B motif coordinates the stability of this complex. The key residues of LuxO that increases its stability to its DNA binding site are four aliphatic amino acid residues and two negatively charged residues [62]. Also, the Arginine finger motif with the conserved Arg residues probably found to aid ATP binding and hydrolysis as it is required to remodel the closed DNA complex. The critical role of LuxO includes to accept phosphate group then activated to bind to its target site in DNA enhanced by Fis and with the hydrolysis of ATP [53]. Its efficiency of activation/deactivation entirely depends on the concentration of the signal, AI-2 and CAI-2 as it is directly proportional to the cell density state. So, LuxO is activated in the LCD state and vice versa in the HCD state. Recent studies have also shown to characterize novel inhibitors (ML366 and 5-thio-6-azauracil derivatives) to selectively inhibit the ATPase domain of LuxO (response regulatory protein) and reverse the LCD to a HCD condition [31, 67].

Qrrl

Qrrls are the small regulatory RNAs that control the transcription of target genes involved in establishing *Vibrio cholera*e pathogenicity. The cells at LCD state, the signals (AI-2) integrate to various regulators like, LuxO enhances the expression of the small regulatory RNAs (Qrrl), likely to antisense with the HapR mRNA most probably protecting its 5′UTR- ribosome binding site (RBS) from preventing its initiation of translation and also repress its action of regulating the target gene expression. It has also been observed that the

