World Journal of Clinical Cases

Submit a Manuscript: http://www.wjgnet.com/esps/
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
DOI: 10.12998/wjcc.v3.i7.575

World J Clin Cases 2015 July 16; 3(7): 575-598
ISSN 2307-8960 (online)

REVIEW

Role of quorum sensing in bacterial infections

Israel Castillo-Juárez, Toshinari Maeda, Edna Ayerim Mandujano-Tinoco, María Tomás, Berenice Pérez-Eretza, Silvia Julieta García-Contreras, Thomas K Wood, Rodolfo García-Contreras

Israel Castillo-Juárez, Colegio de Postgraduados, Campus Montecillo, Texcoco 56230, México

Toshinari Maeda, Department of Biological Functions and Engineering, Kyushu Institute of Technology, Kitakyushu 808-0196, Japan

Edna Ayerim Mandujano-Tinoco, Epigenetics of Cancer Laboratory, Division of Basic Research, National Institute of Genomic Medicine, Tlalpan 14610, Mexico

María Tomás, Department of Microbiology, Instituto de Investigación Biomédica de A Coruña (INIBIC), Complexo Hospitalario Universitario de A Coruña (CHUAC), Sergas, Universidade da Coruña (UDC), 15006 A Coruña, Spain

Berenice Pérez-Eretza, Rodolfo García-Contreras, Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, México DF 04510, Mexico

Silvia Julieta García-Contreras, Centro Médico Coyoacán, Coyoacán CP 04890, México

Thomas K Wood, Department of Chemical Engineering, Pennsylvania State University, University Park, PA 16802-440, United States

Thomas K Wood, Department of Biochemistry and Molecular Biology, Pennsylvania State University, University Park, PA 16802-440, United States

Author contributions: All authors designed the review and wrote the paper.

Supported by JSPS grant Challenging Exploratory Research, No. 25660062; SEP-CONACyT grant, No. 152794 (García-Contreras R); Fideicomiso COLPOS 167304 and Programa Cátedras-CONACyT 2112 (Castillo-Juárez); the Miguel Servet Program (C.H.U.A Coruña and ISCIII) (Tomás M); the CONACyT grant number 441393/269132 (Mandujano-Tinoco EA); and the Biotechnology Endowed Chair at the Pennsylvania State University.

Conflict-of-interest statement: The authors declare no conflict of interest.

Correspondence to: Rodolfo García-Contreras, Associate Professor, Departamento de Microbiología y Parasitología, Facultad de Medicina, Universidad Nacional Autónoma de México, Av Universidad 3000, Coyoacán, México DF 04510, Mexico. rgarc@bq.unam.mx
Telephone: +52-55-22514217
Fax: +52-55-56232468

Received: October 9, 2014
Peer-review started: October 13, 2014
First decision: November 14, 2014
Revised: November 30, 2014
Accepted: April 16, 2015
Article in press: April 20, 2015
Published online: July 16, 2015

Abstract

Quorum sensing (QS) is cell communication that is widely used by bacterial pathogens to coordinate the expression of several collective traits, including the production of multiple virulence factors, biofilm formation, and swarming motility once a population threshold is reached. Several lines of evidence indicate that QS enhances virulence of bacterial pathogens in animal models as well as in human infections; however, its relative importance for bacterial pathogenesis is still incomplete. In this review, we discuss the present evidence from *in vitro* and *in vivo* experiments in animal models, as well as from clinical studies, that link QS

systems with human infections. We focus on two major QS bacterial models, the opportunistic Gram negative bacteria *Pseudomonas aeruginosa* and the Gram positive *Staphylococcus aureus*, which are also two of the main agents responsible of nosocomial and wound infections. In addition, QS communication systems in other bacterial, eukaryotic pathogens, and even immune and cancer cells are also reviewed, and finally, the new approaches proposed to combat bacterial infections by the attenuation of their QS communication systems and virulence are also discussed.

Key words: Quorum sensing; Virulence; Infections; *Pseudomonas aeruginosa*; *Staphylococcus aureus*; Animal models

Core tip: In this manuscript we discuss the basics aspects of quorum sensing (QS) and its relationship with human infections, focusing in two major QS bacterial models, the opportunistic Gram negative bacteria *Pseudomonas aeruginosa* and *Staphylococcus aureus*.

Castillo-Juárez I, Maeda T, Mandujano-Tinoco EA, Tomás M, Pérez-Eretza B, García-Contreras SJ, Wood TK, García-Contreras R. Role of quorum sensing in bacterial infections. *World J Clin Cases* 2015; 3(7): 575-598 Available from: URL: http://www.wjgnet.com/2307-8960/full/v3/i7/575.htm DOI: http://dx.doi.org/10.12998/wjcc.v3.i7.575

INTRODUCTION

Several important bacterial pathogens, like *Pseudomonas aeruginosa* (*P. aeruginosa*), *Staphylococcus aureus* (*S. aureus*), and *Vibrio cholerae,* utilize quorum sensing (QS) cell communication to coordinate the expression of multiple virulence factors and associated behaviors such as swarming and biofilm formation, once a population size threshold is reached. QS systems consist of an enzyme that catalyzes the synthesis of the signal (such as acyl-homoserine lactones or cyclic peptides) and a receptor that binds the signal and reprograms the expression of several genes, including those encoding the enzyme that produces the signal, creating a positive feedback loop. In bacterial pathogens, most of the QS controlled genes codify several different virulence factors, such as proteases, toxins, and adhesins[1]. The expression of QS controlled phenotypes is energetically costly to the cells and only provides an advantage if it is expressed when cells reach high densities[2,3]; hence, in the context of bacterial infections, the expression of QS regulated virulence factors is delayed until a sufficient bacterial load is achieved and once this threshold is reached, bacteria coordinate their attack against the host, which maximizes the probability of establishing the infections and disseminating them, hence increasing the pathogen fitness. In fact, QS along with subversion of the immune system are the main factors that determine the bacterial infectious doses. Hence those bacterial pathogens that need small doses to infect, generally lack QS systems but are very effective at inactivating the immune response by killing professional phagocytes. In contrast, those bacterial pathogens that need high infectious doses rely in QS for the coordination of the expression of virulence[4]. In this review, the current knowledge about QS control of virulence factors in two main model bacterial pathogens, *P. aeruginosa* and *S. aureus* (which are also responsible for nosocomial and wound infections), will be discussed along with the relationship of their QS systems, its virulence in animal infection models, and the data available from human infections. Furthermore, the role of QS in other important infections and the role of QS in immune and cancer cells are discussed. Finally, proposed novel approaches of blocking QS/virulence as an alternative in fighting recalcitrant bacterial infections are also reviewed.

QS-CONTROLS OF THE EXPRESSION OF VIRULENCE FACTORS *IN VITRO*

P. aeruginosa

P. aeruginosa possesses at least three functional QS circuits; two of them are mediated by N-acyl homoserine lactones (HSL) signals and the other mediated by quinolones (Figure 1). The HSL-QS systems were first described and they were named after the virulence factors that were first identified under their control; hence, the Las system was discovered as a positive regulator for elastase production through the expression of the structural elastase gene lasB[5]. This system (by LasI HSL-acyl-synthase) produces the 3-oxo-C12-homoserine lactone (3-oxo-C12-HSL), that binds its receptor LasR which then dimerizes and binds promoters that contain *las* boxes, turning on the expression of several genes, including lasI, which then in a positive feedback loop increases the production of *3*-oxo-*C*12-HSL, the other HSL mediated QS system was named Rhl since it controls the expression of the biosurfactant rhamnolipids[6]. This system (RhlI) produces N-butyryl-L-homoserine lactone that is sensed by RhlR and also shows positive autoregulation[7]. The third QS system is mediated by different kinds of signals, alkyl quinolones, specifically 2-heptyl-3-hydroxy-4-quinolone(Pseudomonas quinolone signal or PQS) which is synthesized from anthranilate by the products of *pqsABCDEH* genes and sensed by PqsR (MvfR)[8,9]. The three systems are interconnected and function in a hierarchical way[10]; the Las system is the first to become activated, and it in turn it stimulates the Rhl and PQS systems[11,12], while PQS activates Rhl[13] and RhL inhibits PQS[11,14]. Moreover, 3-oxo-*C*12-HSL, the Las signal, is able to bind functional

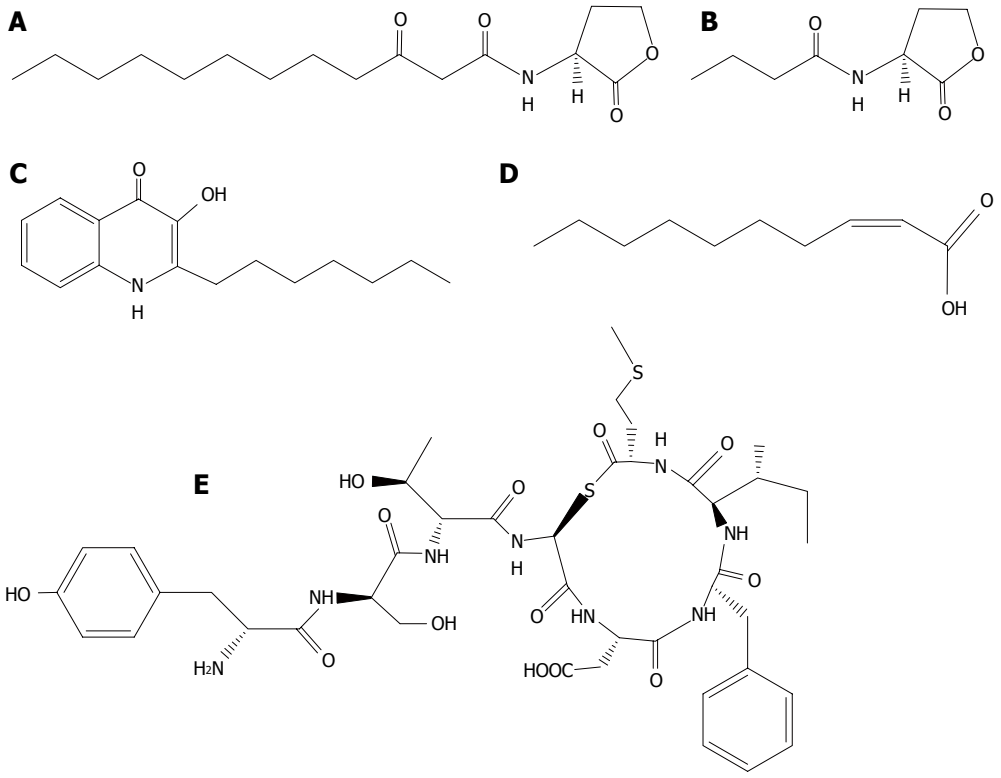


Figure 1 Structures of representative quorum sensing signal molecules of *Pseudomonas aeruginosa*. A: 3-oxo-C12-homoserine lactone; B: N-butyryl-L-homoserine lactone; C: 2-heptyl-3-hydroxy-4-quinolone; D: DSF-like fatty acids, cis-2-decenoic acid) and *S. aureus* (E: AIP group I).

RhlR dimmers, promoting their dissociation and inactivation[15]. In addition to control *lasB* elastase, the Las system also controls the expression of *lasA* elastase, exotoxin A (PA1148), and alkaline protease (PA1246)[16], and the Rhl also controls the expression of the phenazine pyocyanin a pigment able to cause oxidative damage to the eucaryotic host, promoting the production of reactive oxygen species and depleting the host antioxidant defense mechanisms[17], while the PQS system increases the expression of *lasB* elastase and pyocyanin[9]. In fact, the regulation of virulence factors by these 3 QS systems is complex and often overlaps[18]; for example, RhlR is apparently enough to compensate the absence of LasR at least in stationary phase cells in which it promotes the production of exoproteases, pyocyanin, PQS, and the 3-oxo-*C*12-HSL[18,19]. To add even more complexity, recently the role of environmental signals, such as the availability of iron and phosphate in influencing QS systems has been beginning to be explored[20]. In addition, other ions such as calcium strongly influence the production of QS modulated virulence factors such as pyocyanin, and proteases[21] and in fact there is solid evidence that indicates that the chemical composition of the sputum in cystic fibrosis patients promotes the use of the PQS system for communication, preferentially over the HSL systems[22]. Moreover the presence of metabolites like 2,3-butanediol (end product of bacterial fermentation from species that coexist with *P. aeruginosa* in the lung of cystic fibrosis patients) enhance the production of QS controlled virulence factors (phenazines and exotoxin) and improve biofilm formation *via* the Las QS system[23]; hence, the expression of QS-virulence factors *in vivo* is likely influenced by several variables, related with the state of the host as well as the presence or absence of other bacterial species. Indeed, the simultaneous utilization of several QS systems in bacteria, may serve different purposes like identifying community composition[24] or distinguish phases in population development[25], and a recent study shows that the concomitant utilization of Las and Rhl systems allows *P. aeruginosa* to simultaneously assess their population density and the presence of nutrients by combinatorial communication. Therefore, the secretion of QS controlled factors is subjected to "AND-gate" like responses to multiple signal inputs, allowing effective expression of secreted factors in high-density and low mass-transfer environments[26]. Another important role of QS systems in regulating bacterial physiology is that they are implicated in the tolerance against stress[27-29] that allow them to maximize their chances to effectively contend and survive the immune system attack[30], which may be a major determinant for the establishment and progression of *P. aeruginosa* and other pathogens infections.

S. aureus

S. aureus produces several virulence factors and many

of them are regulated by QS. In Gram positive bacteria, regulation by QS is generally mediated by autoinducing cyclic peptides. Specifically for *S. aureus,* QS controls the expression of virulence factors such as hemolysins, leukocidins, cell surface adhesins, exoenzymes, and biofilm formation *via* the Agr system, which relies on the autoinducing peptide (AIP) (Figure 1E). AIPis encoded by *agrD* and consists of 7-9 amino acids, and has a 5-membered thiolactone ring[31-33]; this peptide is secreted by the membrane protein AgrB and activated by the AgrC sensor kinase[1]. The Agr system regulates the expression of several genes by the production of two regulatory RNAs, RNAII and RNAIII[34], which are produced from promoters P2 and P3 respectively[34,35]. Transcription from the *agr* operon (*agrA*, *agrB*, *agrC* and *agrD*) is regulated by a phosphorylated AgrA homodimer from P2[36], while RNAIII is produced by AgrA from P3. RNAIII, which is the effector of the system, upregulates *α*-haemolysin, and increases the production of proteases, toxins, and the synthesis of capsule, while it repress protein A (which allows *S. aureus* to evade opzonization), and the expression of surface adhesions[1,31,34,35,37,38]. Such modulation of the expression of several virulence factors by the Agr system allows *S. aureus* to express a different repertoire of those determinants according to the kind of disease and the environmental conditions including the host status. Noteworthy is that *in vitro* the appearance of clones with diminished QS had been observed; these clones are apparently social cheaters which exploit cooperative individuals without contributing with the production of virulence factors. The presence of cheaters during infections may be very relevant for disease progression, since in controlled experiments, the ratio between cheaters and cooperating individuals strongly affects the mortality rate and extent of infection; *i.e.*, the severity of the infections are inversely proportional to the percentage of cheaters in the population[39]. Among the QS-controlled virulence factors in *S. aureus*, RNAIII is very important since it regulates biofilm formation, and resistance to antibiotics as well as the establishment of chronic infections is intimately related to the biofilm formation abilities of pathogens[40]. However, the *in vivo* biofilms in which *S. aureus* exists can be very complex environments, due the presence of several other bacterial species and their multiple interactions with each other and with the host, hence *in vitro* models for studying *S. aureus* virulence may have the disadvantage of not revealing the real expression of virulence factors. This hypothesis is supported by significant differences in the expression of several virulence factors in *S. aureus* grown in calf serum compared with those grown in defined CDM medium, since in serum the expression of hemolysins, enterotoxins, proteases, iron acquisition factors, and of RNAIII is significantly higher than in standard growth medium, and such differences are partially due the low iron concentration in serum[41,42].

QS-CONTROLS OF THE EXPRESSION OF VIRULENCE FACTORS *IN VIVO P. AERUGINOSA* QS AND VIRULENCE IN ANIMAL MODELS

A number of animal models including the nematode (*Caenorhabditis elegans*), fruit fly (*Drosophila melanogaster*), zebrafish (*Danio rerio*) and mouse (*Mus musculus*), have been used to identify and define the role of virulence determinants in the pathogenesis of *P. aeruginosa*[43,44]. The attenuation of its QS is achieved by two basic strategies: (1) the utilization of mutant strains with QS genes disrupted; and (2) the quenching of QS by treatments that interfere with it; these methods have shown that QS systems as well as QS-independent virulence determinants are required for *P. aeruginosa* infections in animals.

