Review

Bench-to-bedside review: Quorum sensing and the role of cell-to-cell communication during invasive bacterial infection

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Abstract

Bacteria communicate extensively with each other and employ a communal approach to facilitate survival in hostile environments. A hierarchy of cell-to-cell signaling pathways regulates bacterial growth, metabolism, biofilm formation, virulence expression, and a myriad of other essential functions in bacterial populations. The notion that bacteria can signal each other and coordinate their assault patterns against susceptible hosts is now well established. These signaling networks represent a previously unrecognized survival strategy by which bacterial pathogens evade antimicrobial defenses and overwhelm the host. These quorum sensing communication signals can transgress species barriers and even kingdom barriers. Quorum sensing molecules can regulate human transcriptional programs to the advantage of the pathogen. Human stress hormones and cytokines can be detected by bacterial quorum sensing systems. By this mechanism, the pathogen can detect the physiologically stressed host, providing an opportunity to invade when the patient is most vulnerable. These rather sophisticated, microbial communication systems may prove to be a liability to pathogens as they make convenient targets for therapeutic intervention in our continuing struggle to control microbial pathogens.

Introduction

When first encountering a new host, every potential microbial pathogen is presented with three possible options. The microorganism can stay and play (colonize and establish biofilms), or can scoop and run (transiently colonize, acquire nutrients, and then seek another host). Alternatively, the pathogen could throw caution to the wind, express its full complement of virulence factors, and invade the host. It may come as a surprise to learn that this decision is often not made in isolation. The notion that a bacterium survives essentially as a lone soldier whose success or failure is dependent upon mere happenstance alone has given way to a more complex and nuanced view of microbial pathogenesis. Successful invasion of a host is now understood to be a collective process, predicated upon microbial information sharing and active collaboration [1,2].

Pathogens employ a series of chemical signals and sensing systems that jointly engage bacterial communities to genetically respond in concert to specific conditions existing in their immediate microenvironment. An emerging field termed sociomicrobiology is beginning to unravel the evolutionary, ecologic, and functional advantages of communal living among bacterial populations. A central component of bacterial communication is known as quorum sensing (QS).

QS is defined as the capacity to detect extracellular, small-molecule signals and to alter gene expression in response to bacterial population densities. Elements of the QS apparatus of bacteria are now known to serve a wide variety of functions beyond a simple estimate of cell density [3,4]. Bacteria use QS signals to coordinate gene expression within their own kind. Moreover, these same sensing signals are used to either inhibit or activate transcriptional programs among competing bacterial strains and other species existing within the same microenvironment [5]. Communication can even cross kingdom boundaries, as bacterial QS effector molecules can alter transcriptional programs found in eukaryotic epithelial cells and immune effector cells [4,6].

Potential microbial pathogens face very long odds when attempting to successfully invade a human host. An impressive repertoire of antibacterial defenses awaits any microorganism that transgresses the human epithelial barrier [7-9]. In response, a myriad of rather ingenious defensive and offensive weaponry is expressed by microbial invaders. A pathogen must evade host innate and acquired cellular and humoral immune responses, replicate at a sufficient rate to overwhelm host clearance mechanisms, and cause tissue

ABC = ATP-binding cassette; *agr* = accessory gene regulator in *Staphylococcus aureus*; AHL = *N*-acyl homoserine lactone; AI-2 = autoinducer type 2; AI-3 = autoinducer type 3; AIP = autoinducer peptide; IFN = interferon; QS = quorum sensing.

injury. In the present brief review we shall discuss the mechanisms by which bacteria communicate, and discuss how this capability is exploited by pathogens to successfully invade the host.

The discovery of QS is attributable to the pioneering work of three marine microbiologists – Nealson, Platt and Hastings [10]. An unusual form of symbiosis exists between the halophilic bacterium *Vibrio fischeri* and Hawaiian bobtail squid (*Euprymna scolopes*). The bioluminescent *V. fischeri* is taken up by strategically placed light organs along the outer surface of the squid. When the bacterial population reaches a threshold concentration, the bacterium activates its luciferase operon to generate visible light. The bacteria benefit from its association with the squid, which provides a safe haven and a steady source of nutrients. The light source created by the bacterial enzymes provides the squid with an ingenious form of camouflage. The dark outline of the squid is silhouetted against the starlit sky on clear nights, rendering them readily visible from below by predatory fish [11]. The light organs of the squid provide a starry sky camouflage thanks to the light source provided by the large aggregates of *V. fischeri*.