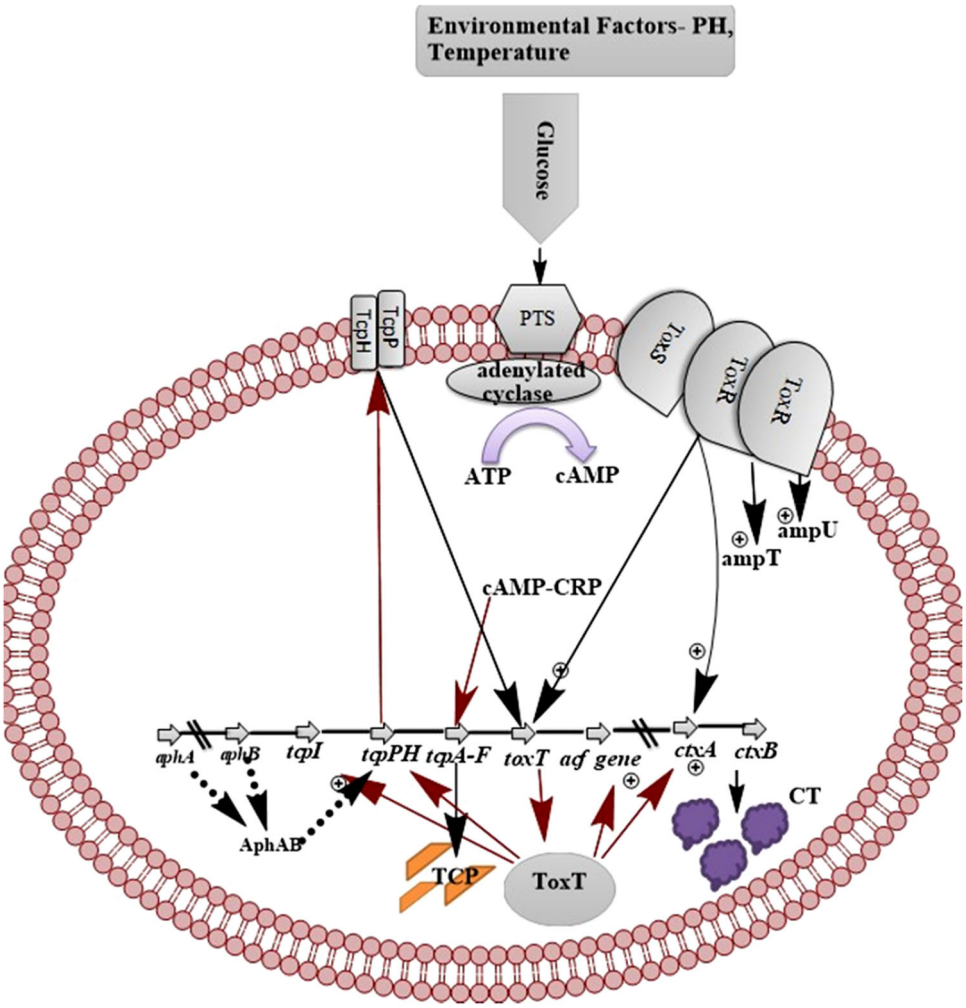


Fig. 3 *Vibrio cholerae* quorum sensing circuit response to environmental cues

Qrrls shows negative feedback regulation of repressing the translation of LuxO protein and their interaction to its target mRNA are being stabilized by an RNA chaperone, Hfq [68, 69]. The Qrrl protection of its target mRNA would act as signal sequence to be either degraded or to prevent them from translation. Base pairing of Qrrl RNA to its target mRNA is aided with the first two stem loops (S_1) and (S_2) the third stem loop (S_3) stabilizes the complex and the fourth stem loop (S_4) act as terminator probably it means to provide an intrinsic strength to respond to the QS response [70].

ToxR

Vibrio cholerae respond to environmental factors as it triggers the expression of membrane bound regulatory protein, ToxR and elicit QS response. The regulator, ToxR has two separate N-terminal cytoplamic domain (binds to DNA molecules) and C- terminal periplasmic domain (interact with transmembrane protein) [71]. The role of ToxS protein is very essential for the activation of *ToxR* gene, although its functional significance is not clear other than it is known to stabilize and dimerise the Tox R protein [71, 72]. Combined effect of ToxR-ToxS complex is well known to activate *OmpU*, *OmpT* and porins molecules [73]. The cells should have an equal balance of porin molecules to resist against bile and intestinal colonization. The main target of *ToxR-ToxS* is the *ToxT,* as it is triggered with the factor complex, TcpP-TcpH that result in the CT toxin. It has been well documented that the factor complex, TcpP-TcpH is regulated by the *apha* gene at the LCD state. The *ToxR* (control of virulence) gene expression has been related to have influenced with the environmental cues where the cAMP-CRP system was found to be critical in its regulation. When the concentration of cAMP increases, the cAMP-CRP