The main animal model that was used to discover the relationship between QS and virulence of *P. aeruginosa* is the nematode *C. elegans*. In 1999 Tan and coworkers, first described conditions to test the role of QS in virulence using this model, showing that the reference strain PA14 kills the nematode either after days (slow killing) or quickly after a few hours (fast killing)[45]. Their evidence indicate that fast and slow killing occur by distinct mechanisms; the slow killing involves an infection-like process and correlates with accumulation of PA14 within worm intestines, while the fast killing is mediated by the production of phenazines(regulated by QS); that increase active oxygen species[45,46]. A third mode by which *P. aeruginosa* can kill *C. elegans is lethal paralysis; this mechanism is mediated by QS since* Darby and coworkers, using QS-less mutant strains of *P. aeruginosa*, found that the lethal effect is associated with a rapid neuromuscular paralysis, caused by the action of diffusible unidentified factors whose production requires the *las* and *rhl* genes, since the infection with a *lasR* mutant and with a *rhlR* reduces the paralysis (by 28%-100% and 100% respectively)[47]. A potential target of these diffusible factors is the EGL-9 worm protein, which is expressed in the neuronal muscle tissues[47]. In a recent study, a reduction of 83% in the death of the nematodes by the double mutant (PA14*rhlRlasR*) was reported; however, the analysis of individual mutants, revealed that only the *rhlR* mutant reduced death 69%, implying that the RhlR system is crucial for infection under their experimental conditions[48]. In addition to lethal paralysis and slow and fast killing, a fourth kind of *C. elegans* death induced by *P. aeruginosa* is the "red death", characterized by the formation of red precipitate (PQS + Fe^{3+} complex) within the intestine of the nematodes. This mode of death is mediated by the quinolone dependant QS system Mvfr-PQS in coordination with the PhoB phosphate sensor and the pyoverdine iron acquisition system[49]. The role of QS in *P. aeruginosa* infectivity and virulence in *C.*

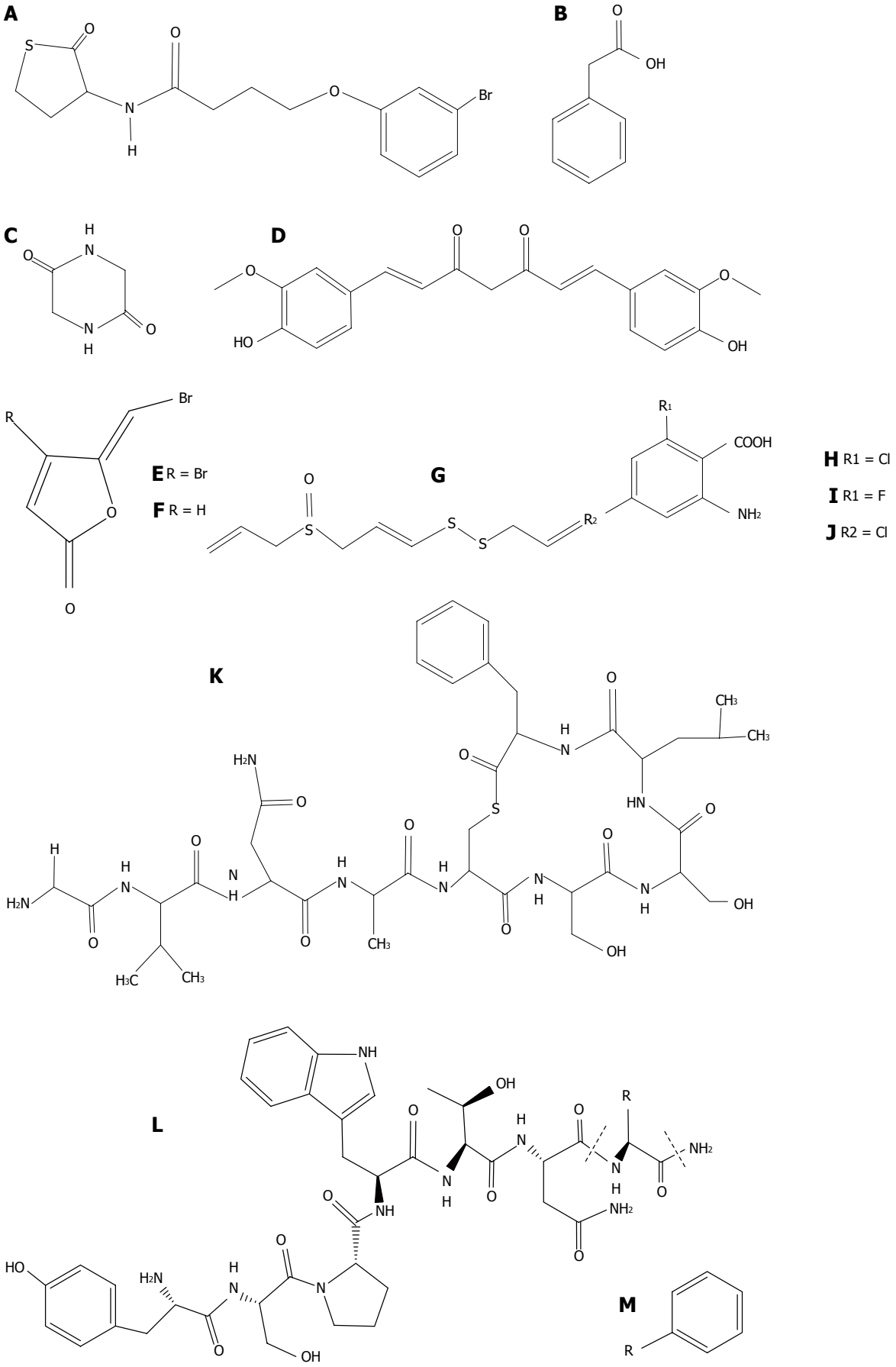


Figure 2 Structures of quorum sensing inhibitors evaluated in animal models. A: Meta-bromo-thiolactone; B: Phenylacetic acid; C: 2,5-piperazinedione; D: Cucurmin; E: Furanone C-30; F: Furanone C-56; G: Ajoene 4,5,9,-trithiadodeca-1,6,11-triene-9-oxide; H: 2-amino-6-chlorobenzoic acid; I: 2-amino-6-fluorobenzoic acid; J: 2-amino-4-chlorobenzoic acid; K: AIP-II; L: RIP (H-Tyr-Ser-Pro-Trp-Thr-Asn-Phe-NH_2); M: FS8 (H-Tyr-Ser-Pro-Trp-Thr-Asn-Ala-NH_2).

elegans is also evidenced by the effect of QS inhibitors, since a synthetic analog of HSL, meta-bromo-thiolactone (Figure 2A) that partially inhibits *in vitro* the LasR and RhrlR systems also reduces the death of worms infected with PA14, to 60% at 24 h. Interestingly, the *in vivo* action of the quencher in the worm model occurs mainly through the RhrlR system[48]. Moreover, phenylacetic acid (Figure 2B), which is a byproduct of the degradation of antibiotics such as penicillin G and cephalosporin Gby G acylase[50], increases the survival of PAO1 infected nematodes by 53%, while untreated worms die within 72 h. This protective activity is perhaps a consequence of interfering with the LasR and RhrlR systems, due to the structural similarity of phenylacetic acid with salicylic acid, a quorum quencher[51]. Another compound,2,5-piperazinedione (Figure 2C), increases the survival of worms by 66%, compared to untreated ones, and it was shown by molecular docking that it interacts with an amino acid residue (E145) in LasR, which is required for correct binding of the natural HSL ligand[52]. Similarly cucurmin (Figure 2D), a secondary metabolite from *Curcuma longa,* increases the survival of worms by 28%; this compound decreases the expression of genes involved in biofilm formation and attenuates HSL production in PAO1. Thus, it was suggested that it may act as a quorum quencher, delaying the synthesis of HSL molecules or by impairing autoinducers perception[53]. Moreover, various enzymes that degrade natural autoinducers are able to decrease the pathogenicity of *P. aeruginosa*; for example, adding the purified acylase PvdQ to *C. elegans* infected with *P. aeruginosa* PAO1, strongly reduces their pathogenicity and increases the nematodes life span[54]. Although the utilization of *C. elegans* as a model for studying *P. aeruginosa* infections has been very fruitful, recently it was proposed to use the fruit fly (*D. melanogaster*) as an animal model for the study of the *P. aeruginosa* pathogenesis, since the fly has a higher similarity to human[55-57]. The importance of both *P. aeruginosa* HSL QS pathways for infection was also demonstrated in *D. melanogaster* using the feeding assay, in which the bacteria are ingested and a local infection type is established in the intestine. In this assay the PA103 (*las*R), PDO100 (*rhl*I), PDO111 (*rhl*R), PAOR1 (*las*R) and PAOJP2 (*las*I/*rhl*I) mutant strains were avirulent with respect to wild-type PAO1 whose infected flies were killed at 14 d post-infection. Similarly, using the nicking assay (needle pricking), in which an injury is produced in the dorsum of the flies and *P. aeruginosa* is added to the wound, all mutant strains showed a lower death rate than wild-type, including the PDO100 mutant (*rhlI*) with 50% survival of the flies compared to 90% death for the PAO1 wild-type 35 d post-infection[57]. However, in contrast to the work with *C. elegans,* to date the effect of quorum quenching in *P. aeruginosa* virulence in the fly was not yet evaluated. With regard of these two infection models, Clatworthy and colleagues pointed out that a drawback to study *P. aeruginosa* infections using invertebrate hosts are the differences between their immune response and the one of vertebrates. For example, *C. elegans* and *D. melanogaster* do not have an adaptive immunity, or complex multilineage immune cells, such as those present in vertebrates[58]. Thus it is important to analyze the participation of QS in the pathogenesis of *P. aeruginosa* in vertebrate animal models, like zebrafish and mice. Specifically for zebrafish, the microinjection of PA14 QS$^-$ mutant strains (*lasR* and *mvfR*) during two different stages of fish development [28 and 50 h post-fertilization (hpf)], revealed that the participation of these two transcriptional activators during the infections is different and is influenced by the maturity of the immune system at different stages of the embryo development, since for the *lasR* mutant, only a 40% decrease in the death of the embryos at 50 hpf (a developmental stage when both macrophages and neutrophils are present) was recorded, whereas the *mvfR* mutant showed a moderate effect by decreasing death by 20% to 28 hpf (an stage in which only macrophages are present), but a higher effect of 60% decrease at 50 hpf[58].

For murine models, different protocols have been used to determine the participation of QS in *P. aeruginosa* pathogenicity[43,44]. The thermal induced injury model is frequently used and consists of producing a burn of second or third degree on the dorsal side of the mouse using water at 90 ℃ and subsequent inoculation of *P. aeruginosa.* Several experiments using this model have linked QS and virulence; for example, a PAO1-R1 (Δ*las*R) mutant has a diminished ability to spread systemically, as well as lower dispersion through the lesion at early stages[59,60]. Also, mice infected with PA14 *pqsA* show a 75% survival rate in contrast to 10% survival with wild-type PA14[61]. Similarly, virulence is reduced in PAO1 *lasR, lasI*, and *rhlI* mutants, with the greatest effect seen for the double mutant *lasI-rhlI* that decreased the mortality of animals by approximately 88%, significantly reduced the number of c.f.u in the lesion, liver and spleen, and delayed the spread of the bacteria from the lesion[59]. In agreement, similar results were found using the pneumonia model in neonatal mice, in which *lasR* mutants showed reduced virulence and are unable to replicate efficiently in the lung tissue; as a consequence, less damage occurs and the bacterial infection does not spread[62]. A third kind of experimental infection, the foreign-body infection model, consists in introducing a fragment of *P. aeruginosa* infected silicone into the peritoneal cavity of mice. This model was successfully used to determine the participation of QS in biofilm formation[43]. In this system, the mutant strain *lasR-rhlR* disappears from the silicone fragments during the first 7 d of infection, in contrast with wild-type PAO1 cells which remain in the silicon implant for at least 14 and up to 21 d. Critically, the establishment of the PAO1 infection in the implants depends in the mouse strain that is used, since for Balb/c, bacterial counts in the implants decayed constantly from day one and several of the implants were completely cleared after 21 d, while for the NMRI strain, bacterial counts initially decreased

(day 1 to 4), then remained constant and finally increased at levels similar to the initials at day 15[63].

Regarding studies testing the quorum quenching effect on virulence, by using the foreign body model, it was found that the intraperitoneal addition of furanone C-30 greatly increased the bacterial clearance rate (Figure 2E)[63]. In agreement a similar effect was observed with the lung infection model in mice in which a related compound, C-furanone 56 (Figure 2F) accelerated the bacterial clearance from the lungs, reducing the severity of the damage and significantly increasing mice survival[64]. Another quorum quencher, the synthetic molecule ajoene (ajoene 4,5,9,-trithiadodeca-1,6,11-triene-9-oxide) (Figure 2G), is able to attenuate the production of various *P. aeruginosa* QS-controlled virulence factors *in vitro*, while in the pulmonary infection model in mice infected with PAO1, its prophylactic administration from two days before the infection and during its course, reduced the bacterial c.f.u. in the lungs 500-fold relative to the non-treated mice[65]. Moreover, in the burn mice model, the intravenous administration of three related quorum quenchers, the anthranilic acid analogues: 2-amino-6-chlorobenzoic acid, 2-amino-6-fluorobenzoic acid, and 2-amino-4-chlorobenzoic acid (Figure 2H-J), that inhibit the biosynthesis of quinolone signals and disrupt the MvfR-dependant gene expression, restrict the systemic spread of the PA14 strain and decreases the animals death by 30% to 50%[61].

QS IN *P. AERUGINOSA* HUMAN INFECTIONS

The importance of the QS systems of *P. aeruginosa* in human infections is highlighted by their presence in most clinical strains that were isolated during the moment of the infection. This was demonstrated in 2004 by Schaber *et al*[66], by screening 200 isolates from patients with urinary tract, lower respiratory tract, and wound infections. Of those isolates, 97.5% (195 isolates) had robust functional HSL-QS communication systems and hence were able to produce elastase (codified by the genes *lasB* and *lasA,* which expression is QS dependent through LasR) and high levels of both HSL autoinducers while only 5 isolates failed to satisfy those criteria; however, 2 isolates were identified as being the same bacteria, but isolated at two different times from the same patient, and for one isolate there was no clinical data available to support that it was implicated in an infective process[66]. Hence only approximately 1% of the isolates that were implicated in infections appeared to be QS deficient. Critically, one of these isolates had no *lasR* and *rhlR* functional genes. In addition the authors demonstrated that those isolates deficient in HSL-QS systems produced high levels of non-QS controlled virulence factors, such as the ExoS and ExoT proteins that are components of the type three secretion system. Hence, perhaps this was an adaptive response that potentially could compensate for the decrease in virulence caused by QS deficiency., Nevertheless, the production of those proteins in the QS proficient isolates was not evaluated, and the virulence of the isolates was not tested using infection models. Other studies have reported similar results;, for example, the characterization of 442 *P. aeruginosa* isolates colonizing the respiratory tract of 13 intubated patients identified 9 genotypically different strains and of these, 6 strains produced both HSL-autoinducers and the virulence factors: elastase, exoproteases, rhamnolipid, hydrogen cyanide, and pyocyanin *in vitro*, and two of them had mutations in both *lasR* and *rhlR* genes, while the third had a mutant *lasR* gene[67]. Another study performed with 100 isolates from patients with respiratory infections that were collected from sputum, tracheal aspirate, and bronchoalveolar lavage identified 11 HSL-QS deficient isolates, six of them with absent *QS* genes (one isolate negative for *rhlR*, two isolates negative for *rhlI* and *rhlR,* and three isolates were negative for *rhlI*, *rhlR*, *lasI* and *lasR*). Interestingly, this study found a negative correlation between the expression of QS controlled virulence factors and antibiotic resistance[68]. Furthermore, the analysis of 82 *P. aeruginosa* clinical strains isolated from urinary tract infections identified 6 isolates deficient in the production of both HSL autoinducers, biofilm, rhamnolipids, and elastase, correlating with the absence of the *lasR* gene in one isolate and the absence of *lasI, lasR, rhlR* in another isolate, while the other 4 isolates harbored point mutations that probably inactivated their *lasI, lasR, rhlR*, and *rhlI* genes[69].

Taken together, these independent studies indicate that about 90% of *P. aeruginosa* isolates that cause infection generally preserve active HSL-QS systems, although clearly a small percentage of the isolates have those systems impaired by mutations or loss of the important QS regulatory genes, nevertheless, in all these studies, the third QS system of *P. aeruginosa*, the quinolone dependent system, was not evaluated; hence, it is not reliable to conclude that these isolates were indeed 100% QS deficient. In addition, the existence of cell communication systems not yet described in this organism cannot be ruled out, and indeed in the reference strain PAO1, cell communication by fatty acids was recently discovered (DSF-like fatty acids, cis-2-decenoic acid) (Figure 1D)[70]. Another possibility that may explain the isolation of QS deficient strains from infections is the presence of multiple *P. aeruginosa* strains in the infection site and that the QS deficient isolates coexist with QS proficient strains; this was demonstrated recently in 8 patients with cystic fibrosis (CF), in which a complex mixture of QS-proficient and deficient isolates were found. Interestingly, among all the patients, the deficiency of the isolates in individual QS regulated phenotypes (LasA and LasB elastase, rhamnolipids, growth in adenosine, and HSL signals) ranged from 0 to approximately 90% and no single

patient with 100% QS deficient isolates was found.

Such high diversity in isolates from the same patient likely is the result of a complex and multifactorial selective process, perhaps including social components like the advantages accrued by QS-deficient clones that use the resources made by the QS positive strains (siderophores, proteases, *etc.*), without contributing to the generation of the public goods; these bacteria are termed social cheaters[71]. Regarding the importance of QS for infections, these results indicate that at the population level, QS may be essential for CF infections; however, more studies increasing the number of CF patients and including other kinds of infections are necessary to better understand the importance of *P. aeruginosa* QS in the infective process. In addition, the elucidation of factors that shape the mosaic-like composition of isolates in patients or in animal models need to be determined in order to design better anti-QS therapies since the current ones are focused on laboratory strains with QS-proficient systems rather than clinical strains recently isolated from infections[72,73]. Although such factors are still unknown, some variables like: the severity and progression of the infection, the nutritional, health, and immunological status of the patients, the exposure of the susceptible individuals to only one, a few, or several strains and the bacterial loads during the infections could be involved. In this sense, animal models would be useful to evaluate the role of these and other valuables in the colonization diversity in the patients, for example experiments comparing the colonization of well feed animals and animals with a deficient nutrition, immune competent animals and immunosuppressed ones, or healthy animals compared to animals harboring important disorders such as the alpha-1-antitrypsin deficiency that promotes major pulmonary inflammation, degradation of lung tissue, and eventually manifestations of pulmonary emphysema, *etc*. using several bacterial strains (QS proficient and QS deficient) alone or in combination could be very valuable to determine the factors involved in the *in vivo* bacterial ecology in infections.

In addition, although *P. aeruginosa* virulence is multifactorial[74], the individual importance of QS controlled virulence factors in different kinds of infections is a current research area, and the role of molecules from the HSL autoinducers themselves, to extracellular factors like rhamnolipids, elastase, pyocyanin, *etc*. have been established. For example several independent studies have shown that the main *P. aeruginosa* autoinducer N-(3-oxododecanoyl)-HSL is readily detected in sputum samples collected from patients with cystic fibrosis[75-77], which correlates with a QS dependent gene expression during the infections[78-80]. However, besides its role as a signal, the autoinducer is also able to inhibit lymphocyte proliferation as well as secretion of tumor necrosis alpha by macrophages and interferon gamma by T-cells[27]. Moreover QS controlled secreted factors such as alkaline protease can interfere with the classical and the lectin pathway-mediated complement activation *via* cleavage of C2, blocking phagocytosis and killing of *P. aeruginosa* by neutrophils[81]. Also elastase, by cleaving the pulmonary surfactant protein-A, can contribute to phagocytosis evasion[82]. Furthermore, rhamnolipids are able to disrupt calcium-regulated pathways and protein kinase C activation, preventing the induction of human beta-defensin-2 in keratinocytes[83]. Remarkably, the production of rhamnolipids in mechanically ventilated patients is associated with the development of life-threatening ventilator-associated pneumonia (VAP), while elastase production and QS independent production of the cytotoxins ExoU and ExoS are not[84]. Another QS controlled virulence factor, the polysaccharide alginate, protects *P. aeruginosa* biofilm cells from IFN-gamma-mediated macrophage killing[85]. Surprisingly, the importance of pyocyanin, a blue redox-active compound which is one of the main *P. aeruginosa* virulence factors studied *in vitro* and one of the more notorious in infections (present in large quantities in sputum from patients with cystic fibrosis infected by *P. aeruginosa)* during clinical infection is still underexplored[86].