Bioluminescence by this *Vibrio* species, and a closely related organism *Vibrio harveyi*, is activated only when large concentrations of bacteria are present. But how do individual bacterial cells sense when their population density is sufficient to generate bioluminescence? The answer came when *V. fischeri* was found to produce a soluble QS molecule that only induces transcription of the luciferase operon when neighboring bacterial populations are above a preset threshold concentration [4,11,12].

Quorum sensing systems in microbial pathogens

The practical relevance of these initial, astute observations in *V. fischeri* was largely overlooked by clinical microbiologists, whose primary interest revolves around the study of human pathogens. Biologists certainly appreciated QS as a remarkable adaptation and prime example of mutualism in the field of marine ecology, but this work did not attract much attention until researchers began to find homologous, autoinducer systems among many clinically relevant, microbial pathogens [1-6,11-14].

Many common bacterial pathogens, including *Escherichia coli*, *Pseudomonas aeruginosa*, *Bacteroides*, *Yersinia*, *Burkholderia* and *Enterococcus* spp., and many clinically important staphylococcal and streptococcal pathogens contain QS genes. Cell-to-cell communication via QS systems might contribute to the global regulation of multiple bacterial genes, including virulence genes, under specific environmental and growth conditions. Up to 15% of the open reading frames of bacteria are controlled by QS molecules [4]. QS can promote the growth of related strains of bacteria (referred to as alloinduction) and can simultaneously inhibit the growth of other bacterial [15], or even fungal, organisms competing for the same ecologic niche [6,16].

QS gene products regulate a multitude of transcriptional programs in bacteria *in vitro* and probably *in vivo*. QS systems control biofilm formation [17-27], growth potential, sporulation, antibiotic resistance expression, DNA transfer, virulence expression, autolysis, oxidative stress tolerance, metabolic activity, motility, antibiotic synthesis by antibiotic-producing bacteria, sessile versus planktonic behavior, and – most importantly – genetic determinants of virulence [1-3,11-14].

Bacterial pathogens possess an array of genes on pathogenicity islands scattered around their chromosome, plasmids and lysogenic bacteriophages, which function together to mediate microbial virulence [28,29]. This total complement of virulence genes is now referred to as the virulome [30]. How global regulation of the virulome is accomplished by QS mechanisms will be the primary focus of the present review.

Quorum sensing signal molecules and sensor systems

A remarkable array of signaling molecules can function as local sensors to communicate population densities in Gram-negative and Gram-positive bacteria. These molecular signals and their receptors are broadly grouped into three, and possibly four, QS systems. The general features of each type of the QS systems identified thus far are presented in Table 1.

Autoinducer type 1 system

The autoinducer type 1 system is widely used in multiple genera of Gram-negative bacteria and is highly homologous to the original *luxR*/*luxI* autoinducer type 1 system in *Vibrio* spp. first defined in *V. fischeri* [8]. A highly soluble and freely diffusible sensor molecule uses a series of *N*-acyl homoserine lactone (AHL) molecules for signaling (Figure 1).

AHL molecules vary in the *N*-acyl chain length (from 4 to 18 carbons), the degree of saturation, and the number of oxygen substitutions. The L-isomeric form of the homoserine lactone ring is common to all AHLs. The *luxI* gene, or its homologues, encodes the sequences that mediate the formation of the AHL signaling molecule. AHLs freely pass though cell membranes and, when bacterial population densities are low, limited amounts of the AHL are diluted away and the genes under QS control remain turned off. When population densities increase beyond a predetermined threshold level, enough AHL accumulates to interact with its cytosolic receptor.

The receptor for AHL is mediated by the *luxR* gene in *V. fischeri* or related genes in other bacterial species. The translated product of *luxR* is the LuxR receptor molecule, which together with its AHL partner functions as a coactivator complex at the promoter sites for QS responsive operons in bacterial genome. In *V. fischeri* the end result is biolumines-