complex is formed and thus repress the expression of the gene, *tcpPH* as both *CRP* and *AphAB* binding site overlap at *tcpPH* promoter [59]. Since the global regulator, cAMP-CRP exhibit combinatorial control and its influence over ToxR regulon under various environmental condition provides an insight to explore the involvement of multiple overlapping systems on its control (Fig. 3).

Conclusion

The review has established that the signals AI-2/CAI-1/environmental cues elicit QS response via QS molecules (regulators, small regulatory RNA etc.) to establish *Vibrio cholerae* pathogenicity. Notably, exploiting QS molecules might be a novel strategy to discover novel anti-infectives or anti-virulent molecules for cholera therapy. Earlier reports accounted to use similar approach for the development of anti-pathogenic compounds from *Terminalia chebula* Retz to quench AHL mediated QS in *Pseudomonas aeruginosa* PAO1 and sponge derived compounds against *Serratia marcescens* [74, 75]. Our earlier studies have identified CAI-1 mimic molecules from *Melia dubia* leaves and its potency to establish QS in *Vibrio cholerae* has opened the roads to a new anti-virulent Cholera therapy employing QS mimic molecules [76]. We could also prove that the methanol extract of *Melia dubia* seed will competitively inhibit SdiA (quorum regulator) in *E.coli* thus inhibits the hemolysin, biofilm production and motility of the bacteria [77]. Any QS based anti-virulent therapy aims at reversal of the CqsS and LuxP/Q molecular switch from kinase to phosphatase i.e. mimics HCD conditions in an LCD state. This anti-virulent strategy is highly efficacious since it completely eliminates the possibility of development of MDR strains, making the therapy effective and also eliminates the subsequent need for strain tracking. Hence, interfering with *Vibrio cholerae* murmurs is a way employed currently to combat Cholera and recover Cholera victims.