S. AUREUS QS AND VIRULENCE IN ANIMAL MODELS

The participation of SarA and Agr *S.aureus* QS systems in pathogenicity has been evaluated using numerous animal models, in which the bacteria induce diseases such as osteomyelitis, septic arthritis, endocarditis, endophthalmitis and soft tissue abscesses. By using the mutant strains *agr* and *sarA,* as well as QS inhibition, the participation of these systems in the infectivity of the bacterium and the damage of tissues has been proved. Intravenous inoculation of the bacteria in mice induces the development of septic arthritis. In this model, *agr* mutants showed a reduced ability to induce the pathology since it is produced in only approximately 10% of the animals while the wild-type strain produces it in approximately 60% of the inoculated mice. Furthermore, in mice infected with the mutant strain, the arthritis severity is less, and only a few developed erosive arthropathy in contrast to those infected with wild-type[87]. Similarly in the endophthalmitis-rabbit model, which is established by the intraocular injection of the bacteria, *agr* mutants produced a smaller loss of neuroretinal function during the first 3 d of the infection, with respect to the wild-type strain. In addition, those infected with the mutant strain had normal eye histology, whereas those infected with the wild-type strain showed focal retinal destruction and mild vitritis[88]. In a subsequent study employing the same animal model, no significant differences in the rabbits eyes infected with the mutant strain (*sarA*) and with the wild-type were found; however, the simultaneous deletion of genes *agr* and *sarA* resulted in a near to complete attenuation of virulence[89]. Moreover, employing the model of endocarditis in rabbits, which consists in introducing a

catheter into the ventricle and subsequently colonizing it by intravenous administration of the bacteria, it was observed that single mutations (*sarA* and *agr*) diminish the bacterial ability to induce the pathology, while a *agr*/*sarA* double mutant was incapable of inducing endocarditis in 100% of the animals inoculated with 10^3 or 10^4 c.f.u.[90]. Another infection model in mammals is the murine brain abscess model in which lesions are produced by embedding bacteria in agarose beads that are later inoculated in the cranial cavity. In this model, the *agr*/*sarA* double mutant, but not the single mutants, had reduced virulence, lower proliferation in the brain and poorly developed abscesses that were drastically smaller than those produced by the wild-type strain. Furthermore, the double mutation attenuates the expression of pro-inflammatory cytokines and chemokines[91]. Similarly invertebrate models such as the nematode *C. elegans*, which is killed by feeding on *S. aureus,* showed similar outcomes, since mutating *sarA* or *agr* increased the survival of the worms with respect to the wild-type strain[92].

Regarding the effect of quorum quenching in *S. aureus* animal infections, several inhibitory peptides have been evaluated; for example, in the murine subcutaneous abscess model, the administration of the synthetic autoinducer analog of AIP-Ⅱ peptide (Figure 2K) in a single dose was able to decrease the formation of abscesses, and although AIP-Ⅱ prevents expression of the *S. aureus agr* QS regulon for only a short time period, this transient inhibition is sufficient to achieve significant effects[93]. Other QS inhibitory peptides such as the RNAⅢ-inhibiting peptide (RIP) and its analogues (Figure 2K and L), that inhibit the phosphorylation of a target protein called "target of RNAⅢ-activating protein" (TRAP), leading to the suppression of virulence factor production *in vitro*[94,95], are also effective *in vivo.* For example, in the vascular-graft rat model, the administration of RIP (Figure 2L), both locally and systemically, is able to completely inhibit the formation of biofilms in graft and in polymethylmethacrylate beads infected with methicillin-susceptible and resistant *S. aureus*. Similarly, in the mouse sepsis model, administering RIP significantly reduced the bacterial load and mice mortality; this effect is potentiated by co-administration of antibiotics like cefazolin, imipenem, or vancomycin[96]. In addition, using the graft rat model, a RIP treatment increases its effectiveness in combination with antibiotics rifampin and temporin, and the complete elimination of infection is achieved by combining it with temporin A[97]. The same phenomenon has been documented with a derivative of RIP, termed FS3, which contains a substitution of alanine in the second position, since FS3 in combination with daptomycin has higher efficiency than single compounds, in the rat model of vascular graft staphylococcal infection[98]. Also in this model, a similar effect was obtained, by combining tigecycline and the RIP analogue called FS8 which contains a terminal alanine (Figure 2M)[99]. Taken together these extensive studies demonstrate the participation of *S. aureus* QS systems in its pathogenicity and indicate that QS inhibition in combination with antibiotics is a promising new strategy that may be effective to treat the infections produced by this important pathogen.

QS IN *S. AUREUS* HUMAN INFECTIONS

Although it is not always pathogenic to humans, the Gram (+) bacterium *S. aureus,* is frequently found in the human respiratory tract and on the skin and it is considered as a transient member of the human microbial flora[32], it is able to cause several kind of infections with a plethora of clinical manifestations. There are risk factors that complicate the infection caused by *S. aureus*, including the presence of prosthetic material and immunosuppression[100], and it is considered one of the three main causes of nosocomial bacterial infections. Among the many kinds of *S. aureus* infections, skin ones are very common; for instance, in children it is the main cause of impetigo, a superficial skin infection that according to its clinical manifestations is divided into non-bullous and bullous impetigo, the non-bullous is the most common form, the lesions begin as papules that progress to vesicles with erythema on its periphery. These become pustules that later form adherent crusts with a golden appearance and can be accompanied by regional lymphadenitis, although systemic symptoms are usually absent. Bullous impetigo is seen in young children in which the vesicles enlarge to form flaccid blisters with clear yellow liquid, which later becomes darker and turbid, leaving a thin brown crust[3,4]. Other common infections produced by *S. aureus* are hair follicle infection or folliculitis, furunculosis, and cellulitis. In addition to skin infections, *S. aureus* also causes respiratory tract infections such as nosocomial and septic pneumonia, septic pulmonary emboli and post viral empyema. It also infects the apocrine glands, causes musculoskeletal infections, produces bacteremia and its complications like sepsis, septic shock, and infective endocarditis and is able to produce toxic shock syndrome and food poisoning. Therefore, *S. aureus* is a major health concern worldwide.

Although the role of QS in regulating the expression of several virulence determinants including toxin production, and biofilm formation have been extensively studied *in vitro* and in animal models, its importance in actual human infections is yet under studied; nevertheless, it is known that as in the case of *P. aeruginosa* the great majority of *S. aureus* clinical isolates implicated in human infections possess active QS systems (agr^+). Although some agr^- strains are also commonly found in *S. aureus* infections[101-103], the presence of both kind of strains during infection indicates that agr^+ and agr^- variants may have a cooperative interaction[103] and also raises the possibility that social interactions like QS cheating may exist during the

infections[39]. In addition, the link of QS and biofilm formation in *S. aureus* strongly suggests that this QS is important for the development and establishment of its chronic infections[32]; however, further work in this area is needed to define the importance and the specifics of QS in regulating *S. aureus* virulence in human infections.

QS ANTIVIRULENCE DRUGS

P. aeruginosa

To date, there are a large number of quorum quenching (QQ) compounds reported. In general, the three types of QQ compounds are degraders of AHL autoinducers, synthase inhibitors, and receptor inhibitors. Here the QQ compounds used against *P. aeruginosa* are sorted into seven categories.

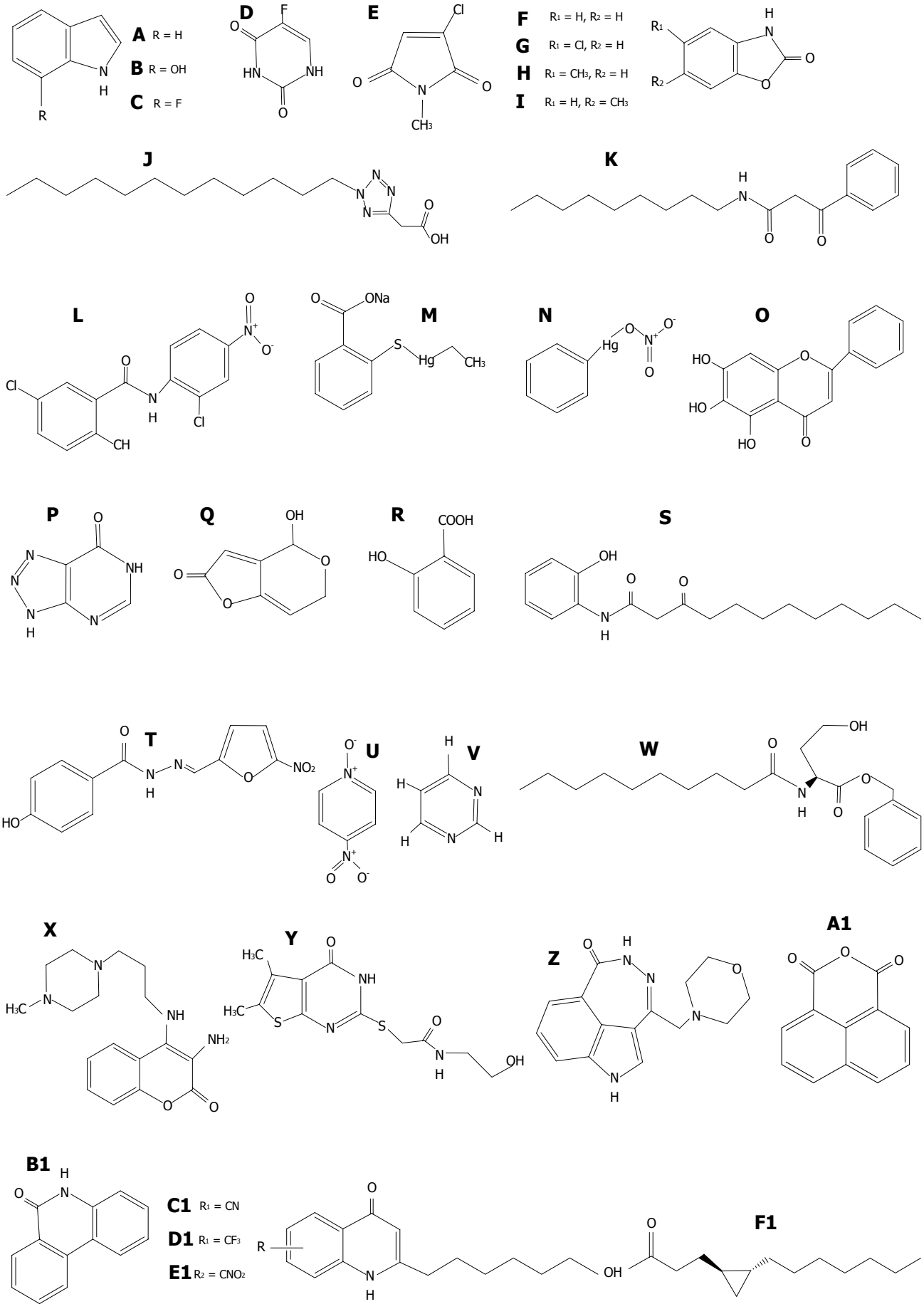
Halogenated compounds

One of the best characterized QQ compounds is the synthetic brominated furanone 4-bromo-5-(bromomethylene)-2(5*H*)-furanone known as C-30 (Figure 2E)[104]. This compound was synthetically modified from the natural brominated furanone (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone of the algae *Delisea pulchra*. Another furanone compound is 5-(bromomethylene)-2(5*H*)-furanone, also called furanone C-56 (Figure 2F) which is a derivative of the secondary metabolites produced by the algae[105]. Interestingly, although C-30 is effective for the attenuation of several QS-dependent virulence factors *in vitro* and in animal models, resistance against this compound has been found both in laboratory PA14 derived mutants and in clinical isolates; to date, the only resistance described mechanism is the active efflux of this compound by the MexAB-OmpR pump but the existence of other mechanisms cannot be ruled out[72,106,107]. Furanone C-56 affects the processes of biofilm formation and dispersal although it does not influence initial attachment to abiotic substrata. In addition, indole (Figure 3A) produced from L-tryptophan by a variety of bacteria and 7-hydroxy indole (7HI) (Figure 3B), an oxidized compound of indole created by bacterial oxygenases, are extracellular signals that attenuate the production of biofilm and virulence factors in *P. aeruginosa*[108]. In addition, among 31 natural and synthetic indole analogs, 7-fluoroindole (7FI) (Figure 3C) was identified to be a QQ compound capable of reducing the production of virulence factors such as 2-heptyl-3-hydroxy-4(1*H*)-quinolone, pyocyanin, rhamnolipid, pyoverdin, and pyochelin[109]. 7FI shows higher inhibition toward biofilm formation than indole or 7HI.As another fluorine compound which shows the QQ ability, 5-fluorouracil (5-FU) (Figure 3D), an anticancer uracil analog, also is a potent inhibitor of *P. aeruginosa* virulence[110]. Since 5-FU is basically used for clinical purposes as a chemotherapeutic approach in patients with cancer, it holds promise as a QQ compound for clinical use. However, clinical strains resistant against this compound have been identified[72,107].

Based on the previous report that chlorolactone (CL) is an inhibitor to the QQ receptor[111], three other synthetic CL analogs were tested for QQ effects. As a result, meta-bromo-thiolactone (mBTL) (Figure 2A) was the most effective QQ compound since pyocyanin production and biofilm formation were inhibited in the presence of mBTL, in addition, mBTL moderately protected *C. elegans* and human lung epithelical cells from killing by *P. aeruginosa*[48]. Other halogenated QQ compounds include the derivatives of anthranilic acid which is the primary precursor of 4-hydroxy-2-alkylquinolines (Figure 2H-J)[61]. The halogenated anthranilic acid analogs inhibit quinoline biosynthesis and the expression of QS-related genes. Beyond that, halogenated maleimide analogs also are QQ compounds; in particular, bromo- and iodo-substituted maleimides decrease bacterial attachment and biofilm formation whereas chloro-N-methyl-maleimide has bacteriocidal action rather a QQ effect[112]. In addition to this, 5-chloro-1,3-benzoxazol-2(3H)-one[113] also called chlorzoxazone[114] are QQ compounds that contain a halogen group (Figure 3G).

Lactonases and acylases

Degrading enzymes such as lactonases and acylases are another class of QQ compounds. Their effect on QQ is due to the degradation of AHL-based autoinducers. To date, some unique lactonases have been characterized; for example, the halotolerant lactonases derived from *Bacillus* spp[115] and a thermally-stable lactonase from *Bacillus weihenstephanensis* P65[116], which may be useful for future applications. Lactonase itself or in combination with ciprofloxacin prevented systemic spread of the bacteria in murine burn wounds infected with *P. aeruginosa*, while for the combination mice mortality was completely abolished and skin regeneration was promoted[117]. In addition, immobilized esterases and acylases embeded on medical plastic materials inhibit biofilm formation[118]. Another unique approach using a lactonase is to utilize an engineered *Lactobacillus plantarum* strain expressing the lactonase AiiA from *Bacillus thuringiensis* 4A3[119]. Extracellular virulence factors such as pyocyanin, protease, elastase, and rhamnolipids of multi-drug resistant clinical isolates of *P. aeruginosa* were inhibited and the attachment to uroepithelial cells was reduced by co-culturing *P. aeruginosa* with the engineered strain. The original trial was performed using a *P. aeruginosa* strain capable of expressing a lactonase derived from *Microbacterium testaceum*, which led to reduced production of virulence factors and attenuated cytotoxicity against human lung epithelial cells[120]. A new trial using genetic engineering has been recently reported; this is by an engineered T7 bacteriophage expressing a lactonase with activity for a broad-range of bacterial hosts[121]. The engineered T7 bacteriophage was able to inhibit the biofilm formation of a consortium of *P. aeruginosa* and *Escherichia coli*.


A R = H
B R = OH
C R = F
D
E
F R1 = H, R2 = H
G R1 = Cl, R2 = H
H R1 = CH3, R2 = H
I R1 = H, R2 = CH3
J
K
L
M
N
O
P
Q
R
S
T
U
V
W
X
Y
Z
A1
B1
C1 R1 = CN
D1 R1 = CF3
E1 R2 = CNO2
F1

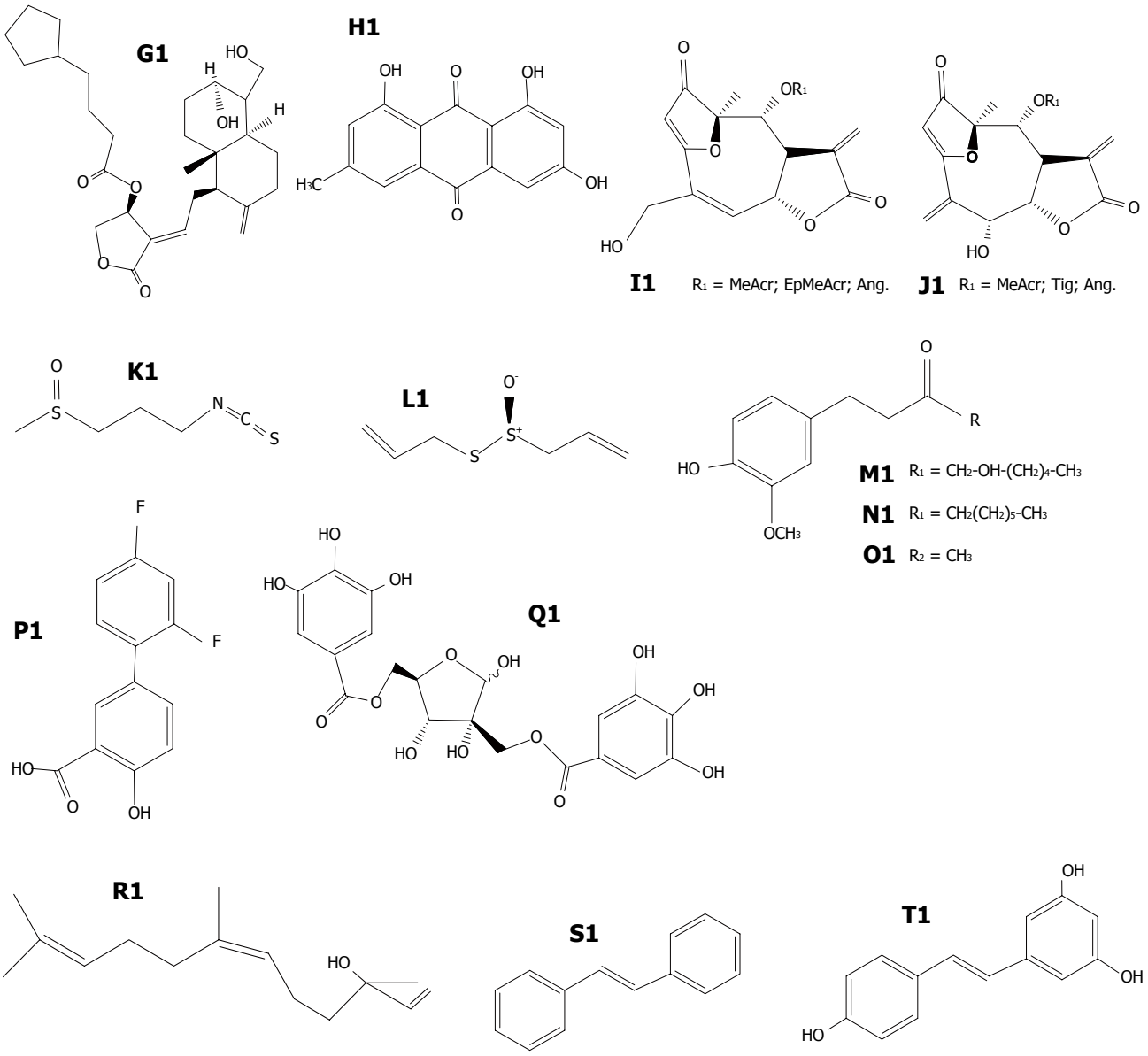


Figure 3 Structures of representative quorum quenching molecules of *Pseudomonas aeruginosa*. A: Indole; B: 7-hidroxy indole; C: 7-fluoroindole; D: 5-fluorouracil; E: 2-chloro-N-methyl-maleimide; F: 1,3-benzoxazol-2(3H)-one; G: 5-cloro-1,3-benzoxazol-2(3H)-one (clorzoxazone); H: 5-methyl-1,3-benzoxazol-2(3H)-one; I: 6-methyl-1,3-benzoxazol-2(3H)-one; J: PD12; K: V-06-018; L: Niclosamide; M: Thimerosal; N: Phenylmercuric nitrate; O: Baicalein; P: 5-imino-4,6-dihydro-3H-1,2,3-triazolo[5,4-*d*]pyrimidin-7-one; Q: Patulin; R: Salicylic acid; S: 3-oxo-C12-(2-aminophenol); T: Nifuroxazide; U: 4-nitropyridine-N-oxide; V: Pyrimidine; W: N-decanoyl-L-homoserine benzyl ester; X: V23; Y: V30; Z: P1; A1: NAP; B1: PJ97A; C1: 6-CN; D1: 6-CF3; E1: 6-NO2; F1: Lyngbyoic acid; G1: Andrographolide 14-(5-cyclopentylvaleryl); H1: Emodin; I1: Goyazensolide-type; J1: Isogoyazensolide-type; K1: Iberin; L1: Allicin; M1: [6]-gingerol; N1: [6]-shogaol; O1: Zingerone and *S. aureus*; P1: Diflunisal; Q1: Hamamelitannin; R1: Cis-nerolidol; S1: Trans-stilbene; T1: Resveratrol.