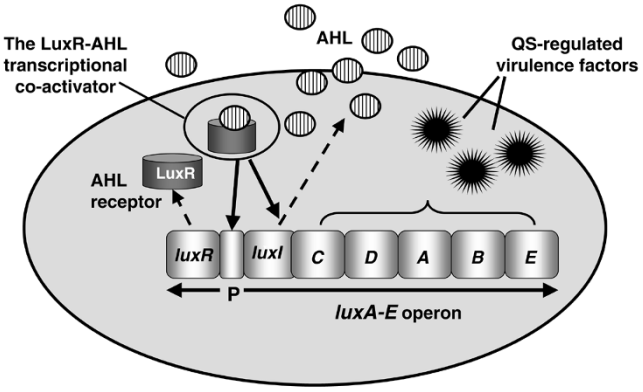
Table 1

Basic elements of the quorum sensing systems in bacteria

Type	Sensing molecules	Receptor(s)	Special features
Autoinducer type 1, LuxR-I type	*N*-acyl-homoserine lactones	Intracellular Lux-R homologues as transcriptional coactivator	Found in Gram-negative bacteria (*Burkholderia*, *Vibrio*, *Pseudomonas* spp.); might affect human genes
Autoinducer type 2, LuxS type	Heterocyclic furanosyl-borate	Two-component membrane receptor-cytoplasmic kinase complex	Widespread in Gram-negative and Gram-positive bacteria; might be a primary metabolic system rather than a communication system
Autoinducer type 3, epinephrine/norepinephrine signaling system	Catecholamine-like molecules	Two-component membrane-sensor kinase/response regulator (QseBC)	Found in Gram-negative, enteric bacteria enterohemorrhagic *Escherichia coli*, enteropathogenic *E. coli*, *Shigella*, *Salmonella* spp.; functional role unclear at present
Cyclic short-peptide systems (AgrC/AgrA, staphylococci; competence stimulating peptide, pneumococci; *Enterococcus faecalis* regulator, enterococci)	Small cyclic peptides with thiolactone ring	Two-component sensor kinase (AgrC)-response regulator (AgrA)	Gram-positive bacteria, *Staphylococcus*, *Bacillus*, *Enterococcus*, *Streptococcus* spp.

Agr, accessory gene regulator; QseBC, autoinducer type 3 system in enteric bacteria.

Figure 1



Autoinducer type 1 quorum sensing signal system in Gram-negative bacteria. The autoinducer type 1 quorum sensing (QS) system found in *Vibrio fischeri*, and many homologous variations on the system in other Gram-negative bacteria. AHL, acyl homoserine lactone; luxR/luxI, autoinducer type 1 system in *Vibrio* spp.; P, promoter site.

cence; for certain bacterial pathogens, the end product is the activation of the virulome for microbial invasion.

The autoinducer type 1 system is found in many medically important, Gram-negative bacterial pathogens [1-5,31]. Some the bacterial genera use the same AHL molecule, indicating some level of interspecies cross-talk probably exists [4]. Other bacteria including *E. coli* do not synthesize AHLs but express a LuxR biosensor homologue (SdiA). It is speculated that this sensing system allows *E. coli* to listen in on communication signals from other Gram-negative bacteria and exploit this information to its own advantage [32].

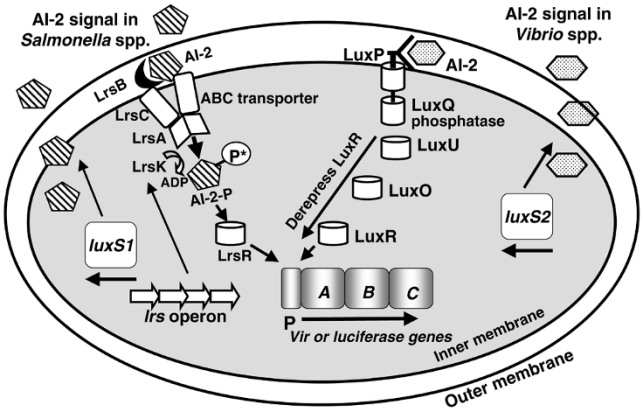
There are many variations on this central theme among the QS apparatus of specific bacterial pathogens. *P. aeruginosa*, for example, actually deploys at least two such AHL signaling systems simultaneously (the autoinducer type 1 system in *P. aeruginosa*, the *lasR/lasI* (3-oxo-C_{12}-homoserine lactone) system; and the autoinducer type 1 system in *P. aeruginosa*, the *rhlR/rhlI* (C_4-homoserine lactone) system) to jointly regulate biofilm synthesis and virulence [31].