References

1. Lippi D, Gotuzzo E (2014) The greatest steps towards the discovery of *Vibrio cholerae*. Clin Microbiol Infec 20:191–195. doi:10.1111/1469-0691.12390
2. Miller MB, Skorupski K, Lenz DH, Taylor RK, Bassler BL (2002) Parallel quorum sensing systems converge to regulate virulence in *Vibrio cholerae*. Cell 110:303–314
3. Shapiro RL, Otieno MR, Adcock PM, Phillips-Howard PA, Hawley WA, Kumar L, Waiyaki P, Nahlen BL, Slutsker L (1999) Transmission of epidemic *Vibrio cholerae* O1 in rural western Kenya associated with drinking water from Lake Victoria: an environmental reservoir for cholera? Am J Trop Med Hyg 60:271–276
4. Organisation WH (2011) Weekly epidemiological record—Cholera 2010, vol 31
5. Organisation WH (2012) Weekly epidemiological record—Cholera 2011, vol 87
6. Smith GD (2002) Commentary: behind the broad street pump: aetiology, epidemiology and prevention of cholera in mid-19th century Britain. Int J Epidemiol 31:920–932. doi:10.1093/ije/31.5.920
7. Kanungo SSB, Lopez AL, Sung JS, Paisley AM, Sur D, Clemens JD, Balakrish Nair G (2010) Cholera in India: an analysis of reports. Bull World Health Organ 88:185–191. doi:10.2471/BLT.09.073460
8. UNICEF technical bulletin no. 9: new formulation of oral rehydration salts (ORS) with reduced osmolarity
9. Faruque AS, Alam K, Malek MA, Khan MG, Ahmed S, Saha D, Khan WA, Nair GB, Salam MA, Luby SP (2007) Emergence of multidrug-resistant strain of *Vibrio cholerae O1* in Bangladesh and reversal of their susceptibility to tetracycline after two years. J Health Popul Nutr 25:241
10. Khan W, Bennish ML, Seas C, Khan EH, Ronan A, Dhar U, Busch W, Salam MA (1996) Randomised controlled comparison of single-dose ciprofloxacin and doxycycline for cholera caused by *Vibrio cholerae* O1 or O139. Lancet 348:296–300. doi:10.1016/S0140-6736(96)01180-4
11. Saha D, Karim MM, Khan WA, Ahmed S, Salam MA, Bennish ML (2006) Single-dose azithromycin for the treatment of cholera in adults. New Engl J Med 354:2452–2462. doi:10.1056/NEJMoa054493
12. Khan WA, Saha D, Rahman A, Salam MA, Bogaerts J, Bennish ML (2002) Comparison of single-dose azithromycin and 12-dose, 3-day erythromycin for childhood cholera: a randomised, double-blind trial. Lancet 360:1722–1727. doi:10.1016/S0140-6736(02)11680-1
13. Kalia VC, Wood TK, Kumar P (2013) Evolution of resistance to quorum-sensing inhibitors. Microb Ecol 68:13–23. doi:10.1007/s00248-013-0316-y
14. Chakraborty S, Garg P, Ramamurthy T, Thungapathra M, Gautam J, Kumar C, Maiti S, Yamasaki S, Shimada T, Takeda Y (2001) Comparison of antibiogram, virulence genes, ribotypes and DNA fingerprints of *Vibrio cholerae* of matching serogroups isolated from hospitalised diarrhoea cases and from the environment during 1997–1998 in Calcutta, India. Indian J Med Microbiol 50:879–888
15. Garg P, Chakraborty S, Basu I, Datta S, Rajendran K, Bhattacharya T, Yamasaki S, Bhattacharya S, Takeda Y, Balakrish Nair G (2000) Expanding multiple antibiotic resistance among clinical strains of *Vibrio cholerae* isolated from 1992–1997 in Calcutta, India. Epidemiol Infect 124:393–399
16. Spicknall IH, Foxman B, Marrs CF, Eisenberg JN (2013) A modeling framework for the evolution and spread of antibiotic resistance: literature review and model categorization. Am J Epidemiol 178:508–520. doi:10.1093/aje/kwt017
17. Kalia VC, Purohit HJ (2011) Quenching the quorum sensing system: potential antibacterial drug targets. Crit Rev Microbiol 37:121–140. doi:10.3109/1040841X.2010.532479
18. Kalia VC (2013) Quorum sensing inhibitors: an overview. Biotechnol Adv 31:224–245. doi:10.1016/j.biotechadv.2012.10.004
19. Kalia V (2014) Microbes, antimicrobials and resistance: the battle goes on. Indian J Microbiol 54:1–2. doi:10.1007/s12088-013-0443-7
20. Kumar P, Patel SK, Lee J-K, Kalia VC (2013) Extending the limits of *Bacillus* for novel biotechnological applications. Biotechnol Adv 31:1543–1561. doi:10.1016/j.biotechadv.08.007
21. Kalia V, Kumar P, Pandian S, Sharma P (2015) Biofouling control by quorum quenching. Hb25_Springer Handbook of Marine Biotechnology, pp 431–440
22. Kalia VCRS, Purohit HJ (2011) Genomic analysis reveals versatile organisms for quorum quenching enzymes: acyl-homoserine lactone-acylase and -lactonase. Open Microbiol J 5:1–13. doi:10.2174/1874285801105010001
23. Kumar P, Koul S, Patel SK, Lee J-K, Kalia VC (2015) Heterologous expression of quorum sensing inhibitory genes in diverse organisms. In: Quorum sensing vs quorum quenching: a battle with no end in sight. Springer, New York, pp 343–356. doi:10.1007/978-81-322-1982-8_28
24. Kalia VC, Kumar P (2015) Potential applications of quorum sensing inhibitors in diverse fields. In: Quorum sensing vs