QQ compounds found by several screening approaches

For searching for novel QQ compounds, structure-based computational screens and high-throughput screens have been conducted. An ultra-high-throughput, cell-based assay to screen a library of approximately 200000 compounds was used to find an inhibitor which can decrease the gene expression regulated by the Las system[122]. As a result, PD12 (Figure 3J), a tetrazole with a 12-carbon alkyl tail and V-06-018, a phenyl ring with a 12-carbon alkyl tail (Figure 3K), which have both similarity with the structure of 3OC12-HSL, were identified as QQ compounds.In addition, a compound having QQ ability was also found among a series of 1,3-benzoxazol-2(3H)-one derivatives[113]; thereby, 1,3-benzoxazol-2(3H)-one (Figure 3F), 5-chloro-1,3-benzoxazol-2(3H)-one (Figure 3G), 6-methyl-1,3-benzoxazol-2(3H)-one (Figure 3I), and 5-methyl-1,3-benzoxazol-2(3H)-one (Figure 3H) have QQ ability. As another approach for clinical application of QQ compounds, the thousands of drugs clinically used in the treatment of different diseases were screened to find drugs with QQ properties which can be applicable to humans. By the screening, it was found that an anthelmintic drug, niclosamide (Figure 3L) strongly inhibits the QS response by *P. aeruginosa*[123], although the active compound was demonstrated to be 5-FU

which was already described as a QQ agent[107,110]. Moreover, since antibiotics are also robust compounds for clinical use, inhibition of QS by antibiotics was surveyed. As a result, it was found that low concentrations of azithromycin, ceftazidime, and ciprofloxacin inhibit QS in *P. aeruginosa*[124]. In addition, QS in a *P. aeruginosa* environmental isolate was inhibited at sub-inhibitory concentrations of tobramycin[125] although other studies demonstrated that a low concentration of tobramycin induces biofilm formation[126].

Screening using a computational approach and molecular docking analysis has also been useful for evaluating the binding capacity of QQ compounds to receptor proteins; thereby, new potential QQ compounds were identified. Pharmacophore modeling and *in silico* screening to find an antagonist for QS in *P. aeruginosa* indicated that a compound with tetravalent lead has QQ ability[127]. Another two compounds thimerosal (Figure 3M) and phenyl mercuric nitrate (Figure 3N) were selected as QQ compounds based on their similarity to the Pb-QQ compound. Also, the automated docking program by which the docking capability of a ligand to a receptor can be analyzed identified 5 potential new QQ compounds; among the candidates, baicalein (Figure 3O) has the strongest QQ ability as it inhibits biofilm formation of *P. aeruginosa* and the QQ effect by baicalein increases synergistically in the presence of ampicillin[128]. Also, another 5 compounds were identified to be QQ by using a structure-based virtual screening approach targeting the QS receptor LasR; of the 5 compounds, the most promising was 5-imino-4,6-dihydro-3H-1,2,3-triazolo[5,4-d]pyrimidin-7-one also called G1 (Figure 3P)[129].

Other AHL antagonists

Some of QQ compounds described above are antagonists of AHL molecules; hence their QS inhibition effect is triggered by interrupting the binding (interaction) between AHL molecules and receptors. To date, there are a large number of AHL antagonists; for example, patulin (Figure 3Q)[130], salicylic acid (Figure 3R)[114], 3-oxo-C12-(2-aminophenol) (Figure 3S)[131], and nifuroxazide (Figure 3T)[114] as well as C-30 (Figure 2E)[104]. In addition, 4-nitropyridine-N-oxide (Figure 3U) is a QQ compound[132], which also reduces bacterial adhesion to silica-coated surfaces[133]. Other QQ compounds are pyrimidine (Figure 3V)[134], N-decanoyl-L-homoserine benzyl ester (C2) (Figure 3W)[135], 2,5-piperazinedione (Figure 2C)[52], and phenylacetic acid (Figure 2B)[51]. The bacterial sensitivities to several antibiotics (tobramycin, gentamycin, cefepime, and meropenem) in the presence of C2 were higher than those without C2[135]. This may be due to the synergistic interactions between C2 and the antibiotics. In addition, QQ by the cyclic dipeptide 2,5-piperazinedione (Figure 2C) might be due to interference with the binding of the natural ligand 3-oxo-C12-HSL to its receptor protein based on the molecular docking analysis[52]. Phenylacetic acid (Figure 2B), which is similar to salicylic acid, has been reported to be a QQ compound[51].

Inhibitors with different QS targets

There are some reports on inhibitors with QS targets different than AHLs and receptors.A new class of antivirulence compounds was reported by Shouldice *et al*[136]; the QQ compounds interact with the bacterial periplasmic protein DsbA, which is essential for the folding and function of exported virulence factors. Another target of QQ compounds is mono-ADP-ribosyltransferase which functions as a bacterial toxin[137]. Some newly-identified QQ compounds were found by using a virtual screen of commercially available compounds combined with a directed poly(ADP-ribose) polymerase; thereby, V23 (Figure 3X), V30 (Figure 3Y), and P1 (Figure 3Z) compounds as well as NAP (Figure 3A1)[137] and PJ97A (Figure 3B1)[138] were identified as inhibitors of toxin production[137]. Other antagonists are a series of compounds targeting PqsR, the receptor of the *pqs* system[139]. Among the analogs of 2-heptyl-4-hydroxyquinoline (HHQ) synthesized, three HHQ analog with 6-CN (Figure 3C1), 6-CF_3 (Figure 3D1), or 6-NO_2 (Figure 3E1) along with n-C_7H_{15} are the best competitors[139], which are promising starting compounds for further drug design.

Cell extracts and secretion products from isolated microorganisms

Based on the concept that microbial interaction (inhibition, repression, acceleration, and dependence) is a complex phenomenon due the large numbers of microbes, a new approach to isolate unique microorganisms with QQ ability and to utilize cell extracts and secretion products has been recently reported. Among the 46 marine bacterial isolates, 11 extracts from *Bacillus*, *Marinobacter*, *Halobacillus*, *Staphylococcus*, or *Ferrimonas* species showed antibiofilm activity against *P. aeruginosa*[140]. The partially-purified antibiofilm compound from S6-15 (similarity with *Bacillus pumilus*) is stable up to 60 ℃ and under neutral and alkaline conditions. In addition, its QQ ability was inactivated by the treatment by enzymes such as proteinase K, trypsin and lysozyme[140]. Also, bacteria able to utilize AHL molecules as a sole source of carbon and nitrogen have been isolated and characterized as AHL-degrading bacteria[141]. Among 41 isolates which retained QQ activity after heat treatment, some of the isolates showed impaired QS inhibition after the treatment by proteinase K whereas the other isolates remained active. In addition, actinomycetes with QQ activity were also isolated from marine sponge. In this study, methanol extracts of 12 actinomycetes had an inhibitory effect on the production of QS-mediated virulence factors[142]; in particular, of the three strains which showed very good anti-QS activity, the most promising strain is NIO 10068 (*Streptomyces* sp.) that secretes cinnamic acid and/or linear Pro-Gly dipeptide which may be QQ compounds. Further bacteria capable of having QQ ability were also isolated from healthy coral species[143]; of 120 bacterial

isolates, up to 24% of the isolates showed anti-QS activity. In particular, a *Favia* sp. coral isolate inhibits the biofilm formation of *P. aeruginosa* by secreting a low-molecular mass compound which is not inactivated by heat and proteinase K[143]. Also, a cell-free lysate of endophytic bacteria isolated from *Pterocarpus santalinus* Linn. also showed QQ activity[144]. *Bacillus firmus* PT18 and *Enterobacter asburiae* PT39 isolated as the endophytic bacteria exhibit potent AHL degrading ability by inhibiting about 80% violacein production in a biosensor strain. QQ activity by the cell lysate was effective against biofilm formation rather than to planktonic cells, and the QQ activity was due to the presence of AHL lactonase in cell-free lysate[144].

Moreover, a small cyclopropane-containing fatty acid, lyngbyoic acid (Figure 3F1), a major metabolite produced by the marine cyanobacterium, *Lyngbya* cf. *majuscule* has been identified to be a QQ compound capable of strongly inhibiting Las-QS system[145]. In addition, the biosurfactant, lunasan produced by *Candida sphaerica* UCP 0995 is also a QQ compound[146]. Recently, it was discovered that a conditioned high density lipoprotein is also a QQ compound capable of reducing the virulence of *P. aeruginosa* by influencing *las*-and *rhl*-QS systems as well as biofilm formation[147]. Furthermore, ultra-small solid lipid nanoparticles for the pulmonary delivery, which are prepared by using various pharmaceutical lipids, are fabricated to deliver QQ compounds to a target site without any penetrable cellular barrier[148]. In this study, plain small solid lipid nanoparticles exhibited anti-virulence properties themselves.

QS inhibitors from food and plant sources

Since biocompatibility of QQ compounds to higher organisms is one of the important requirements for clinical use, there are a lot of trials to find QS inhibitors from food and plants. The anti-QS activity of aqueous extracts from edible plants and fruits, like pineapple, plantain, and sapodilla, was evaluated; most of these extracts showed QQ activity without inhibiting bacterial growth in *P. aeruginosa*[149]. Also, analogs from a natural bicyclic diterpeniod lactone, andrographolide which is the main phytoconstituent from *Andrographis paniculata* Nees (herb), were screened to evaluate QQ activity[150]. An andrographolide-based compound, 14-(5-cyclopentylvaleryl) andrographolide (compound 11b) (Figure 3G1) had the best QQ activity among all the new compounds. In addition, some QQ compounds were found from traditional Chinese medicine by using a molecular docking analysis and QS assays[151]. As a result, emodin (Figure 3H1) had a certain antibiofilm activity as well as the ability to increase the activity of ampicillin against *P. aeruginosa*.

Furthermore, five sesquiterpene lactones of the goyazensolide (Figure 3I1)and isogoyazensolide-type (Figure 3J1)isolated from the Argentine herb *Centratherum punctatum*[152], iberin (Figure 3K1)from horseradish[153], allicin (Figure 3L1)from garlic[154], and phenolic components {([6]-gingerol (Figure 3M1),[6]-shogaol (Figure 3N1), zingerone (Figure 3O1)} from ginger[155] and anacardic acids[156] are QQ compounds, which might be suitable for further development of antivirulence and antibacterial agents.

S. aureus

Since QS regulates the expression of multiple *S. aureus* virulence determinants, and since the frequency of drug resistant clinical strains causing infections is rising (like methicillin-resistant *S.aureus* "MRSA"), several compounds aiming to disrupt these regulatory interactions have been identified; among them, perhaps the best characterized is the QS inhibitor RIP (Figure 2L), an endogenous *S. aureus* peptide that is able to decrease the damage of *S. aureus* in several animal models as discussed before[94,157]. At the molecular level, the production of several toxins is activated in a cell density manner by the RNAIII-activating protein (RAP) and by the autoinducing peptide (AIP), and is inhibited by RIP and by inhibitory AIPs; RAP participation in the pathogenesis consists in inducing the phosphorylation of a 21-kDa protein (known as target of RAP or TRAP). While RIP inhibits its phosphorylation, the phosphorylation of TRAP is essential to create the autoinducing loop since it leads to the activation of RNAIII synthesis[94]. In addition to decreasing the damage of *S. aureus* during infection, RIP treatment also is able to prevent its adhesion to human kidney cells and its biofilm formation on dialysis catheters[158]. Other effective peptides analogues to RIP are FS3 and FS8 (Figure 2M) discussed previously[98,99]. Moreover, recently four AIP non-functional peptide analogues were identified; these peptides have an ample spectrum since they can repress many AgrC receptors (type I-IV) and have a very high affinity. For example, treatment with the peptides block hemolysis (at picomolar concentrations)and attenuate the production of toxic shock syndrome toxin-1 by 80% at nanomolar concentrations; hence, these compounds are the most potent synthetic inhibitors of QS in *S. aureus* to date[159].

Beyond the QS inhibitory peptides, several other interesting molecules able to block the expression of *S. aureus* virulence factors have been discovered, among them, the small molecule biaryl compounds in which the aromatic rings either are either fused or separated by a short linker. This result is particularly interesting, since they are able to inhibit the production of the alpha-hemolysin and the modulin-α toxin in a dose-dependent manner without inhibiting bacterial growth, since they are effective against methicillin-resistant *S. aureus*, and since one of the effective compounds is diflunisal (Figure 3P1), an Federal Drug Administration-approved nonsteroidal anti-inflammatory drug[160]. Diflunisal has the clear advantage that it could be easily repurposed for treating *S. aureus* infections or could be used to coat catheters and other medical devices just as was recently done for 5-FU (Figure 3D)[161], which has QS inhibitory activity against *P. aeruginosa*[110], *Escherichia coli* (*E. coli*)[162] and perhaps several other

pathogens. In addition, some natural products with QS inhibition activity against *S. aureus* like 2,5-di-*O*-galloyl-dhamamelose (hamamelitannin) (Figure 3Q1), a non-peptide analog of RIP found in the bark of the plant *Hamamelis virginiana* had been identified. This compound is effective *in vitro* to inhibit virulence without affecting growth, and *in vivo* in a rat graft model, preventing device-associated infections[163]. Moreover, recently the screening of 83 essential oils led to the identification that black pepper, cananga, and myrrh oils and their common constituent cis-nerolidol (Figure 3R1) strongly attenuate *S. aureus* biofilm formation, its hemolytic activity, and protect *C. elegans* against its infection. Although their mechanism is not fully understood yet, transcriptional analyses showed that at least black pepper oil treatment inhibited the expression of the α-toxin gene (*hla*), nuclease genes, and QS regulatory genes[164]; similar effects can be observed with treatments with trans-stilbene (Figure 3S1)and resveratrol (Figure 3T1)[165].

QS SYSTEMS ARE PRESENT IN SEVERAL OTHER IMPORTANT BACTERIAL PATHOGENS

In addition to *P. aeruginosa* and *S. aureus,* several other bacterial pathogens utilize QS systems to control the expression of multiple virulence factors during infection. Among the more relevant for human health are *Vibrio* spp, *Acinetobacter* spp**,** *Burkholderia cepacea,* and enteric bacteria like *Escherichia* spp. and *Salmonella typhimurium*. The following section is an overview of their known QS systems and their relationship with virulence.

Vibrio spp.

Vibrio is a genus of facultative anaerobic Gram-negative bacteria possessing a curved rod shape (comma shape) typically found in saltwater. Several species are pathogenic to animals including humans and are responsible for food borne infections that are usually associated with eating contaminated food or water. In addition, they also cause wound infections and septicemia. The first QS system was described in the bioluminescent marine bacterium *Vibrio fischeri*, considered the paradigm for QS found in most Gram-negative bacteria. *Vibrio fischeri* colonizes the light-emitting organs of the squid *Euprymna scalopes*, in which it multiplies and reaches a high population density and induces the expression of luminescence genes. This gene expression occurs in a coordinated fashion[166]. The squid uses the light conferred by the bacteria to hide its own shadow in shallow waters and thus avoid predators[167]. To date several QS systems have been described in *Vibrio* spp.

In Vibrio harveyi, the following three QS systems are known: (1) LuxM (synthase), LuxN (receptor) and 3OHC4HSL (signal); (2) LuxS (synthase), LuxP (receptor) and AI-2 (signal); and (3) CqsA (synthase), CqsS (receptor) and CAI-1 (signal).

These systems are associated with bioluminescence, siderophores, protease and extracellular polysaccharide (EPS) production, and other virulence factors[168-170].

In Vibrio cholerae, two QS systems have been described: (1) LuxS (synthase), LuxP (receptor) and AI-2 (signal); (2) CqsA (synthase), CqsS (receptor) and CAI-1 (signal).

These systems have been associated with biofilm formation, EPS production, and other virulence factors[169].

Finally, in *Vibrio fischeri* three QS systems are known: (1) LuxI (synthase), LuxR (receptor) and 3OC6HSL (signal); (2) AinS (synthase), AinR (receptor) and C8HSL (signal); and (3) LuxS (synthase), LuxP (receptor) and AI-2 (signal).

These systems are associated with bioluminescence, host colonization, and motility[168,170]. Other QS systems found in various *Vibrio* spp. and in *Legionella pneumophila* utilize hydroxyketones (AHKs) as signalling molecules[170].

Acinetobacter spp.

Acinetobacter is a genus of aerobic, non-motile Gram-negative bacteria that are widely distributed in nature, commonly occurring in soil. Among them, some species like *Acinetobacter baumannii* (*A. baumannii*) are frequently isolated in nosocomial infections, especially in intensive care units, since they attack debilitated and immunocompromised patients; in addition they have a high tolerance against antibiotics and an inherent ability to acquire antibiotic resistance genes, being therefore a serious emergent health problem. Their QS systems consist of homologues of the LuxR and LuxI proteins of *Vibrio fischeri* known as AbaR (receptor) and AbaI (synthase) and play a role in biofilm formation and motility in *Acinetobacter* spp[171] and in *Acinetobacter baumannii*[172]. This QS system is an important virulence factor responsible for the outstanding antibiotic resistance and survival properties in the latter species[173]. However, the role of QS systems in the regulation of other virulence factors implicated in the development of infection has not yet been established[174].

Synthesis of N-(3-hydroxydodecanoyl)-L-HSL (3-hydroxy-C12-HSL) is catalyzed by AbaI from *Acinetobacter* strain M2 (initially characterized as *Acinetobacter baumannii*, although genomic sequencing studies have distinguished this strain as *Acinetobacter nosocomiales*[174,175]). The completed genome sequence of *A. baumannii* strain ATCC 17978 indicates that autoinducer synthase AbaI (gene *A1S_110*) and acyltransferases may be the sole participants in the synthesis of AHL signals of variable chain length by the organism[176]. Many strains of *Acinetobacter* (63%) produce more than one AHL. However, none of the AHL signals can be specifically assigned to a particular species of the genus[177]. *Acinetobacter* quorum signals are not homogenously distributed, and therefore

distinction between virulent and non-virulent strains on the basis of QS signals is difficult. Communication between bacteria with respect to cell density is integral to the maturation of *Acinetobacter* spp. Biofilm[176,178]. Mutation of *abaI*, which produces the acyl-homoserine lactone molecule, resulted in a 30%-40% reduction in biofilm production relative to that of the isogenic parental strain[173]. Exogenous addition of purified *Acinetobacter* acyl homoserine lactone restored biofilm maturation in the *abaI* mutant[176].