LuxS autoinducer type 2 signaling system

A second QS pathway was initially discovered in the *V. harveyi* bioluminescence system and is mediated by the *luxS* gene locus and related homologues [1,5]. Elements of the autoinducer type 2 (AI-2) system are detectable in almost one-half of all sequenced bacterial genomes, and this system is now recognized as the most ubiquitous signaling system employed by both Gram-negative and Gram-positive bacteria [3,9,33]. The AI-2 pathway uses a more complex, two-component, receptor-kinase network to accomplish efficient signaling among bacteria (see Figure 2). Structurally, the AI-2 signal in *Vibrio* spp. is composed of rather complex, multiple-ringed, cyclical furanosyl molecules containing the highly unusual presence of a boron atom [5].

The receptor for the AI-2 apparatus is also complex, with a series of gene products that function as the receptor-kinase signal transcription complex. In *Vibrio* species the receptor is a membrane-bound, two-domain, sensor kinase and response regulator (LuxQ). In enteric bacteria, a soluble receptor binds

Figure 2



Two types of autoinducer type 2 quorum sensing systems in different genera of Gram-negative bacteria. The two autoinducer type 2 (AI-2) quorum sensing signal systems found in *Salmonella* spp. and in *Vibrio* spp. ABC, ATP-binding cassette transporter; Lrs, LuxS regulated; P, promoter site; P*, phosphorylated; Vir, virulence.

to the AI-2 signal molecule in the periplasmic space and then transports the AI-2 molecule across the membrane via a specific ABC-type transporter system [17]. The internalized AI-2 molecule is phosphorylated and then complexes with an intracellular receptor that acts as the transcriptional activator. Multiple variations of this AI-2 system are found in bacteria [6]. The AI-2 signaling molecule in Salmonella spp. is a furane molecule that lacks boron. Many bacteria apparently do not express the *luxS* gene but do express the AI-2 receptor complex [17,19]. Such an arrangement has been proposed to allow some bacterial strains to sense and use AI-2 signals generated by other bacteria to regulate their own coordinated transcriptional responses [6,19]. While this is an intriguing hypothesis, there is as yet no convincing, direct evidence that AI-2 signals from one species can regulate the gene expression of an unrelated species.

The precise role of AI-2 signaling in bacterial pathogenesis is not clear, as much of the transcriptional activity of AI-2 systems is directed toward regulation of metabolic pathways [3,17]. In addition to its potential role as an AI-2 synthase, the *luxS* gene encodes a dual-function enzyme that mediates the conversion of the toxic intermediate molecule *S*-adenosyl-L-homocysteine to homocysteine. This enzymatic activity is central to an activated methyl cycle in which methyl groups are attached to nucleic acid precursors, proteins and other metabolites. Byproducts of LuxS enzyme activity are furanose structures that act as AI-2 signals [34,35].

Many of the QS signaling events ascribed to AI-2 signaling may, in fact, be the consequence of LuxS as a detoxifying enzyme in the activated methyl cycle rather than a consequence of QS effects. Recent, direct genetic evidence in *luxS* mutants of *Streptococcus mutans* indicates that many but not all of the phenotypic effects of AI-2 can be ascribed to the metabolic impact of LuxS rather than its QS effects [36]. A total of 585 genes (30% of the transcriptome) were altered by deletion of *luxS*; however, only 59 gene transcripts were upregulated by addition of AI-2. These data assign a primary metabolic role of LuxS, with a potential secondary role in QS signaling [36]. As seen in some examples provided in the following section, however, AI-2 signaling might play a facilitating role in bacterial virulence with some pathogens [19,37,38].

Autoinducer type 3 system

This newly discovered QS system is perhaps the most complicated of all signaling pathways thus far discovered. The autoinducer type 3 (AI-3) system shares many characteristics of the AI-2 system as it uses a two-component, receptor kinase-intracellular signaling complex to activate genes of the virulome; but, in contrast to AI-2, the AI-3 system can use the human stress hormones epinephrine or norepinephrine to signal the system (see Figure 3) [11,39-41]. The natural microbial ligand molecule that activates the AI-3 receptor complex has not been clearly defined but is probably similar to catecholamines. In fact, α-adrenergic receptor blocking agents can block AI-3 signaling in *E. coli* [17].