quorum quenching: a battle with no end in sight. Springer, New York, pp 359–370. doi:10.1007/978-81-322-1982-8_29
25. Dunny GM, Leonard BA (1997) Cell-cell communication in gram-positive bacteria. Ann Rev Microbiol 51:527–564. doi:10.1146/annurev.micro.51.1.527
26. Bassler BL (2002) Small talk: cell-to-cell communication in bacteria. Cell 109:421–424. doi:10.1016/S0092-8674(02)00749-3
27. Nusrat H, Kushwah J, Joshi J, Raju SC, Kalia VC (2011) Diversity and polymorphism in AHL-lactonase gene (aiiA) of *Bacillus*. J Microbiol Biotechnol 21:1001–1011. doi:10.4014/jmb.1105.05056
28. Kalia VC (2014) In search of versatile organisms for quorum-sensing inhibitors: acyl homoserine lactones (AHL)-acylase and AHL-lactonase. FEMS Microbiol Letts 359:143. doi:10.1111/1574-6968.12585
29. Kalia VC, Kumar P (2015) The battle: quorum-sensing inhibitors versus evolution of bacterial resistance. In: Quorum sensing vs quorum quenching: a battle with no end in sight. Springer, New York, pp 385–391. doi:10.1007/978-81-322-1982-8_31
30. Hammer BK, Bassler BL (2003) Quorum sensing controls biofilm formation in *Vibrio cholerae*. Mol Microbiol 50:101–104. doi:10.1046/j.1365-2958.2003.03688.x
31. Ng W-L, Perez L, Cong J, Semmelhack MF, Bassler BL (2012) Broad spectrum pro-quorum-sensing molecules as inhibitors of virulence in Vibrios. PLoS Pathog 8:e1002767. doi:10.1371/journal.ppat.1002767
32. Lenz DH, Miller MB, Zhu J, Kulkarni RV, Bassler BL (2005) CsrA and three redundant small RNAs regulate quorum sensing in *Vibrio cholerae*. Mol Microbiol 58:1186–1202. doi:10.1111/j.1365-2958.2005.04902.x
33. Kelly RC, Bolitho ME, Higgins DA, Lu W, Ng W-L, Jeffrey PD, Rabinowitz JD, Semmelhack MF, Hughson FM, Bassler BL (2009) The *Vibrio cholerae* quorum-sensing autoinducer CAI-1: analysis of the biosynthetic enzyme CqsA. Nat Chem Biol 5:891–895. doi:10.1038/nchembio.237
34. Ng W-L, Wei Y, Perez LJ, Cong J, Long T, Koch M, Semmelhack MF, Wingreen NS, Bassler BL (2010) Probing bacterial transmembrane histidine kinase receptor–ligand interactions with natural and synthetic molecules. Proc Natl Acad Sci 107:5575–5580. doi:10.1073/pnas.1001392107
35. Wei Y, Ng WL, Cong J, Bassler BL (2012) Ligand and antagonist driven regulation of the Vibrio cholerae quorum-sensing receptor CqsS. Mol Microbiol 83:1095–1108. doi:10.1111/j.1365-2958.2012.07992.x
36. Lenz DH, Mok KC, Lilley BN, Kulkarni RV, Wingreen NS, Bassler BL (2004) The small RNA chaperone Hfq and multiple small RNAs control quorum sensing in *Vibrio harveyi* and *Vibrio cholerae*. Cell 118:69–82. doi:10.1016/j.cell.2004.06.009
37. Waters CM, Lu W, Rabinowitz JD, Bassler BL (2008) Quorum sensing controls biofilm formation in *Vibrio cholerae* through modulation of cyclic di-GMP levels and repression of vpsT. J Bacteriol 190:2527–2536. doi:10.1128/JB.01756-07
38. Tsou AM, Zhu J (2010) Quorum sensing negatively regulates hemolysin transcriptionally and posttranslationally in *Vibrio cholerae*. Infect Immun 78:461–467. doi:10.1128/IAI.00590-09
39. Finkelstein RA, Boesman-Finkelstein M, Chang Y, Häse C (1992) *Vibrio cholerae* hemagglutinin/protease, colonial variation, virulence, and detachment. Infect Immun 60:472–478
40. Joelsson A, Kan B, Zhu J (2007) Quorum sensing enhances the stress response in *Vibrio cholerae*. Appl Environ Microbol 73:3742–3746. doi:10.1128/AEM.02804-06
41. Schauder S, Shokat K, Surette MG, Bassler BL (2001) The LuxS family of bacterial autoinducers: biosynthesis of a novel quorum-sensing signal molecule. Mol Microbiol 41:463–476. doi:10.1046/j.1365-2958.2001.02532.x