Burkhordelia cepacea

The *Burkholderia cepacia* complex is a group of Gram-negative bacteria composed of at least 18 different species; they are important human pathogens which produce pneumonia in immunocompromised individuals that are affected by lung diseases such as cystic fibrosis. All *Burkhordelia cepacea* complex members encode at least one QS system that consists of homologues of the LuxR and LuxI proteins of *Vibrio fischeri* [CepI(synthase), CepR (receptor), and AHLs C8-HSL and C6-HSL (signal)]. AHL production in the *Burkhordelia cepacea* complex is strain-dependent with respect to both the quantity and type of AHL molecules[179,180]. Another QS system in the *Burkhordelia cepacea* complex is the CciIR system [CciI (synthase), CciR (receptor), and/AHLs C8-HSL and C6-HSL (signal)][181]. Phenotypic assays and global transcript and protein analysis with *cepIR* and *cciIR* mutant strains have shown that AHL-mediated QS controls various functions, including swarming motility, biofilm formation and the production of virulence factors, such as proteases (*e.g.,* the metal proteases ZmpA and ZmpB), siderophores, toxins and antifungal agents[179].

In 2008, Boon *et al*[182] reported the identification of a novel fatty acid signal molecule that is produced by several *B. cenocepacia* strains. The structure of the molecule synthesized by *B. cenocepacia* J2315 was identified as *cis*-2-dodecenoic acid, referred to as BDSF (*Burkholderia* diffusible signal factor). BDSF is structurally related to DSF (diffusible signal factor, *cis*-11-methyl-2-dodecenoic acid), which was first isolated from supernatants of *Xanthomonas campestris* pv *campestris.* The BDSF-regulated QS system is involved in the control of several functions. Mutation of *rpfFBc* resulted in decreased motility, reduced adherence to porcine mucin, diminished exopolysaccharide (EPS) production and lowered protease activity. In addition, the BDSF mutant strains were found to be more susceptible to antimicrobial agents, and their ability to form biofilms was shown to be strongly reduced[179].

Escherichia spp./Salmonella typhimurium

E. coli and *S. typhimurium* are related enteric Gram-negative, facultative anaerobic bacteria. Although most *E. coli* strains are commensal for warm-blooded organisms, such as mammals, some serotypes cause serious food poisoning and other kinds of infections like urinary tract infections and neonatal meningitis, while *S. typhimurium* and other *Salmonella* pathogenic serovars are responsible for Salmonellosis, an infection that causes diarrhea, fever, vomiting, and abdominal cramps. Although usually the illness resolves after four to seven days without medical treatment, several million people are infected by this bacterium each year. In *E. coli* and *S. typhimurium*, three QS systems have been described: (1) Unknown (synthase), SdiA (receptor) and 3OC8HSL (signal). This system has been associated with motility and acid resistance[183]; (2) LuxS (synthase), LsrB (receptor) and AI-2 (signal). Lsr operon expression (AI-2 uptake)[184]; and (3) Unknown (synthase), QseC (receptor) and AI-3 (signal). This system has been implicated in virulence, motility and biofilm formation[185].

QS SYSTEMS BEYOND BACTERIA

QS systems have been extensively studied in bacteria and are of great interest for understanding the development of clinically-significant infections, but whether eukaryotes have cell signaling systems similar to bacterial QS mechanisms is a question that has recently drawn the attention of research worldwide. In this section, we will discuss some examples of eukaryotic microorganisms and human cells that use QS for the development of certain biological functions. The first report of a QS system in eukaryotes was carried out more than 40 years ago, when it was observed that dense cultures of the fungi *Candida albicans* show a reduced tendency towards the morphological transition from yeast to hypha, which is considered a key virulence factor for this opportunistic fungal pathogen[186]. To date, several compounds have been identified as responsible for this phenomenon, such as 2-phenylethanol, tryptophol, farnesol, farnesoic acid, and tyrosol[187]; these QS molecules are secreted by *C. albicans* and when they accumulate over a threshold level, they trigger changes in: (1) fungal dimorphisms[186]; (2) biofilm formation[188]; and (3) expression of virulence genes[189]. In addition, in other dimorphic fungi (*Mucor rouxii, Histoplasma capsulatum, Ceratocystis ulmi*), the "inoculum size effect" is usually observed; however, QS autoinducers in these organisms have not been identified[187]. In 1997, it was discovered that in the protozoan parasite of humans *Trypanosoma cruzi,* the differentiation of replicating and slender forms to non-dividing and stumpy ones is also a density-dependent (quorum) response that limits the population size[190]. This phenomenon is mediated by the soluble factor SIF (stumpy induction factor) that is released by trypanosomes, and a recent study revealed that the QS signaling in *T. cruzi* shares components with the quiescence pathways of mammalian stem cells, providing novel therapeutic targets *via* QS interference[191]. Like those parasitic species described thus far, in 2006 it was demonstrated that the budding yeast *Saccharomyces cerevisiae* endure morphological transition from the yeast form to

Table 1 Quorum sensing systems and quorum sensing-virulence associated phenotypes in the reviewed organisms

Organism	QS systems	Regulated phenotypes
Gram (-) bacteria		
Pseudomonas aeruginosa	(1) LasI (S)-LasR (R)-HSL (s) (2) HSL RhlI-RhlR (3) Alkyl quinolones (PQS)	Expression of several virulence factors including: Pyocyanin, pyoverdine, elastase, alkaline protease, HCN, rhamnolipids and biofilm formation
Vibrio harveyi	(1) LuxM (S)-LuxN (R)- 3OHC4HSL (s) (2) LuxS (S)-LuxP (R)-AI-2 (s) (3) CqsA (S)-CqsS (R)-CAI-1 (s)	Expression of bioluminescence genes and several virulence factors including: Siderophores, protease, EPS production
Vibrio cholerae	(1) LuxS (S)-LuxP (R)-AI-2 (s) (2) CqsA (S)-CqsS (R)-CAI-1 (s)	Expression of several virulence factors including: Biofilm formation and EPS production.
Vibrio fischeri	(1) LuxI (S)-LuxR (R)-3OC6HSL (s) (2) AinS (S)-AinR (R)-C8HSL (s) (3) LuxS (S)-LuxP (R)-AI-2 (s)	Expression of bioluminescence, host colonization and motility genes
Acinetobacter spp	(1) Abal (S)-AbaR (R)-3OHC12HSL (s)	Expression of virulence factors including biofilm formation.
Burkhordelia cepacea	(1) Cep1 (S)-CepR (R)-C8HSL,C6HSL (s) (2) Ccil (S)-CciR (R)-C8HSL,C6HSL (s)	Expression of swarm motility genes and several virulence factors including: proteases, siderophores, toxins, antifungal agents and biofilm formation
Escherichia coli *Salmonella typhimurium*	(1) Unknown (S)-SdiA (R)-3OC8HSL (s) (2) LuxS (S)-LsrB (R)-AI-2 (s) (3) Unknown (S)-QseC (R)-AI-3 (s)	Expression of motility genes, acid resistance and virulence factors including biofilm formation
Gram (+) bacteria		
Staphylococcus aureus	(1)AgrB (S)-AgrC (R)-AIP(s)	Expression of several virulence factors including: hemolysins, leukocidins, cell surface adhesins, exoenzymes, and biofilm formation
Fungi		
Candida albicans	2-phenylethanol, tryptophol, farnesol, farnesoic acid, and tyrosol as (s)	Fungal dimorphism, biofilm formation and expression of virulence genes
Mucor rouxii, Histoplasma capsulatum, Ceratocystis ulmi	QS (s) unknown	Fungal dimorphism
Saccharomyces cerevisiae	Phenylethanol and tryptophol as (s)	Transition from yeast to filamentous form
Protozoa		
Trypanosoma cruzi	SIF soluble factor as (s)	Differentiation of replicating to non-dividing forms
Mammal cells		
$CD4^+$T cells	IL-2 (s) and IL-2Rα (R)	Regulation of the $CD4^+$T cells population
Cancer cells	Multiple (S) and (R) and paracrine factors as (s)	Regulation of the metastatic process

IL-2: Interleukin 2; SIF: Soluble inhibitory factor; QS: Quorum sensing; PQS: Pseudomonas quinolone signal; EPS: Exopolysaccharide.

a filamentous form in response to both cell density and the nutritional state of the environment. This induction is mediated by the phenylethanol and tryptophol auto signaling molecules, that regulate the transcription of a set of approximately 150 genes and which include *FLO11*, an essential gene for filamentous growth as well as several others genes that may play a role in the transition from the exponential to the stationary growth phase[192,193]. However, not only parasitic infections or traits present in unicellular eukaryotes are controlled by QS, since surprisingly in our body the number of cells of the immune system is maintained throughout a similar mechanism, where IL-2 is produced and secreted by activated $CD4^+$ T cells and sensed with high affinity (IL-2Rα) by a population of $CD4^+$ Treg, which in turn can regulate the number of total $CD4^+$ T population[194] by competition for the IL-2 factor[195]. Failure of QS due to the absence of IL-2 or by defects on the sensor IL-2Rα leads to lymphoid hyperplasia and autoimmune diseases[196]. Furthermore, in 2009 Hickson *et al*[197] proposed that cancer cells may use QS mechanisms to operate as communities and regulate different multicellular functions as the metastatic process resembles bacterial biofilm formation and dispersion. This idea emerged based on several lines of evidence that suggested a close relationship between high cancer cell densities and high metastatic ability[197]. This relationship can be possibly explained by the fact that the cells secrete paracrine factors or autoinducers that increase their metastatic efficiency; these observations have been made since the 90's[198] and recently by combining mathematical modeling with experimental evidence, the presence of QS systems in cancer was confirmed[199]. Such interesting findings are now opening new areas of study, including the development of future clinical applications (a summary of all the QS reviewed is provided in Table 1).

REFERENCES

1 **Antunes LC**, Ferreira RB, Buckner MM, Finlay BB. Quorum sensing in bacterial virulence. *Microbiology* 2010; **156**: 2271-2282 [PMID: 20488878 DOI: 10.1099/mic.0.038794-0]

2 **Diggle SP**, Griffin AS, Campbell GS, West SA. Cooperation and conflict in quorum-sensing bacterial populations. *Nature* 2007; **450**: 411-414 [PMID: 18004383 DOI: 10.1038/nature06279]

3 **Pai A**, Tanouchi Y, You L. Optimality and robustness in quorum sensing (QS)-mediated regulation of a costly public good enzyme. *Proc Natl Acad Sci USA* 2012; **109**: 19810-19815 [PMID: 23144221 DOI: 10.1073/pnas.1211072109]

4 **Gama JA**, Abby SS, Vieira-Silva S, Dionisio F, Rocha EP. Immune subversion and quorum-sensing shape the variation in infectious dose among bacterial pathogens. *PLoS Pathog* 2012; **8**: e1002503 [PMID: 22319444 DOI: 10.1371/journal.ppat.1002503]

5 **Gambello MJ**, Iglewski BH. Cloning and characterization of the Pseudomonas aeruginosa lasR gene, a transcriptional activator of elastase expression. *J Bacteriol* 1991; **173**: 3000-3009 [PMID: 1902216]

6 **Ochsner UA**, Koch AK, Fiechter A, Reiser J. Isolation and characterization of a regulatory gene affecting rhamnolipid biosurfactant synthesis in Pseudomonas aeruginosa. *J Bacteriol* 1994; **176**: 2044-2054 [PMID: 8144472]

7 **Croda-García G**, Grosso-Becerra V, Gonzalez-Valdez A, Servín-González L, Soberón-Chávez G. Transcriptional regulation of Pseudomonas aeruginosa rhlR: role of the CRP orthologue Vfr (virulence factor regulator) and quorum-sensing regulators LasR and RhlR. *Microbiology* 2011; **157**: 2545-2555 [PMID: 21719541 DOI: 10.1099/mic.0.050161-0]

8 **Pesci EC**, Milbank JB, Pearson JP, McKnight S, Kende AS, Greenberg EP, Iglewski BH. Quinolone signaling in the cell-to-cell communication system of Pseudomonas aeruginosa. *Proc Natl Acad Sci USA* 1999; **96**: 11229-11234 [PMID: 10500159 DOI: 10.1073/pnas.96.20.11229]

9 **Déziel E**, Gopalan S, Tampakaki AP, Lépine F, Padfield KE, Saucier M, Xiao G, Rahme LG. The contribution of MvfR to Pseudomonas aeruginosa pathogenesis and quorum sensing circuitry regulation: multiple quorum sensing-regulated genes are modulated without affecting lasRI, rhlRI or the production of N-acyl-L-homoserine lactones. *Mol Microbiol* 2005; **55**: 998-1014 [PMID: 15686549 DOI: 10.1111/j.1365-2958.2004.04448.x]

10 **Rutherford ST**, Bassler BL. Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb Perspect Med* 2012; **2**: [PMID: 23125205]

11 **Xiao G**, He J, Rahme LG. Mutation analysis of the Pseudomonas aeruginosa mvfR and pqsABCDE gene promoters demonstrates complex quorum-sensing circuitry. *Microbiology* 2006; **152**: 1679-1686 [PMID: 16735731 DOI: 10.1099/mic.0.28605-0]

12 **Latifi A**, Foglino M, Tanaka K, Williams P, Lazdunski A. A hierarchical quorum-sensing cascade in Pseudomonas aeruginosa links the transcriptional activators LasR and RhlR (VsmR) to expression of the stationary-phase sigma factor RpoS. *Mol Microbiol* 1996; **21**: 1137-1146 [PMID: 8898383 DOI: 10.1046/j.1365-2958.1996.00063.x]

13 **McKnight SL**, Iglewski BH, Pesci EC. The Pseudomonas quinolone signal regulates rhl quorum sensing in Pseudomonas aeruginosa. *J Bacteriol* 2000; **182**: 2702-2708 [PMID: 10781536 DOI: 10.1128/JB.182.10.2702-2708.2000]

14 **Wade DS**, Calfee MW, Rocha ER, Ling EA, Engstrom E, Coleman JP, Pesci EC. Regulation of Pseudomonas quinolone signal synthesis in Pseudomonas aeruginosa. *J Bacteriol* 2005; **187**: 4372-4380 [PMID: 15968046 DOI: 10.1128/JB.187.13.4372-4380.2005]

15 **Ventre I**, Ledgham F, Prima V, Lazdunski A, Foglino M, Sturgis JN. Dimerization of the quorum sensing regulator RhlR: development of a method using EGFP fluorescence anisotropy. *Mol Microbiol* 2003; **48**: 187-198 [PMID: 12657054 DOI: 10.1046/j.1365-2958.2003.03422.x]

16 **Passador L**, Cook JM, Gambello MJ, Rust L, Iglewski BH. Expression of Pseudomonas aeruginosa virulence genes requires cell-to-cell communication. *Science* 1993; **260**: 1127-1130 [PMID: 8493556 DOI: 10.1126/science.8493556]

17 **Gloyne LS**, Grant GD, Perkins AV, Powell KL, McDermott CM, Johnson PV, Anderson GJ, Kiefel M, Anoopkumar-Dukie S. Pyocyanin-induced toxicity in A549 respiratory cells is causally linked to oxidative stress. *Toxicol In Vitro* 2011; **25**: 1353-1358 [PMID: 21596130 DOI: 10.1016/j.tiv.2011.05.004]

18 **Dekimpe V**, Déziel E. Revisiting the quorum-sensing hierarchy in Pseudomonas aeruginosa: the transcriptional regulator RhlR regulates LasR-specific factors. *Microbiology* 2009; **155**: 712-723 [PMID: 19246742 DOI: 10.1099/mic.0.022764-0]

19 **Cabeen MT**. Stationary phase-specific virulence factor overproduction by a lasR mutant of Pseudomonas aeruginosa. *PLoS One* 2014; **9**: e88743 [PMID: 24533146 DOI: 10.1371/journal.pone.0088743]

20 **Jensen V,** Lons D, Zaoui C, Bredenbruch F, Meissner A, Dieterich G, Munch R, Haussler S. RhlR expression in Pseudomonas aeruginosa is modulated by the Pseudomonas quinolone signal via PhoB-dependent and -independent pathways. *J Bacteriol* 2006; **188**: 8601-8606 [PMID: 17028277 DOI: 10.1128/JB.01378-06]

21 **Sarkisova SA**, Lotlikar SR, Guragain M, Kubat R, Cloud J, Franklin MJ, Patrauchan MA. A Pseudomonas aeruginosa EF-hand protein, EfhP (PA4107), modulates stress responses and virulence at high calcium concentration. *PLoS One* 2014; **9**: e98985 [PMID: 24918783 DOI: 10.1371/journal.pone.0098985]

22 **Palmer KL**, Aye LM, Whiteley M. Nutritional cues control Pseudomonas aeruginosa multicellular behavior in cystic fibrosis sputum. *J Bacteriol* 2007; **189**: 8079-8087 [PMID: 17873029 DOI: 10.1128/JB.01138-07]

23 **Venkataraman A**, Rosenbaum MA, Werner JJ, Winans SC, Angenent LT. Metabolite transfer with the fermentation product 2,3-butanediol enhances virulence by Pseudomonas aeruginosa. *ISME J* 2014; **8**: 1210-1220 [PMID: 24401856 DOI: 10.1038/ismej.2013.232]

24 **Nadell CD**, Xavier JB, Foster KR. The sociobiology of biofilms. *FEMS Microbiol Rev* 2009; **33**: 206-224 [PMID: 19067751 DOI: 10.1111/j.1574-6976.2008.00150.x]

25 **Anetzberger C**, Reiger M, Fekete A, Schell U, Stambrau N, Plener L, Kopka J, Schmitt-Kopplin P, Hilbi H, Jung K. Autoinducers act as biological timers in Vibrio harveyi. *PLoS One* 2012; **7**: e48310 [PMID: 23110227 DOI: 10.1371/journal.pone.0048310]

26 **Cornforth DM**, Popat R, McNally L, Gurney J, Scott-Phillips TC, Ivens A, Diggle SP, Brown SP. Combinatorial quorum sensing allows bacteria to resolve their social and physical environment. *Proc Natl Acad Sci USA* 2014; **111**: 4280-4284 [PMID: 24594597 DOI: 10.1073/pnas.1319175111]

27 **Telford G**, Wheeler D, Williams P, Tomkins PT, Appleby P, Sewell H, Stewart GS, Bycroft BW, Pritchard DI. The Pseudomonas aeruginosa quorum-sensing signal molecule N-(3-oxododecanoyl)-L-homoserine lactone has immunomodulatory activity. *Infect Immun* 1998; **66**: 36-42 [PMID: 9423836]

28 **Hassett DJ**, Ma JF, Elkins JG, McDermott TR, Ochsner UA, West SE, Huang CT, Fredericks J, Burnett S, Stewart PS, McFeters G, Passador L, Iglewski BH. Quorum sensing in Pseudomonas aeruginosa controls expression of catalase and superoxide dismutase genes and mediates biofilm susceptibility to hydrogen peroxide. *Mol Microbiol* 1999; **34**: 1082-1093 [PMID: 10594832 DOI: 10.1046/j.1365-2958.1999.01672.x]

29 **García-Contreras R**, Nuñez-López L, Jasso-Chávez R, Kwan BW, Belmont JA, Rangel-Vega A, Maeda T, Wood TK. Quorum sensing enhancement of the stress response promotes resistance to quorum quenching and prevents social cheating. *ISME J* 2015; **9**: 115-125 [PMID: 24936763]