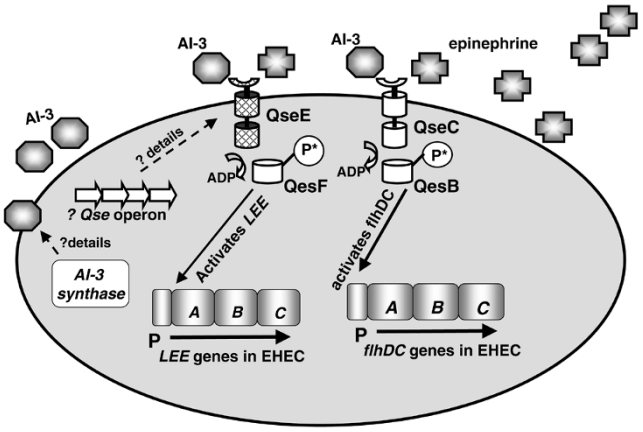
The periplasmic receptor for the AI-3 system has recently been characterized and is known as the QseBC complex [42]. QseC is the sensor kinase and QseB is the phosphorylated response regulator that alters transcription of virulence genes. The AI-3 system is essential in the pathogenesis of enterohemorrhagic *E. coli* infections and shigellosis [17]. Components of the AI-3 signaling network have been detected in enteropathogenic *E. coli* strains, commensal *E. coli* strains, and a number of other Gram-negative, enteric organisms, but thus far not in Gram-positive bacteria.

Short peptide signaling system in Gram-positive bacteria

Some Gram-positive bacterial pathogens also possess another structurally dissimilar, but functionally similar, system of global regulation of genes based upon cell densities [20,43]. This system is referred to as the accessory gene regulator system (*agr*) in staphylococci [43] and as the *Enterococcus faecalis* regulator-QS (*frs*) system in enterococci [44]. Gram-positive pathogens rely upon short, cyclical peptides known as autoinducer peptides (AIPs). The AIPs of *Staphylococcus aureus* usually feature cyclical short peptides from seven to nine amino acids in size with terminal thiolactone ring structures. The ring formation is created by linkage of the carboxyl terminus with a conserved cysteine moiety within the peptide sequences.

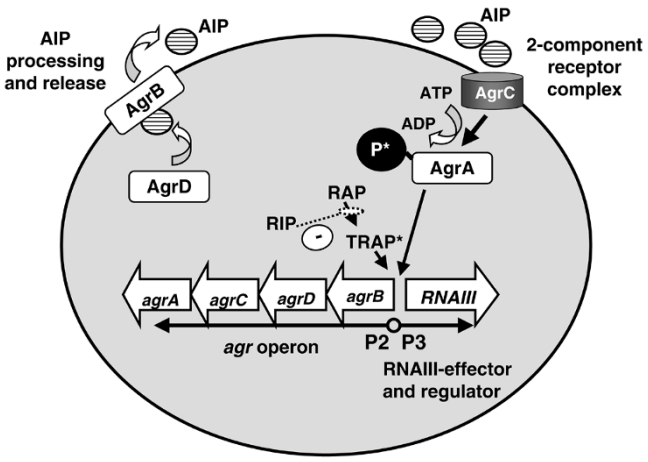
Cell surface receptors sense these peptides and activate a histidine kinase that generates transcriptional activators for multiple gene loci. The *agr* genetic locus is best characterized in *S. aureus* (see Figure 4) and consists of two operons denoted as RNAII and RNAIII. RNAII contains four open

Figure 3



Autoinducer type 3/epinephrine quorum sensing system in enteric Gram-negative bacteria. AI-3, autoinducer type 3; EHEC, enterohemorrhagic *Escherichia coli*; *LEE*, locus for enterocyte effacement; *flh*, flagella regulon; P, promoter site; P*, phosphorylated; Qse, AI-3 system in enteric bacteria.

Figure 4



Accessory gene regulator quorum sensing system in *Staphylococcus aureus.* Homologous short peptide signaling systems exist in other Gram-positive bacterial species and genera. *agr*, accessory gene regulator; AIP, autoinducer peptide; P, promoter site; P*, phosphorylated protein; RAP, RNAIII-activating protein; RIP, RNAIII-inhibiting peptide; TRAP, target of RNAIII-activating protein.

reading frames designated *agrA* to *agrD* [20,30]. The current working model suggests that the autoinducer peptide is generated from *agrD* and then processed, post-translationally modified, and secreted by a membrane-bound protein bearing an ABC-type transporter system known as AgrB. The sensing apparatus consists of the transmembrane receptor-histidine kinase AgrC. If sufficient quantities of the appropriate AIP ligand have accumulated, AgrC phosphorylates AgrA. Then AgrA acts as a transcriptional activator with binding to the promoter sites of both RNAII and RNAIII [30].