42. Chen X, Schauder S, Potier N, Van Dorsselaer A, Pelczer I, Bassler BL, Hughson FM (2002) Structural identification of a bacterial quorum-sensing signal containing boron. Nature 415:545–549. doi:10.1038/415545a
43. Jahan N, Potter JA, Sheikh MA, Botting CH, Shirran SL, Westwood NJ, Taylor GL (2009) Insights into the biosynthesis of the *Vibrio cholerae* major autoinducer CAI-1 from the crystal structure of the PLP-dependent enzyme CqsA. J Mol Biol 392:763–773. doi:10.1016/j.jmb.2009.07.042
44. Ng WL, Perez LJ, Wei Y, Kraml C, Semmelhack MF, Bassler BL (2011) Signal production and detection specificity in Vibrio CqsA/CqsS quorum-sensing systems. Mol Microbiol 79:1407–1417. doi:10.1111/j.1365-2958.2011.07548.x
45. Duan F, March JC (2008) Interrupting *Vibrio cholerae* infection of human epithelial cells with engineered commensal bacterial signaling. Biotechnol Bioeng 101:128–134. doi:10.1002/bit.21897
46. Krell T, Lacal J, Busch A, Silva-Jiménez H, Guazzaroni M-E, Ramos JL (2010) Bacterial sensor kinases: diversity in the recognition of environmental signals. Ann Rev Microbiol 64:539–559. doi:10.1146/annurev.micro.112408.134054
47. Casino P, Rubio V, Marina A (2010) The mechanism of signal transduction by two-component systems. Curr Opin Struct Biol 20:763–771. doi:10.1016/j.sbi.2010.09.010
48. Dutta R, Qin L, Inouye M (1999) Histidine kinases: diversity of domain organization. Mol Microbiol 34:633–640. doi:10.1046/j.1365-2958.1999.01646.x
49. Swem LR, Swem DL, Wingreen NS, Bassler BL (2008) Deducing receptor signaling parameters from in vivo Analysis: LuxN/AI-1 quorum Sensing in *Vibrio harveyi*. Cell 134:461–473. doi:10.1016/j.cell.2008.06.023
50. Tyagi V (2011) Modification of *Vibrio cholerae* quorum sensing receptors. Princeton University (Ph.D Dissertation)
51. Quiocho FA, Ledvina PS (1996) Atomic structure and specificity of bacterial periplasmic receptors for active transport and chemotaxis: variation of common themes. Mol Microbiol 20:17–25. doi:10.1111/j.1365-2958.1996.tb02484.x
52. Dwyer MA, Hellinga HW (2004) Periplasmic binding proteins: a versatile superfamily for protein engineering. Curr Opin Struct Biol 14:495–504. doi:10.1016/j.sbi.2004.07.004
53. Freeman JA, Lilley BN, Bassler BL (2000) A genetic analysis of the functions of LuxN: a two-component hybrid sensor kinase that regulates quorum sensing in Vibrio harveyi. Mol Microbiol 35:139–149. doi:10.1046/j.1365-2958.2000.01684.x
54. Taylor BL, Zhulin IB (1999) PAS domains: internal sensors of oxygen, redox potential, and light. Microbiol Mol Biol Rev 63:479–506
55. Freeman JA, Bassler BL (1999) A genetic analysis of the function of LuxO, a two-component response regulator involved in quorum sensing in Vibrio harveyi. Mol Microbiol 31:665–677. doi:10.1046/j.1365-2958.1999.01208.x
56. Neiditch MB, Federle MJ, Miller ST, Bassler BL, Hughson FM (2005) Regulation of LuxPQ receptor activity by the quorum-sensing signal autoinducer-2. Mol Cell 18:507–518. doi:10.1016/j.molcel.2005.04.020
57. Tsou AM, Cai T, Liu Z, Zhu J, Kulkarni RV (2009) Regulatory targets of quorum sensing in *Vibrio cholerae*: evidence for two distinct HapR-binding motifs. Nucleic Acids Res 37:2747–2756. doi:10.1093/nar/gkp121
58. De Silva RS, Kovacikova G, Lin W, Taylor RK, Skorupski K, Kull FJ (2007) Crystal structure of the *Vibrio cholerae* quorum-sensing regulatory protein HapR. J Bacteriol 189:5683–5691. doi:10.1128/JB.01807-06
59. Liang W, Pascual-Montano A, Silva AJ, Benitez JA (2007) The cyclic AMP receptor protein modulates quorum sensing, motility and multiple genes that affect intestinal colonization in *Vibrio*