30 **Bjarnsholt T**, Jensen PØ, Burmølle M, Hentzer M, Haagensen JA, Hougen HP, Calum H, Madsen KG, Moser C, Molin S, Høiby N, Givskov M. Pseudomonas aeruginosa tolerance to tobramycin, hydrogen peroxide and polymorphonuclear leukocytes is quorum-sensing dependent. *Microbiology* 2005; **151**: 373-383 [PMID: 15699188 DOI: 10.1099/mic.0.27463-0]

31 **Roux A**, Payne SM, Gilmore MS. Microbial telesensing: probing the environment for friends, foes, and food. *Cell Host Microbe* 2009; **6**: 115-124 [PMID: 19683678 DOI: 10.1016/j.chom.2009.07.004]

32 **Yarwood JM**, Schlievert PM. Quorum sensing in Staphylococcus infections. *J Clin Invest* 2003; **112**: 1620-1625 [PMID: 14660735 DOI: 10.1172/JCI200320442]

33 **Bernardo K**, Pakulat N, Fleer S, Schnaith A, Utermöhlen O, Krut O, Müller S, Krönke M. Subinhibitory concentrations of linezolid reduce Staphylococcus aureus virulence factor expression. *Antimicrob Agents Chemother* 2004; **48**: 546-555 [PMID: 14742208 DOI: 10.1128/AAC.48.2.546-555.2004]

34 **Novick RP**, Geisinger E. Quorum sensing in staphylococci. *Annu Rev Genet* 2008; **42**: 541-564 [PMID: 18713030 DOI: 10.1146/annurev.genet.42.110807.091640]

35 **Morfeldt E**, Taylor D, von Gabain A, Arvidson S. Activation of alpha-toxin translation in Staphylococcus aureus by the trans-

encoded antisense RNA, RNAIII. *EMBO J* 1995; **14**: 4569-4577 [PMID: 7556100]
36 **George EA**, Muir TW. Molecular mechanisms of agr quorum sensing in virulent staphylococci. *Chembiochem* 2007; **8**: 847-855 [PMID: 17457814 DOI: 10.1002/cbic.200700023]
37 **Kong KF**, Vuong C, Otto M. Staphylococcus quorum sensing in biofilm formation and infection. *Int J Med Microbiol* 2006; **296**: 133-139 [PMID: 16487744 DOI: 10.1016/j.ijmm.2006.01.042]
38 **Novick RP**, Projan SJ, Kornblum J, Ross HF, Ji G, Kreiswirth B, Vandenesch F, Moghazeh S. The agr P2 operon: an autocatalytic sensory transduction system in Staphylococcus aureus. *Mol Gen Genet* 1995; **248**: 446-458 [PMID: 7565609 DOI: 10.1007/BF02191645]
39 **Pollitt EJ**, West SA, Crusz SA, Burton-Chellew MN, Diggle SP. Cooperation, quorum sensing, and evolution of virulence in Staphylococcus aureus. *Infect Immun* 2014; **82**: 1045-1051 [PMID: 24343650 DOI: 10.1128/IAI.01216-13]
40 **Morrison JM**, Miller EW, Benson MA, Alonzo F, Yoong P, Torres VJ, Hinrichs SH, Dunman PM. Characterization of SSR42, a novel virulence factor regulatory RNA that contributes to the pathogenesis of a Staphylococcus aureus USA300 representative. *J Bacteriol* 2012; **194**: 2924-2938 [PMID: 22493015 DOI: 10.1128/JB.06708-11]
41 **Li YH**, Tian X. Quorum sensing and bacterial social interactions in biofilms. *Sensors* (Basel) 2012; **12**: 2519-2538 [PMID: 22736963 DOI: 10.3390/s120302519]
42 **Oogai Y**, Matsuo M, Hashimoto M, Kato F, Sugai M, Komatsuzawa H. Expression of virulence factors by Staphylococcus aureus grown in serum. *Appl Environ Microbiol* 2011; **77**: 8097-8105 [PMID: 21926198 DOI: 10.1128/AEM.05316-11]
43 **Bjarnsholt T**, van Gennip M, Jakobsen TH, Christensen LD, Jensen PØ, Givskov M. In vitro screens for quorum sensing inhibitors and in vivo confirmation of their effect. *Nat Protoc* 2010; **5**: 282-293 [PMID: 20134428 DOI: 10.1038/nprot.2009.205]
44 **Papaioannou E**, Utari PD, Quax WJ. Choosing an appropriate infection model to study quorum sensing inhibition in Pseudomonas infections. *Int J Mol Sci* 2013; **14**: 19309-19340 [PMID: 24065108 DOI: 10.3390/ijms140919309]
45 **Tan MW**, Mahajan-Miklos S, Ausubel FM. Killing of Caenorhabditis elegans by Pseudomonas aeruginosa used to model mammalian bacterial pathogenesis. *Proc Natl Acad Sci USA* 1999; **96**: 715-720 [PMID: 9892699 DOI: 10.1073/pnas.96.2.715]
46 **Mahajan-Miklos S**, Tan MW, Rahme LG, Ausubel FM. Molecular mechanisms of bacterial virulence elucidated using a Pseudomonas aeruginosa-Caenorhabditis elegans pathogenesis model. *Cell* 1999; **96**: 47-56 [PMID: 9989496 DOI: 10.1016/S0092-8674(00)80958-7]
47 **Darby C**, Cosma CL, Thomas JH, Manoil C. Lethal paralysis of Caenorhabditis elegans by Pseudomonas aeruginosa. *Proc Natl Acad Sci USA* 1999; **96**: 15202-15207 [PMID: 10611362 DOI: 10.1073/pnas.96.26.15202]
48 **O'Loughlin CT**, Miller LC, Siryaporn A, Drescher K, Semmelhack MF, Bassler BL. A quorum-sensing inhibitor blocks Pseudomonas aeruginosa virulence and biofilm formation. *Proc Natl Acad Sci USA* 2013; **110**: 17981-17986 [PMID: 24143808 DOI: 10.1073/pnas.1316981110]
49 **Zaborin A**, Romanowski K, Gerdes S, Holbrook C, Lepine F, Long J, Poroyko V, Diggle SP, Wilke A, Righetti K, Morozova I, Babrowski T, Liu DC, Zaborina O, Alverdy JC. Red death in Caenorhabditis elegans caused by Pseudomonas aeruginosa PAO1. *Proc Natl Acad Sci USA* 2009; **106**: 6327-6332 [PMID: 19369215 DOI: 10.1073/pnas.0813199106]
50 **Chandel AK**, Rao LV, Narasu ML, Singh OV. The realm of penicillin G acylase in β-lactam antibiotics. *Enzyme and Microbial Technology* 2008; **42**: 199-207 [DOI: 10.1016/j.enzmictec.2007.11.013]
51 **Musthafa KS**, Sivamaruthi BS, Pandian SK, Ravi AV. Quorum sensing inhibition in Pseudomonas aeruginosa PAO1 by antagonistic compound phenylacetic acid. *Curr Microbiol* 2012; **65**: 475-480 [PMID: 22782469 DOI: 10.1007/s00284-012-0181-9]
52 **Musthafa KS**, Balamurugan K, Pandian SK, Ravi AV. 2,5-Piperazinedione inhibits quorum sensing-dependent factor production in Pseudomonas aeruginosa PAO1. *J Basic Microbiol* 2012; **52**: 679-686 [PMID: 22359266 DOI: 10.1002/jobm.201100292]
53 **Rudrappa T**, Bais HP. Curcumin, a known phenolic from Curcuma longa, attenuates the virulence of Pseudomonas aeruginosa PAO1 in whole plant and animal pathogenicity models. *J Agric Food Chem* 2008; **56**: 1955-1962 [PMID: 18284200 DOI: 10.1021/jf072591j]
54 **Papaioannou E**, Wahjudi M, Nadal-Jimenez P, Koch G, Setroikromo R, Quax WJ. Quorum-quenching acylase reduces the virulence of Pseudomonas aeruginosa in a Caenorhabditis elegans infection model. *Antimicrob Agents Chemother* 2009; **53**: 4891-4897 [PMID: 19721066 DOI: 10.1128/AAC.00380-09]
55 **D'Argenio DA**, Gallagher LA, Berg CA, Manoil C. Drosophila as a model host for Pseudomonas aeruginosa infection. *J Bacteriol* 2001; **183**: 1466-1471 [PMID: 11157963 DOI: 10.1128/JB.183.4.1466-1471.2001]
56 **Apidianakis Y**, Rahme LG. Drosophila melanogaster as a model host for studying Pseudomonas aeruginosa infection. *Nat Protoc* 2009; **4**: 1285-1294 [PMID: 19680242 DOI: 10.1038/nprot.2009.124]
57 **Lutter EI**, Purighalla S, Duong J, Storey DG. Lethality and cooperation of Pseudomonas aeruginosa quorum-sensing mutants in Drosophila melanogaster infection models. *Microbiology* 2012; **158**: 2125-2132 [PMID: 22628480 DOI: 10.1099/mic.0.054999-0]
58 **Clatworthy AE**, Lee JS, Leibman M, Kostun Z, Davidson AJ, Hung DT. Pseudomonas aeruginosa infection of zebrafish involves both host and pathogen determinants. *Infect Immun* 2009; **77**: 1293-1303 [PMID: 19168742 DOI: 10.1128/IAI.01181-08]
59 **Rumbaugh KP**, Griswold JA, Iglewski BH, Hamood AN. Contribution of quorum sensing to the virulence of Pseudomonas aeruginosa in burn wound infections. *Infect Immun* 1999; **67**: 5854-5862 [PMID: 10531240]
60 **Rumbaugh KP**, Griswold JA, Hamood AN. Contribution of the regulatory gene lasR to the pathogenesis of Pseudomonas aeruginosa infection of burned mice. *J Burn Care Rehabil* 1999; **20**: 42-49 [PMID: 9934636 DOI: 10.1097/00004630-199901001-00008]
61 **Lesic B**, Lépine F, Déziel E, Zhang J, Zhang Q, Padfield K, Castonguay MH, Milot S, Stachel S, Tzika AA, Tompkins RG, Rahme LG. Inhibitors of pathogen intercellular signals as selective anti-infective compounds. *PLoS Pathog* 2007; **3**: 1229-1239 [PMID: 17941706 DOI: 10.1371/journal.ppat.0030126]
62 **Tang HB**, DiMango E, Bryan R, Gambello M, Iglewski BH, Goldberg JB, Prince A. Contribution of specific Pseudomonas aeruginosa virulence factors to pathogenesis of pneumonia in a neonatal mouse model of infection. *Infect Immun* 1996; **64**: 37-43 [PMID: 8557368]
63 **Christensen LD**, Moser C, Jensen PØ, Rasmussen TB, Christophersen L, Kjelleberg S, Kumar N, Høiby N, Givskov M, Bjarnsholt T. Impact of Pseudomonas aeruginosa quorum sensing on biofilm persistence in an in vivo intraperitoneal foreign-body infection model. *Microbiology* 2007; **153**: 2312-2320 [PMID: 17600075 DOI: 10.1099/mic.0.2007/006122-0]
64 **Wu H**, Song Z, Hentzer M, Andersen JB, Molin S, Givskov M, Høiby N. Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in Pseudomonas aeruginosa lung infection in mice. *J Antimicrob Chemother* 2004; **53**: 1054-1061 [PMID: 15117922 DOI: 10.1093/jac/dkh223]
65 **Jakobsen TH**, van Gennip M, Phipps RK, Shanmugham MS, Christensen LD, Alhede M, Skindersoe ME, Rasmussen TB, Friedrich K, Uthe F, Jensen PØ, Moser C, Nielsen KF, Eberl L, Larsen TO, Tanner D, Høiby N, Bjarnsholt T, Givskov M. Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. *Antimicrob Agents Chemother* 2012; **56**: 2314-2325 [PMID: 22314537 DOI: 10.1128/AAC.05919-11]
66 **Schaber JA**, Carty NL, McDonald NA, Graham ED, Cheluvappa R, Griswold JA, Hamood AN. Analysis of quorum sensing-deficient clinical isolates of Pseudomonas aeruginosa. *J Med Microbiol* 2004; **53**: 841-853 [PMID: 15314190 DOI: 10.1099/jmm.0.45617-0]
67 **Dénervaud V**, TuQuoc P, Blanc D, Favre-Bonté S, Krishnapillai V,

Reimmann C, Haas D, van Delden C. Characterization of cell-to-cell signaling-deficient Pseudomonas aeruginosa strains colonizing intubated patients. *J Clin Microbiol* 2004; **42**: 554-562 [PMID: 14766816 DOI: 10.1128/JCM.42.2.554-562.2004]

68 **Karatuna O**, Yagci A. Analysis of quorum sensing-dependent virulence factor production and its relationship with antimicrobial susceptibility in Pseudomonas aeruginosa respiratory isolates. *Clin Microbiol Infect* 2010; **16**: 1770-1775 [PMID: 20132256 DOI: 10.1111/j.1469-0691.2010.03177.x]

69 **Senturk S**, Ulusoy S, Bosgelmez-Tinaz G, Yagci A. Quorum sensing and virulence of Pseudomonas aeruginosa during urinary tract infections. *J Infect Dev Ctries* 2012; **6**: 501-507 [PMID: 22706193]

70 **Davies DG**, Marques CN. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. *J Bacteriol* 2009; **191**: 1393-1403 [PMID: 19074399 DOI: 10.1128/JB.01214-08]

71 **Wilder CN**, Allada G, Schuster M. Instantaneous within-patient diversity of Pseudomonas aeruginosa quorum-sensing populations from cystic fibrosis lung infections. *Infect Immun* 2009; **77**: 5631-5639 [PMID: 19805523 DOI: 10.1128/IAI.00755-09]

72 **García-Contreras R**, Martínez-Vázquez M, Velázquez Guadarrama N, Villegas Pañeda AG, Hashimoto T, Maeda T, Quezada H, Wood TK. Resistance to the quorum-quenching compounds brominated furanone C-30 and 5-fluorouracil in Pseudomonas aeruginosa clinical isolates. *Pathog Dis* 2013; **68**: 8-11 [PMID: 23620228 DOI: 10.1111/2049-632X.12039]

73 **Rampioni G**, Leoni L, Williams P. The art of antibacterial warfare: Deception through interference with quorum sensing-mediated communication. *Bioorg Chem* 2014; **55**: 60-68 [PMID: 24823895]

74 **Lee DG**, Urbach JM, Wu G, Liberati NT, Feinbaum RL, Miyata S, Diggins LT, He J, Saucier M, Déziel E, Friedman L, Li L, Grills G, Montgomery K, Kucherlapati R, Rahme LG, Ausubel FM. Genomic analysis reveals that Pseudomonas aeruginosa virulence is combinatorial. *Genome Biol* 2006; **7**: R90 [PMID: 17038190 DOI: 10.1186/gb-2006-7-10-r90]

75 **Singh PK**, Schaefer AL, Parsek MR, Moninger TO, Welsh MJ, Greenberg EP. Quorum-sensing signals indicate that cystic fibrosis lungs are infected with bacterial biofilms. *Nature* 2000; **407**: 762-764 [PMID: 11048725 DOI: 10.1038/35037627]

76 **Geisenberger O**, Givskov M, Riedel K, Høiby N, Tümmler B, Eberl L. Production of N-acyl-L-homoserine lactones by P. aeruginosa isolates from chronic lung infections associated with cystic fibrosis. *FEMS Microbiol Lett* 2000; **184**: 273-278 [PMID: 10713433]

77 **Favre-Bonté S**, Pache JC, Robert J, Blanc D, Pechère JC, van Delden C. Detection of Pseudomonas aeruginosa cell-to-cell signals in lung tissue of cystic fibrosis patients. *Microb Pathog* 2002; **32**: 143-147 [PMID: 11855945 DOI: 10.1006/mpat.2001.0487]

78 **Storey DG**, Ujack EE, Rabin HR, Mitchell I. Pseudomonas aeruginosa lasR transcription correlates with the transcription of lasA, lasB, and toxA in chronic lung infections associated with cystic fibrosis. *Infect Immun* 1998; **66**: 2521-2528 [PMID: 9596711]

79 **Erickson DL**, Endersby R, Kirkham A, Stuber K, Vollman DD, Rabin HR, Mitchell I, Storey DG. Pseudomonas aeruginosa quorum-sensing systems may control virulence factor expression in the lungs of patients with cystic fibrosis. *Infect Immun* 2002; **70**: 1783-1790 [PMID: 11895939 DOI: 10.1128/IAI.70.4.1783-1790.2002]

80 **Li Y**, Qu HP, Liu JL, Wan HY. Correlation between group behavior and quorum sensing in Pseudomonas aeruginosa isolated from patients with hospital-acquired pneumonia. *J Thorac Dis* 2014; **6**: 810-817 [PMID: 24977007]

81 **Laarman AJ**, Bardoel BW, Ruyken M, Fernie J, Milder FJ, van Strijp JA, Rooijakkers SH. Pseudomonas aeruginosa alkaline protease blocks complement activation via the classical and lectin pathways. *J Immunol* 2012; **188**: 386-393 [PMID: 22131330 DOI: 10.4049/jimmunol.1102162]

82 **Kuang Z**, Hao Y, Walling BE, Jeffries JL, Ohman DE, Lau GW. Pseudomonas aeruginosa elastase provides an escape from phagocytosis by degrading the pulmonary surfactant protein-A. *PLoS One* 2011; **6**: e27091 [PMID: 22069491 DOI: 10.1371/journal.pone.0027091]

83 **Dössel J**, Meyer-Hoffert U, Schröder JM, Gerstel U. Pseudomonas aeruginosa-derived rhamnolipids subvert the host innate immune response through manipulation of the human beta-defensin-2 expression. *Cell Microbiol* 2012; **14**: 1364-1375 [PMID: 22500651 DOI: 10.1111/j.1462-5822.2012.01801.x]

84 **Köhler T**, Guanella R, Carlet J, van Delden C. Quorum sensing-dependent virulence during Pseudomonas aeruginosa colonisation and pneumonia in mechanically ventilated patients. *Thorax* 2010; **65**: 703-710 [PMID: 20685744 DOI: 10.1136/thx.2009.133082]

85 **Leid JG**, Willson CJ, Shirtliff ME, Hassett DJ, Parsek MR, Jeffers AK. The exopolysaccharide alginate protects Pseudomonas aeruginosa biofilm bacteria from IFN-gamma-mediated macrophage killing. *J Immunol* 2005; **175**: 7512-7518 [PMID: 16301659 DOI: 10.4049/jimmunol.175.11.7512]

86 **Lau GW**, Hassett DJ, Ran H, Kong F. The role of pyocyanin in Pseudomonas aeruginosa infection. *Trends Mol Med* 2004; **10**: 599-606 [PMID: 15567330 DOI: 10.1016/j.molmed.2004.10.002]

87 **Abdelnour A**, Arvidson S, Bremell T, Rydén C, Tarkowski A. The accessory gene regulator (agr) controls Staphylococcus aureus virulence in a murine arthritis model. *Infect Immun* 1993; **61**: 3879-3885 [PMID: 8359909]

88 **Booth MC**, Atkuri RV, Nanda SK, Iandolo JJ, Gilmore MS. Accessory gene regulator controls Staphylococcus aureus virulence in endophthalmitis. *Invest Ophthalmol Vis Sci* 1995; **36**: 1828-1836 [PMID: 7635657]