RNAII activation functions as a positive feedback loop for more AIP synthesis. There are four recognized *agr* groups in *S. aureus.* The same AIP peptide that autoactivates the producer strain of *S. aureus* will inhibit signaling with other peptide groups of the same species [20,45]. These cross-inhibitory effects of autoinducing peptides are envisioned as a mechanism to reduce competition by multiple strains of *S. aureus* within confined ecologic niches with limited resources [3].

RNAIII is the effector gene with dual roles in virulence expression. The transcribed product of RNAIII mediates delta-toxin synthesis and also functions as a regulatory RNA sequence that alters the expression of multiple other genes of the virulome [20]. Activation of the *agr* system by high concentrations of AIP with some peptide groups of staphylococci will generate RNAIII, which upregulates the expression of a large number of pathogenic exotoxins. These endotoxins include toxic shock syndrome toxin-1, delta toxin, exfoliatin, hemolysins, some but not all the staphylococcal super-antigenic enterotoxins, and the Panton-Valentine leukocidin [20]. RNAIII synthesis simultaneously downregulates a number of surface proteins, clumping factor, and adhesion molecules. The intricate role of the *agr* system in gene regulation and virulence will be discussed in greater detail later (see section Quorum sensing systems in the pathogenesis of *S. aureus* infections) [2,3,20].

Quorum sensing, biofilms, and bacterial communal living

QS is thought to play a role in the production of healthy and fully developed biofilms. These complex, microbially derived, multilayer structures of defined architecture form relatively stable bacterial communities living in a sessile, protected environment [2,11,46-49]. The widespread presence of biofilms on foreign bodies (for example, prosthetic device infections, prolonged use of urinary and vascular catheters) or host surfaces (endovascular and epithelial surfaces) attest to the survival advantages afforded by bacterial biofilms [46,50]. The slow rate of metabolism and their physical location within biofilm exocapsular structures protect bacteria against the bactericidal effect of antibiotics and host clearance mechanisms by opsonins and neutrophils [27]. The clinical consequences of infection associated with mature biofilms are significant. Prolonged antibiotic therapy is often required to eradicate these infections (that is, osteomyelitis, endocarditis, catheter-related infections); regrettably, even this strategy often fails – necessitating surgical excision of the biofilm-contaminated source for long-term cure [1,18,49,51].

The formation of biofilms is a multistep process beginning with microbial surface attachment, cell-to-cell aggregation and proliferation, exopolysaccharide matrix production, growth, maturation, and, finally, biofilm detachment or degradation

[3,20,48,49]. QS systems appear to be involved in all phases of biofilm formation. They regulate the population density and the metabolic activity within the mature biofilm to fit the nutritional demands and resources available. Bacteria residing within biofilms have markedly different transcriptional programs from free-living planktonic bacteria of the same strain. Recent evidence in *Streptococcus pneumoniae* indicates that organisms existing in a sessile state from biofilms are better able to establish localized infections in lung tissue than isogenic organisms in a planktonic physiologic state [46]. The reverse was found in experimental models of primary bacteremia. The planktonic, free-living organisms were significantly more virulent in bacteremic infection than the same organism grown from biofilms [46].

QS networks also appear to be instrumental in the release of bacteria from the extracellular matrix of the biofilm. The protected sanctuary provided by biofilms presents a problem of escape from the extracellular matrix in which the bacteria reside. When population densities in biofilms become high, some evidence suggests that staphylococci might use AI-2 signals to reduce the production of polysaccharide intercellular adhesin to permit bacteria to escape the biofilm [20,30]. Short peptides with detergent qualities that are under QS control are also deployed to release bacteria for biofilms when cell densities are high. Sessile bacteria in biofilms provide a continuous source of planktonic bacteria as bacterial populations expand. This is clearly evidenced by the continued release of bacteria into the bloodstream in patients with endocarditis from endovascular biofilms within vegetation [49].

Biofilm-like communal living may even extend to microbial populations residing within the intracellular space. Intracellular bacterial communities have been identified in epithelial cells in some patients with seemingly uncomplicated urinary tract infections. Rosen and colleagues recently reported detection of intracellular bacterial communities in 18% of young, sexually active women with *E. coli* cystitis [52]. Up to 41% of urine specimens in women with cystitis have evidence of filamentous bacterial forms, a hallmark of sessile bacterial communities. Cytologic evidence of intracellular bacterial communities within exfoliated urinary epithelial cells is readily demonstrable in women with acute cystitis. Apparently some uropathogenic *E. coli* have evolved the capacity to invade epithelial lining cells where they reside in quasi-stable intracellular communities, avoiding immune surveillance and urinary clearance mechanisms [52,53].