cholerae. Micbiology 153:2964–2975. doi:10.1099/mic.0.2007/006668-0
60. Freeman JA, Bassler BL (1999) Sequence and function of LuxU: a Two-component phosphorelay protein that regulates quorum sensing in *Vibrio harveyi*. J Bacteriol 181:899–906
61. Ulrich DL, Kojetin D, Bassler BL, Cavanagh J, Loria JP (2005) Solution structure and dynamics of LuxU from *Vibrio harveyi*, a phosphotransferase protein involved in bacterial quorum sensing. J Mol Biol 347:297–307. doi:10.1016/j.jmb.2005.01.039
62. Fazil MHUT, Kumar S, Rao NS, Selvaraj C, Singh SK, Pandey HP, Singh DV (2012) Comparative structural analysis of two proteins belonging to quorum sensing system in *Vibrio cholerae*. J Biomol Struct Dyn 30:574–584. doi:10.1080/07391102.2012.687523
63. Henke JM, Bassler BL (2004) Three parallel quorum-sensing systems regulate gene expression in *Vibrio harveyi*. J Bacteriol 186:6902–6914. doi:10.1128/JB.186.20.6902-6914.2004
64. Taga ME, Semmelhack JL, Bassler BL (2001) The LuxS-dependent autoinducer AI-2 controls the expression of an ABC transporter that functions in AI-2 uptake in Salmonella typhimurium. Mol Microbiol 42:777–793. doi:10.1046/j.1365-2958.2001.02669.x
65. Sallai L, Tucker PA (2005) Crystal structure of the central and C-terminal domain of the sigma 54-activator ZraR. J Struct Biol 151:160–170
66. Lee S-Y, De La Torre A, Yan D, Kustu S, Nixon BT, Wemmer DE (2003) Regulation of the transcriptional activator NtrC1: structural studies of the regulatory and AAA+ ATPase domains. Genes Dev 17:2552–2563
67. Faloon P, Weiner WS, Matharu DS, Neuenswander B, Porubsky P, Youngsaye W, Bennion M, Ng W-L, Hurley A, Mosher CM (2014) Discovery of ML 370, an inhibitor of vibrio cholerae quorum sensing acting via the LuxO response regulator. Probe reports from the NIH Molecular libraries program [Internet]
68. Hussein R, Lim HN (2011) Disruption of small RNA signaling caused by competition for Hfq. Proc Natl Acad Sci 108:1110–1115. doi:10.1073/pnas.1010082108
69. Svenningsen SL, Waters CM, Bassler BL (2008) A negative feedback loop involving small RNAs accelerates *Vibrio cholerae*'s transition out of quorum-sensing mode. Genes Dev 22:226–238. doi:10.1101/gad.1629908
70. Stauffer LT, Stauffer GV (2013) Multiple roles for the sRNA GcvB in the regulation of Slp levels in *Escherichia coli*. ISRN Bacteriol 2013. doi:10.1155/2013/918106
71. DiRita VJ, Mekalanos JJ (1991) Periplasmic interaction between two membrane regulatory proteins, ToxR and ToxS, results in signal transduction and transcriptional activation. Cell 64:29–37. doi:10.1016/0092-8674(91)90206-E
72. Kolmar H, Hennecke F, Götze K, Janzer B, Vogt B, Mayer F, Fritz H (1995) Membrane insertion of the bacterial signal transduction protein ToxR and requirements of transcription activation studied by modular replacement of different protein substructures. EMBO J 14:3895
73. Sperandio V, Giron JA, Silveira WD, Kaper JB (1995) The OmpU outer membrane protein, a potential adherence factor of *Vibrio cholerae*. Infect Immun 63:4433–4438
74. Sarabhai S, Sharma P, Capalash N (2013) Ellagic acid derivatives from Terminalia chebula Retz. downregulate the expression of quorum sensing genes to attenuate *Pseudomonas aeruginosa* PAO1 virulence. PLoS ONE 8:e53441. doi:10.1371/journal.pone.0053441
75. Annapoorani A, Jabbar AKKA, Musthafa SKS, Pandian SK, Ravi AV (2012) Inhibition of quorum sensing mediated virulence factors production in urinary pathogen *Serratia marcescens* PS1 by marine sponges. Indian J Microbiol 52:160–166. doi:10.1007/s12088-012-0272-0
76. Srikkanth B, Hema M, Santhosh RS, Shanmugam K, Princy SA (2014) Molecular characterization of cholera autoinducer-1 (CAI-1) mimic as a potent CqsS receptor agonist. Biotechnol Indian J 9:56–66
77. Ravichandiran V, Shanmugam K, Anupama K, Thomas S, Princy A (2012) Structure-based virtual screening for plant-derived SdiA-selective ligands as potential antivirulent agents against uropathogenic *Escherichia coli*. Eur J Med Chem 48:200–205. doi:10.1016/j.ejmech.2011.12.015