89 **Booth MC**, Cheung AL, Hatter KL, Jett BD, Callegan MC, Gilmore MS. Staphylococcal accessory regulator (sar) in conjunction with agr contributes to Staphylococcus aureus virulence in endophthalmitis. *Infect Immun* 1997; **65**: 1550-1556 [PMID: 9119503]

90 **Cheung AL**, Eberhardt KJ, Chung E, Yeaman MR, Sullam PM, Ramos M, Bayer AS. Diminished virulence of a sar-/agr- mutant of Staphylococcus aureus in the rabbit model of endocarditis. *J Clin Invest* 1994; **94**: 1815-1822 [PMID: 7962526 DOI: 10.1172/JCI117530]

91 **Kielian T**, Cheung A, Hickey WF. Diminished virulence of an alpha-toxin mutant of Staphylococcus aureus in experimental brain abscesses. *Infect Immun* 2001; **69**: 6902-6911 [PMID: 11598065 DOI: 10.1128/IAI.69.11.6902-6911.2001]

92 **Sifri CD**, Begun J, Ausubel FM, Calderwood SB. Caenorhabditis elegans as a model host for Staphylococcus aureus pathogenesis. *Infect Immun* 2003; **71**: 2208-2217 [PMID: 12654843 DOI: 10.1128/IAI.71.4.2208-2217.2003]

93 **Wright JS**, Jin R, Novick RP. Transient interference with staphylococcal quorum sensing blocks abscess formation. *Proc Natl Acad Sci USA* 2005; **102**: 1691-1696 [PMID: 15665088 DOI: 10.1073/pnas.0407661102]

94 **Balaban N**, Goldkorn T, Gov Y, Hirshberg M, Koyfman N, Matthews HR, Nhan RT, Singh B, Uziel O. Regulation of Staphylococcus aureus pathogenesis via target of RNAIII-activating Protein (TRAP). *J Biol Chem* 2001; **276**: 2658-2667 [PMID: 11160124]

95 **Yang G**, Cheng H, Liu C, Xue Y, Gao Y, Liu N, Gao B, Wang D, Li S, Shen B, Shao N. Inhibition of Staphylococcus aureus pathogenesis in vitro and in vivo by RAP-binding peptides. *Peptides* 2003; **24**: 1823-1828 [PMID: 15019215 DOI: 10.1016/j.peptides.2003.09.017]

96 **Giacometti A**, Cirioni O, Ghiselli R, Dell'Acqua G, Orlando F, D'Amato G, Mocchegiani F, Silvestri C, Del Prete MS, Rocchi M, Balaban N, Saba V, Scalise G. RNAIII-inhibiting peptide improves efficacy of clinically used antibiotics in a murine model of staphylococcal sepsis. *Peptides* 2005; **26**: 169-175 [PMID: 15629527 DOI: 10.1016/j.peptides.2004.09.018]

97 **Cirioni O**, Giacometti A, Ghiselli R, Dell'Acqua G, Gov Y, Kamysz W, Lukasiak J, Mocchegiani F, Orlando F, D'Amato G, Balaban N, Saba V, Scalise G. Prophylactic efficacy of topical temporin A and RNAIII-inhibiting peptide in a subcutaneous rat Pouch model of graft infection attributable to staphylococci with intermediate resistance to glycopeptides. *Circulation* 2003; **108**: 767-771 [PMID:

12885754 DOI: 10.1161/01.CIR.0000083717.85060.16]
98 **Cirioni O**, Mocchegiani F, Cacciatore I, Vecchiet J, Silvestri C, Baldassarre L, Ucciferri C, Orsetti E, Castelli P, Provinciali M, Vivarelli M, Fornasari E, Giacometti A. Quorum sensing inhibitor FS3-coated vascular graft enhances daptomycin efficacy in a rat model of staphylococcal infection. *Peptides* 2013; **40**: 77-81 [PMID: 23262356 DOI: 10.1016/j.peptides.2012.12.002]
99 **Simonetti O**, Cirioni O, Mocchegiani F, Cacciatore I, Silvestri C, Baldassarre L, Orlando F, Castelli P, Provinciali M, Vivarelli M, Fornasari E, Giacometti A, Offidani A. The efficacy of the quorum sensing inhibitor FS8 and tigecycline in preventing prosthesis biofilm in an animal model of staphylococcal infection. *Int J Mol Sci* 2013; **14**: 16321-16332 [PMID: 23965956 DOI: 10.3390/ijms140816321]
100 **Fowler VG**, Justice A, Moore C, Benjamin DK, Woods CW, Campbell S, Reller LB, Corey GR, Day NP, Peacock SJ. Risk factors for hematogenous complications of intravascular catheter-associated Staphylococcus aureus bacteremia. *Clin Infect Dis* 2005; **40**: 695-703 [PMID: 15714415 DOI: 10.1086/427806]
101 **Fowler VG**, Sakoulas G, McIntyre LM, Meka VG, Arbeit RD, Cabell CH, Stryjewski ME, Eliopoulos GM, Reller LB, Corey GR, Jones T, Lucindo N, Yeaman MR, Bayer AS. Persistent bacteremia due to methicillin-resistant Staphylococcus aureus infection is associated with agr dysfunction and low-level in vitro resistance to thrombin-induced platelet microbicidal protein. *J Infect Dis* 2004; **190**: 1140-1149 [PMID: 15319865 DOI: 10.1086/423145]
102 **Sakoulas G**, Eliopoulos GM, Moellering RC, Wennersten C, Venkataraman L, Novick RP, Gold HS. Accessory gene regulator (agr) locus in geographically diverse Staphylococcus aureus isolates with reduced susceptibility to vancomycin. *Antimicrob Agents Chemother* 2002; **46**: 1492-1502 [PMID: 11959587 DOI: 10.1128/AAC.46.5.1492-1502.2002]
103 **Traber KE**, Lee E, Benson S, Corrigan R, Cantera M, Shopsin B, Novick RP. agr function in clinical Staphylococcus aureus isolates. *Microbiology* 2008; **154**: 2265-2274 [PMID: 18667559 DOI: 10.1099/mic.0.2007/011874-0]
104 **Hentzer M**, Wu H, Andersen JB, Riedel K, Rasmussen TB, Bagge N, Kumar N, Schembri MA, Song Z, Kristoffersen P, Manefield M, Costerton JW, Molin S, Eberl L, Steinberg P, Kjelleberg S, Høiby N, Givskov M. Attenuation of Pseudomonas aeruginosa virulence by quorum sensing inhibitors. *EMBO J* 2003; **22**: 3803-3815 [PMID: 12881415 DOI: 10.1093/emboj/cdg366]
105 **Hentzer M**, Riedel K, Rasmussen TB, Heydorn A, Andersen JB, Parsek MR, Rice SA, Eberl L, Molin S, Høiby N, Kjelleberg S, Givskov M. Inhibition of quorum sensing in Pseudomonas aeruginosa biofilm bacteria by a halogenated furanone compound. *Microbiology* 2002; **148**: 87-102 [PMID: 11782502]
106 **Maeda T**, García-Contreras R, Pu M, Sheng L, Garcia LR, Tomás M, Wood TK. Quorum quenching quandary: resistance to antivirulence compounds. *ISME J* 2012; **6**: 493-501 [PMID: 21918575 DOI: 10.1038/ismej.2011.122]
107 **García-Contreras R**, Maeda T, Wood TK. Resistance to quorum-quenching compounds. *Appl Environ Microbiol* 2013; **79**: 6840-6846 [PMID: 24014536 DOI: 10.1128/AEM.02378-13]
108 **Lee J**, Attila C, Cirillo SL, Cirillo JD, Wood TK. Indole and 7-hydroxyindole diminish Pseudomonas aeruginosa virulence. *Microb Biotechnol* 2009; **2**: 75-90 [PMID: 21261883 DOI: 10.1111/j.1751-7915.2008.00061.x]
109 **Lee JH**, Kim YG, Cho MH, Kim JA, Lee J. 7-fluoroindole as an antivirulence compound against Pseudomonas aeruginosa. *FEMS Microbiol Lett* 2012; **329**: 36-44 [PMID: 22251040 DOI: 10.1111/j.1574-6968.2012.02500.x]
110 **Ueda A**, Attila C, Whiteley M, Wood TK. Uracil influences quorum sensing and biofilm formation in Pseudomonas aeruginosa and fluorouracil is an antagonist. *Microb Biotechnol* 2009; **2**: 62-74 [PMID: 21261882 DOI: 10.1111/j.1751-7915.2008.00060.x]
111 **Swem LR**, Swem DL, O'Loughlin CT, Gatmaitan R, Zhao B, Ulrich SM, Bassler BL. A quorum-sensing antagonist targets both membrane-bound and cytoplasmic receptors and controls bacterial pathogenicity. *Mol Cell* 2009; **35**: 143-153 [PMID: 19647512 DOI: 10.1016/j.molcel.2009.05.029]
112 **Carteau D**, Soum-Soutéra E, Faÿ F, Dufau C, Cérantola S, Vallée-Réhel K. Monohalogenated maleimides as potential agents for the inhibition of Pseudomonas aeruginosa biofilm. *Biofouling* 2010; **26**: 379-385 [PMID: 20162472 DOI: 10.1080/08927011003653441]
113 **Miandji AM**, Ulusoy S, Dündar Y, Ozgen S, Onurdağ FK, Boşgelmez-Tınaz G, Noyanalpan N. Synthesis and Biological Activities of Some 1,3-Benzoxazol-2(3H)-One Derivatives as Anti-Quorum Sensing Agents. *Arzneimittelforschung* 2012; **62**: e2 [PMID: 22639381 DOI: 10.1055/s-0032-1312590]
114 **Yang L**, Rybtke MT, Jakobsen TH, Hentzer M, Bjarnsholt T, Givskov M, Tolker-Nielsen T. Computer-aided identification of recognized drugs as Pseudomonas aeruginosa quorum-sensing inhibitors. *Antimicrob Agents Chemother* 2009; **53**: 2432-2443 [PMID: 19364871 DOI: 10.1128/AAC.01283-08]
115 **Easwaran N**, Karthikeyan S, Sridharan B, Gothandam KM. Identification and analysis of the salt tolerant property of AHL lactonase (AiiATSAWB) of Bacillus species. *J Basic Microbiol* 2015; **55**: 579-590 [PMID: 25041996 DOI: 10.1002/jobm.201400013]
116 **Sakr MM**, Aboshanab KM, Aboulwafa MM, Hassouna NA. Characterization and complete sequence of lactonase enzyme from Bacillus weihenstephanensis isolate P65 with potential activity against acyl homoserine lactone signal molecules. *Biomed Res Int* 2013; **2013**: 192589 [PMID: 23936779]
117 **Gupta P**, Chhibber S, Harjai K. Efficacy of purified lactonase and ciprofloxacin in preventing systemic spread of Pseudomonas aeruginosa in murine burn wound model. *Burns* 2015; **41**: 153-162 [PMID: 25015706]
118 **Kisch JM**, Utpatel C, Hilterhaus L, Streit WR, Liese A. Pseudomonas aeruginosa biofilm growth inhibition on medical plastic materials by immobilized esterases and acylase. *Chembiochem* 2014; **15**: 1911-1919 [PMID: 25044227]
119 **Joshi S**, Kaur A, Sharma P, Harjai K, Capalash N. Lactonase-expressing Lactobacillus plantarum NC8 attenuates the virulence factors of multiple drug resistant Pseudomonas aeruginosa in co-culturing environment. *World J Microbiol Biotechnol* 2014; **30**: 2241-2249 [PMID: 24671300 DOI: 10.1007/s11274-014-1645-9]
120 **Migiyama Y**, Kaneko Y, Yanagihara K, Morohoshi T, Morinaga Y, Nakamura S, Miyazaki T, Hasegawa H, Izumikawa K, Kakeya H, Kohrogi H, Kohno S. Efficacy of AiiM, an N-acylhomoserine lactonase, against Pseudomonas aeruginosa in a mouse model of acute pneumonia. *Antimicrob Agents Chemother* 2013; **57**: 3653-3658 [PMID: 23689715 DOI: 10.1128/AAC.00456-13]
121 **Pei R**, Lamas-Samanamud GR. Inhibition of biofilm formation by T7 bacteriophages producing quorum-quenching enzymes. *Appl Environ Microbiol* 2014; **80**: 5340-5348 [PMID: 24951790 DOI: 10.1128/AEM.01434-14]
122 **Müh U**, Schuster M, Heim R, Singh A, Olson ER, Greenberg EP. Novel Pseudomonas aeruginosa quorum-sensing inhibitors identified in an ultra-high-throughput screen. *Antimicrob Agents Chemother* 2006; **50**: 3674-3679 [PMID: 16966394 DOI: 10.1128/AAC.00665-06]
123 **Imperi F**, Massai F, Ramachandran Pillai C, Longo F, Zennaro E, Rampioni G, Visca P, Leoni L. New life for an old drug: the anthelmintic drug niclosamide inhibits Pseudomonas aeruginosa quorum sensing. *Antimicrob Agents Chemother* 2013; **57**: 996-1005 [PMID: 23254430 DOI: 10.1128/AAC.01952-12]
124 **Skindersoe ME**, Alhede M, Phipps R, Yang L, Jensen PO, Rasmussen TB, Bjarnsholt T, Tolker-Nielsen T, Høiby N, Givskov M. Effects of antibiotics on quorum sensing in Pseudomonas aeruginosa. *Antimicrob Agents Chemother* 2008; **52**: 3648-3663 [PMID: 18644954 DOI: 10.1128/AAC.01230-07]
125 **Babić F**, Venturi V, Maravić-Vlahovicek G. Tobramycin at subinhibitory concentration inhibits the RhlI/R quorum sensing system in a Pseudomonas aeruginosa environmental isolate. *BMC Infect Dis* 2010; **10**: 148 [PMID: 20525206 DOI: 10.1186/1471-2334-10-148]
126 **Hoffman LR**, D'Argenio DA, MacCoss MJ, Zhang Z, Jones RA, Miller SI. Aminoglycoside antibiotics induce bacterial biofilm formation. *Nature* 2005; **436**: 1171-1175 [PMID: 16121184 DOI:

10.1038/nature03912]
127 **Taha MO**, Al-Bakri AG, Zalloum WA. Discovery of potent inhibitors of pseudomonal quorum sensing via pharmacophore modeling and in silico screening. *Bioorg Med Chem Lett* 2006; **16**: 5902-5906 [PMID: 16945524 DOI: 10.1016/j.bmcl.2006.08.069]
128 **Zeng Z**, Qian L, Cao L, Tan H, Huang Y, Xue X, Shen Y, Zhou S. Virtual screening for novel quorum sensing inhibitors to eradicate biofilm formation of Pseudomonas aeruginosa. *Appl Microbiol Biotechnol* 2008; **79**: 119-126 [PMID: 18330563 DOI: 10.1007/s00253-008-1406-5]
129 **Tan SY**, Chua SL, Chen Y, Rice SA, Kjelleberg S, Nielsen TE, Yang L, Givskov M. Identification of five structurally unrelated quorum-sensing inhibitors of Pseudomonas aeruginosa from a natural-derivative database. *Antimicrob Agents Chemother* 2013; **57**: 5629-5641 [PMID: 24002091 DOI: 10.1128/AAC.00955-13]
130 **Rasmussen TB**, Skindersoe ME, Bjarnsholt T, Phipps RK, Christensen KB, Jensen PO, Andersen JB, Koch B, Larsen TO, Hentzer M, Eberl L, Hoiby N, Givskov M. Identity and effects of quorum-sensing inhibitors produced by Penicillium species. *Microbiology* 2005; **151**: 1325-1340 [PMID: 15870443 DOI: 10.1099/mic.0.27715-0]
131 **Winsor GL**, Lam DK, Fleming L, Lo R, Whiteside MD, Yu NY, Hancock RE, Brinkman FS. Pseudomonas Genome Database: improved comparative analysis and population genomics capability for Pseudomonas genomes. *Nucleic Acids Res* 2011; **39**: D596-D600 [PMID: 20929876 DOI: 10.1093/nar/gkq869]
132 **Rasmussen TB**, Bjarnsholt T, Skindersoe ME, Hentzer M, Kristoffersen P, Köte M, Nielsen J, Eberl L, Givskov M. Screening for quorum-sensing inhibitors (QSI) by use of a novel genetic system, the QSI selector. *J Bacteriol* 2005; **187**: 1799-1814 [PMID: 15716452 DOI: 10.1128/JB.187.5.1799-1814.2005]
133 **Vanoyan N**, Walker SL, Gillor O, Herzberg M. Reduced bacterial deposition and attachment by quorum-sensing inhibitor 4-nitro-pyridine-N-oxide: the role of physicochemical effects. *Langmuir* 2010; **26**: 12089-12094 [PMID: 20553026 DOI: 10.1021/la101319e]
134 **Khalilzadeh P**, Lajoie B, El Hage S, Furiga A, Baziard G, Berge M, Roques C. Growth inhibition of adherent Pseudomonas aeruginosa by an N-butanoyl-L-homoserine lactone analog. *Can J Microbiol* 2010; **56**: 317-325 [PMID: 20453898 DOI: 10.1139/W10-013]
135 **Yang YX**, Xu ZH, Zhang YQ, Tian J, Weng LX, Wang LH. A new quorum-sensing inhibitor attenuates virulence and decreases antibiotic resistance in Pseudomonas aeruginosa. *J Microbiol* 2012; **50**: 987-993 [PMID: 23274985 DOI: 10.1007/s12275-012-2149-7]
136 **Shouldice SR**, Heras B, Jarrott R, Sharma P, Scanlon MJ, Martin JL. Characterization of the DsbA oxidative folding catalyst from Pseudomonas aeruginosa reveals a highly oxidizing protein that binds small molecules. *Antioxid Redox Signal* 2010; **12**: 921-931 [PMID: 19788398 DOI: 10.1089/ars.2009.2736]
137 **Turgeon Z**, Jørgensen R, Visschedyk D, Edwards PR, Legree S, McGregor C, Fieldhouse RJ, Mangroo D, Schapira M, Merrill AR. Newly discovered and characterized antivirulence compounds inhibit bacterial mono-ADP-ribosyltransferase toxins. *Antimicrob Agents Chemother* 2011; **55**: 983-991 [PMID: 21135177 DOI: 10.1128/AAC.01164-10]
138 **Jagtap P**, Soriano FG, Virág L, Liaudet L, Mabley J, Szabó E, Haskó G, Marton A, Lorigados CB, Gallyas F, Sümegi B, Hoyt DG, Baloglu E, VanDuzer J, Salzman AL, Southan GJ, Szabó C. Novel phenanthridinone inhibitors of poly (adenosine 5'-diphosphate-ribose) synthetase: potent cytoprotective and antishock agents. *Crit Care Med* 2002; **30**: 1071-1082 [PMID: 12006805 DOI: 10.1097/00003246-200205000-00019]
139 **Lu C**, Kirsch B, Zimmer C, de Jong JC, Henn C, Maurer CK, Müsken M, Häussler S, Steinbach A, Hartmann RW. Discovery of antagonists of PqsR, a key player in 2-alkyl-4-quinolone-dependent quorum sensing in Pseudomonas aeruginosa. *Chem Biol* 2012; **19**: 381-390 [PMID: 22444593 DOI: 10.1016/j.chembiol.2012.01.015]
140 **Nithya C**, Begum MF, Pandian SK. Marine bacterial isolates inhibit biofilm formation and disrupt mature biofilms of Pseudomonas aeruginosa PAO1. *Appl Microbiol Biotechnol* 2010; **88**: 341-358 [PMID: 20665017 DOI: 10.1007/s00253-010-2777-y]
141 **Christiaen SE**, Brackman G, Nelis HJ, Coenye T. Isolation and identification of quorum quenching bacteria from environmental samples. *J Microbiol Methods* 2011; **87**: 213-219 [PMID: 21856341 DOI: 10.1016/j.mimet.2011.08.002]
142 **Naik DN**, Wahidullah S, Meena RM. Attenuation of Pseudomonas aeruginosa virulence by marine invertebrate-derived Streptomyces sp. *Lett Appl Microbiol* 2013; **56**: 197-207 [PMID: 23210926 DOI: 10.1111/lam.12034]
143 **Golberg K**, Pavlov V, Marks RS, Kushmaro A. Coral-associated bacteria, quorum sensing disrupters, and the regulation of biofouling. *Biofouling* 2013; **29**: 669-682 [PMID: 23777289 DOI: 10.1080/08927014.2013.796939]
144 **Rajesh PS**, Ravishankar Rai V. Quorum quenching activity in cell-free lysate of endophytic bacteria isolated from Pterocarpus santalinus Linn., and its effect on quorum sensing regulated biofilm in Pseudomonas aeruginosa PAO1. *Microbiol Res* 2014; **169**: 561-569 [PMID: 24268182 DOI: 10.1016/j.micres.2013.10.005]
145 **Kwan JC**, Meickle T, Ladwa D, Teplitski M, Paul V, Luesch H. Lyngbyoic acid, a "tagged" fatty acid from a marine cyanobacterium, disrupts quorum sensing in Pseudomonas aeruginosa. *Mol Biosyst* 2011; **7**: 1205-1216 [PMID: 21258753 DOI: 10.1039/c0mb00180e]
146 **Luna JM**, Rufino RD, Sarubbo LA, Rodrigues LR, Teixeira JA, de Campos-Takaki GM. Evaluation antimicrobial and antiadhesive properties of the biosurfactant Lunasan produced by Candida sphaerica UCP 0995. *Curr Microbiol* 2011; **62**: 1527-1534 [PMID: 21327556 DOI: 10.1007/s00284-011-9889-1]
147 **Deakin SP**, Ducret V, Bioletto S, Perron K, James RW. Modulating reconstituted high density lipoprotein functionality to target the Pseudomonas aeruginosa quorum sensing system. *Life Sci* 2014; **112**: 68-73 [PMID: 25086378 DOI: 10.1016/j.lfs.2014.07.028]
148 **Nafee N**, Husari A, Maurer CK, Lu C, de Rossi C, Steinbach A, Hartmann RW, Lehr CM, Schneider M. Antibiotic-free nanotherapeutics: ultra-small, mucus-penetrating solid lipid nanoparticles enhance the pulmonary delivery and anti-virulence efficacy of novel quorum sensing inhibitors. *J Control Release* 2014; **192**: 131-140 [PMID: 24997276 DOI: 10.1016/j.jconrel.2014.06.055]
149 **Musthafa KS**, Ravi AV, Annapoorani A, Packiavathy IS, Pandian SK. Evaluation of anti-quorum-sensing activity of edible plants and fruits through inhibition of the N-acyl-homoserine lactone system in Chromobacterium violaceum and Pseudomonas aeruginosa. *Chemotherapy* 2010; **56**: 333-339 [PMID: 20720417 DOI: 10.1159/000320185]
150 **Wang Z**, Yu P, Zhang G, Xu L, Wang D, Wang L, Zeng X, Wang Y. Design, synthesis and antibacterial activity of novel andrographolide derivatives. *Bioorg Med Chem* 2010; **18**: 4269-4274 [PMID: 20493714 DOI: 10.1016/j.bmc.2010.04.094]
151 **Ding X**, Yin B, Qian L, Zeng Z, Yang Z, Li H, Lu Y, Zhou S. Screening for novel quorum-sensing inhibitors to interfere with the formation of Pseudomonas aeruginosa biofilm. *J Med Microbiol* 2011; **60**: 1827-1834 [PMID: 21852522 DOI: 10.1099/jmm.0.024166-0]
152 **Amaya S**, Pereira JA, Borkosky SA, Valdez JC, Bardón A, Arena ME. Inhibition of quorum sensing in Pseudomonas aeruginosa by sesquiterpene lactones. *Phytomedicine* 2012; **19**: 1173-1177 [PMID: 22925726 DOI: 10.1016/j.phymed.2012.07.003]
153 **Jakobsen TH**, Bragason SK, Phipps RK, Christensen LD, van Gennip M, Alhede M, Skindersoe M, Larsen TO, Høiby N, Bjarnsholt T, Givskov M. Food as a source for quorum sensing inhibitors: iberin from horseradish revealed as a quorum sensing inhibitor of Pseudomonas aeruginosa. *Appl Environ Microbiol* 2012; **78**: 2410-2421 [PMID: 22286987 DOI: 10.1128/AEM.05992-11]
154 **Lihua L**, Jianhuit W, Jialini Y, Yayin L, Guanxin L. Effects of allicin on the formation of Pseudomonas aeruginosa biofinm and the production of quorum-sensing controlled virulence factors. *Pol J Microbiol* 2013; **62**: 243-251 [PMID: 24459829]
155 **Kumar NV**, Murthy PS, Manjunatha JR, Bettadaiah BK. Synthesis and quorum sensing inhibitory activity of key phenolic compounds of ginger and their derivatives. *Food Chem* 2014; **159**: 451-457 [PMID: 24767081 DOI: 10.1016/j.foodchem.2014.03.039]

156 **Castillo-Juárez I**, García-Contreras R, Velázquez-Guadarrama N, Soto-Hernández M, Martínez-Vázquez M. Amphypterygium adstringens anacardic acid mixture inhibits quorum sensing-controlled virulence factors of Chromobacterium violaceum and Pseudomonas aeruginosa. *Arch Med Res* 2013; **44**: 488-494 [PMID: 24126126 DOI: 10.1016/j.arcmed.2013.10.004]

157 **Balaban N**, Cirioni O, Giacometti A, Ghiselli R, Braunstein JB, Silvestri C, Mocchegiani F, Saba V, Scalise G. Treatment of Staphylococcus aureus biofilm infection by the quorum-sensing inhibitor RIP. *Antimicrob Agents Chemother* 2007; **51**: 2226-2229 [PMID: 17371825 DOI: 10.1128/AAC.01097-06]

158 **Balaban N**, Gov Y, Bitler A, Boelaert JR. Prevention of Staphylococcus aureus biofilm on dialysis catheters and adherence to human cells. *Kidney Int* 2003; **63**: 340-345 [PMID: 12472801 DOI: 10.1046/j.1523-1755.2003.00733.x]

159 **Tal-Gan Y**, Stacy DM, Foegen MK, Koenig DW, Blackwell HE. Highly potent inhibitors of quorum sensing in Staphylococcus aureus revealed through a systematic synthetic study of the group-III autoinducing peptide. *J Am Chem Soc* 2013; **135**: 7869-7882 [PMID: 23647400 DOI: 10.1021/ja3112115]

160 **Khodaverdian V**, Pesho M, Truitt B, Bollinger L, Patel P, Nithianantham S, Yu G, Delaney E, Jankowsky E, Shoham M. Discovery of antivirulence agents against methicillin-resistant Staphylococcus aureus. *Antimicrob Agents Chemother* 2013; **57**: 3645-3652 [PMID: 23689713 DOI: 10.1128/AAC.00269-13]

161 **Walz JM**, Avelar RL, Longtine KJ, Carter KL, Mermel LA, Heard SO. Anti-infective external coating of central venous catheters: a randomized, noninferiority trial comparing 5-fluorouracil with chlorhexidine/silver sulfadiazine in preventing catheter colonization. *Crit Care Med* 2010; **38**: 2095-2102 [PMID: 20711070 DOI: 10.1097/CCM.0b013e3181f265ba]

162 **Attila C**, Ueda A, Wood TK. 5-Fluorouracil reduces biofilm formation in Escherichia coli K-12 through global regulator AriR as an antivirulence compound. *Appl Microbiol Biotechnol* 2009; **82**: 525-533 [PMID: 19172264 DOI: 10.1007/s00253-009-1860-8]

163 **Kiran MD**, Adikesavan NV, Cirioni O, Giacometti A, Silvestri C, Scalise G, Ghiselli R, Saba V, Orlando F, Shoham M, Balaban N. Discovery of a quorum-sensing inhibitor of drug-resistant staphylococcal infections by structure-based virtual screening. *Mol Pharmacol* 2008; **73**: 1578-1586 [PMID: 18314496 DOI: 10.1124/mol.107.044164]

164 **Lee K**, Lee JH, Kim SI, Cho MH, Lee J. Anti-biofilm, anti-hemolysis, and anti-virulence activities of black pepper, cananga, myrrh oils, and nerolidol against Staphylococcus aureus. *Appl Microbiol Biotechnol* 2014; **98**: 9447-9457 [PMID: 25027570]

165 **Lee K**, Lee JH, Ryu SY, Cho MH, Lee J. Stilbenes reduce Staphylococcus aureus hemolysis, biofilm formation, and virulence. *Foodborne Pathog Dis* 2014; **11**: 710-717 [PMID: 25007234 DOI: 10.1089/fpd.2014.1758]

166 **Nealson KH**, Hastings JW. Bacterial bioluminescence: its control and ecological significance. *Microbiol Rev* 1979; **43**: 496-518 [PMID: 396467]

167 **Visick KL**, Foster J, Doino J, McFall-Ngai M, Ruby EG. Vibrio fischeri lux genes play an important role in colonization and development of the host light organ. *J Bacteriol* 2000; **182**: 4578-4586 [PMID: 10913092 DOI: 10.1128/JB.182.16.4578-4586.2000]

168 **Milton DL**. Quorum sensing in vibrios: complexity for diversification. *Int J Med Microbiol* 2006; **296**: 61-71 [PMID: 16487746 DOI: 10.1016/j.ijmm.2006.01.044]

169 **Ng WL**, Bassler BL. Bacterial quorum-sensing network architectures. *Annu Rev Genet* 2009; **43**: 197-222 [PMID: 19686078 DOI: 10.1146/annurev-genet-102108-134304]

170 **LaSarre B**, Federle MJ. Exploiting quorum sensing to confuse bacterial pathogens. *Microbiol Mol Biol Rev* 2013; **77**: 73-111 [PMID: 23471618 DOI: 10.1128/MMBR.00046-12]

171 **Kang YS**, Jung J, Jeon CO, Park W. Acinetobacter oleivorans sp. nov. is capable of adhering to and growing on diesel-oil. *J Microbiol* 2011; **49**: 29-34 [PMID: 21369976 DOI: 10.1007/s12275-011-0315-y]

172 **Stacy DM**, Welsh MA, Rather PN, Blackwell HE. Attenuation of quorum sensing in the pathogen Acinetobacter baumannii using non-native N-Acyl homoserine lactones. *ACS Chem Biol* 2012; **7**: 1719-1728 [PMID: 22853441 DOI: 10.1021/cb300351x]

173 **Gaddy JA**, Actis LA. Regulation of Acinetobacter baumannii biofilm formation. *Future Microbiol* 2009; **4**: 273-278 [PMID: 19327114 DOI: 10.2217/fmb.09.5]

174 **Bhargava N**, Sharma P, Capalash N. Quorum sensing in Acinetobacter: an emerging pathogen. *Crit Rev Microbiol* 2010; **36**: 349-360 [PMID: 20846031 DOI: 10.3109/1040841X.2010.512269]

175 **Carruthers MD**, Harding CM, Baker BD, Bonomo RA, Hujer KM, Rather PN, Munson RS. Draft Genome Sequence of the Clinical Isolate Acinetobacter nosocomialis Strain M2. *Genome Announc* 2013; **1**: pii: e00906-13 [PMID: 24201195]

176 **Niu C**, Clemmer KM, Bonomo RA, Rather PN. Isolation and characterization of an autoinducer synthase from Acinetobacter baumannii. *J Bacteriol* 2008; **190**: 3386-3392 [PMID: 18281398 DOI: 10.1128/JB.01929-07]

177 **González RH**, Dijkshoorn L, Van den Barselaar M, Nudel C. Quorum sensing signal profile of Acinetobacter strains from nosocomial and environmental sources. *Rev Argent Microbiol* 2009; **41**: 73-78 [PMID: 19623895]

178 **Irie Y**, Parsek MR. Quorum sensing and microbial biofilms. *Curr Top Microbiol Immunol* 2008; **322**: 67-84 [PMID: 18453272 DOI: 10.1007/978-3-540-75418-3_4]

179 **Suppiger A**, Schmid N, Aguilar C, Pessi G, Eberl L. Two quorum sensing systems control biofilm formation and virulence in members of the Burkholderia cepacia complex. *Virulence* 2013; **4**: 400-409 [PMID: 23799665 DOI: 10.4161/viru.25338]

180 **Waters CM**, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* 2005; **21**: 319-346 [PMID: 16212498 DOI: 10.1146/annurev.cellbio.21.012704.131001]

181 **Baldwin A**, Sokol PA, Parkhill J, Mahenthiralingam E. The Burkholderia cepacia epidemic strain marker is part of a novel genomic island encoding both virulence and metabolism-associated genes in Burkholderia cenocepacia. *Infect Immun* 2004; **72**: 1537-1547 [PMID: 14977960 DOI: 10.1128/IAI.72.3.1537-1547.2004]

182 **Boon C**, Deng Y, Wang LH, He Y, Xu JL, Fan Y, Pan SQ, Zhang LH. A novel DSF-like signal from Burkholderia cenocepacia interferes with Candida albicans morphological transition. *ISME J* 2008; **2**: 27-36 [PMID: 18049456 DOI: 10.1038/ismej.2007.76]

183 **Soares JA**, Ahmer BM. Detection of acyl-homoserine lactones by Escherichia and Salmonella. *Curr Opin Microbiol* 2011; **14**: 188-193 [PMID: 21353625 DOI: 10.1016/j.mib.2011.01.006]

184 **Kendall MM**, Sperandio V. Quorum sensing by enteric pathogens. *Curr Opin Gastroenterol* 2007; **23**: 10-15 [PMID: 17133078 DOI: 10.1097/MOG.0b013e3280118289]

185 **Moreira CG**, Weinshenker D, Sperandio V. QseC mediates Salmonella enterica serovar typhimurium virulence in vitro and in vivo. *Infect Immun* 2010; **78**: 914-926 [PMID: 20028809 DOI: 10.1128/IAI.01038-09]

186 **Hornby JM**, Jensen EC, Lisec AD, Tasto JJ, Jahnke B, Shoemaker R, Dussault P, Nickerson KW. Quorum sensing in the dimorphic fungus Candida albicans is mediated by farnesol. *Appl Environ Microbiol* 2001; **67**: 2982-2992 [PMID: 11425711 DOI: 10.1128/AEM.67.7.2982-2992.2001]

187 **Kruppa M**. Quorum sensing and Candida albicans. *Mycoses* 2009; **52**: 1-10 [PMID: 18983434 DOI: 10.1111/j.1439-0507.2008.01626.x]

188 **Ramage G**, Saville SP, Wickes BL, López-Ribot JL. Inhibition of Candida albicans biofilm formation by farnesol, a quorum-sensing molecule. *Appl Environ Microbiol* 2002; **68**: 5459-5463 [PMID: 12406738 DOI: 10.1128/AEM.68.11.5459-5463.2002]

189 **Cao YY**, Cao YB, Xu Z, Ying K, Li Y, Xie Y, Zhu ZY, Chen WS, Jiang YY. cDNA microarray analysis of differential gene expression in Candida albicans biofilm exposed to farnesol. *Antimicrob Agents Chemother* 2005; **49**: 584-589 [PMID: 15673737 DOI: 10.1128/AAC.49.2.584-589.2005]

190 **Vassella E**, Reuner B, Yutzy B, Boshart M. Differentiation of African trypanosomes is controlled by a density sensing mechanism which signals cell cycle arrest via the cAMP pathway. *J Cell Sci*

1997; **110** (Pt 21): 2661-2671 [PMID: 9427384]
191 **Mony BM**, MacGregor P, Ivens A, Rojas F, Cowton A, Young J, Horn D, Matthews K. Genome-wide dissection of the quorum sensing signalling pathway in Trypanosoma brucei. *Nature* 2014; **505**: 681-685 [PMID: 24336212 DOI: 10.1038/nature12864]
192 **Chen H**, Fink GR. Feedback control of morphogenesis in fungi by aromatic alcohols. *Genes Dev* 2006; **20**: 1150-1161 [PMID: 16618799 DOI: 10.1101/gad.1411806]
193 **Sprague GF**, Winans SC. Eukaryotes learn how to count: quorum sensing by yeast. *Genes Dev* 2006; **20**: 1045-1049 [PMID: 16651650 DOI: 10.1101/gad.1432906]
194 **Almeida AR**, Legrand N, Papiernik M, Freitas AA. Homeostasis of peripheral CD4+ T cells: IL-2R alpha and IL-2 shape a population of regulatory cells that controls CD4+ T cell numbers. *J Immunol* 2002; **169**: 4850-4860 [PMID: 12391195 DOI: 10.4049/jimmunol.169.9.4850]
195 **Freitas AA**, Rocha B. Homeostasis of naive T cells: the Foxo that fixes. *Nat Immunol* 2009; **10**: 133-134 [PMID: 19148194 DOI: 10.1038/ni0209-133]
196 **Almeida AR**, Amado IF, Reynolds J, Berges J, Lythe G, Molina-París C, Freitas AA. Quorum-Sensing in CD4(+) T Cell Homeostasis: A Hypothesis and a Model. *Front Immunol* 2012; **3**: 125 [PMID: 22654881 DOI: 10.3389/fimmu.2012.00125]
197 **Hickson J**, Diane Yamada S, Berger J, Alverdy J, O'Keefe J, Bassler B, Rinker-Schaeffer C. Societal interactions in ovarian cancer metastasis: a quorum-sensing hypothesis. *Clin Exp Metastasis* 2009; **26**: 67-76 [PMID: 18516689 DOI: 10.1007/s10585-008-9177-z]
198 **Welch DR**, Aeed PA, Earhart RH, McClure SA. Evidence for paracrine regulation of experimental metastasis in 13762NF rat mammary adenocarcinoma cell clones. *Anticancer Res* 1994; **14**: 1743-1751 [PMID: 7847807]
199 **Agur Z**, Kogan Y, Levi L, Harrison H, Lamb R, Kirnasovsky OU, Clarke RB. Disruption of a Quorum Sensing mechanism triggers tumorigenesis: a simple discrete model corroborated by experiments in mammary cancer stem cells. *Biol Direct* 2010; **5**: 20 [PMID: 20406437 DOI: 10.1186/1745-6150-5-20]

P- Reviewer: Akbulut S, Amiri M, Sergi C **S- Editor**: Tian YL
L- Editor: A **E- Editor**: Wu HL

Published by **Baishideng Publishing Group Inc**
8226 Regency Drive, Pleasanton, CA 94588, USA
Telephone: +1-925-223-8242
Fax: +1-925-223-8243
E-mail: bpgoffice@wjgnet.com
Help Desk: http://www.wjgnet.com/esps/helpdesk.aspx
http://www.wjgnet.com