Examples of quorum sensing systems as contributors to microbial pathogenesis among some common human pathogens

Quorum sensing systems in *P. aeruginosa*

P. aeruginosa has a remarkably diverse metabolic capacity to exist in multiple environments and possesses an unusually large chromosome. This extensive array of genetic material is tightly regulated, and *P. aeruginosa* uses QS as one of its global regulators of the virulome and its complement of metabolic enzymes [18,19,54].

Pseudomonas spp. produce at least two distinct AHL signal molecules from the *lasR-I* and *rhlR-I* loci, which are hierarchically controlled and work in concert to control virulence and biofilm formation. The *lasI* product is 3-oxo-C_{12}-homoserine lactone, and the *rhlI* product is a shorter C_4-homoserine lactone. The *lasR-I* signals are the primary determinant of virulence and biofilm formation, and regulate the *rhl* system [31]. A third LuxR homolog (QscR) is known to exist in *P. aeruginosa* that regulates the expression of both the *lasI* and *rhlI* QS gene loci [3]. *P. aeruginosa* also possesses a separate signaling system known as the Pseudomonas quinolone signal that generates a 4-quinolone signaling molecule 2-heptyl-3-hydroxy-4-quinolone [54]. This signaling system is interposed between the *las* and *rhl* QS signaling systems. The *lasR-I* system induces this Pseudomonas quinolone signal, and the Pseudomonas quinolone signal induces the RhlR-I QS system. Pseudomonas quinolone signal is expressed in infection and induces the synthesis of several important virulence factors, including pyocanin, rhamnolipid, lectins, and elastase [54].

These QS systems provide an opportunity for pathogens to minimize early losses and maximize the chances for ultimate success in causing widespread infection and sepsis. The virulome is repressed when population densities are low. This prevents early detection and avoids immune activation against these immunostimulatory virulence factors in the initial phase of colonization of the host. Once the population density expands to critical threshold levels, QS systems activate replication programs and the full expression of virulence programs. Virulence factors include proteases (elastase, alkaline protease), antibiotic resistance, motility, siderophores, adhesins, phospholipases, cytotoxins and other exotoxins, along with type III secretion systems and inducers of neutrophil and macrophage apoptosis [5,16,18,55-57].

Additionally, *P. aeruginosa* expresses the receptor complex of the AI-2 two-component signaling system, but it does not contain the gene of a *luxS* signaling pathway or generate any of its own AI-2 signal molecules. *P. aeruginosa* possibly uses this system to detect AI-2 signals from other competing or commensal bacterial populations within its immediate microenvironment [19].

Site-specific, QS gene deletion experiments have been undertaken in an effort to decipher the role of QS *in vivo* in virulence and biofilm formation. *P. aeruginosa* strains with genetically defined, excision mutations of the QS gene complex exhibit significantly impaired virulence in animal models of invasive infections (for example, burns, pneumonia, and bacteremia) [31,50,58,59]. Biofilms generated from these *lasR-I* mutants display a disordered and blunted architecture [18]. Full virulence and normal biofilms are restored by comple-

mentation experiments in which plasmids are inserted that replace the genes for the *lasR-I* QS into the pathogen [50].

AHLs produced by *P. aeruginosa* may have a further survival advantage by eliminating some of its microbial competitors. The AHL produced by *lasI* inhibits the virulence, growth, and survival potential of some strains of *S. aureus*. This activity is mediated by the inhibition of the *agr* and *sarC* regulatory gene circuitry of the *S. aureus* QS system [15].

The capacity to recognize AI-2 signals is probably advantageous for *Pseudomonas* spp. in assessing the population density of competing bacteria on mucosal surfaces. A previously unappreciated, direct, synergistic interaction exists between *P. aeruginosa* virulence expression and other colonizing bacteria of the oropharynx using cross-species AI-2 signaling. Oropharyngeal bacterial flora release AI-2 signals in the respiratory tract and may be sensed by *P. aeruginosa* in microbial communities such as those found in the airways in cystic fibrosis or in diffuse panbronchiolitis [19,55]. Seemingly innocuous oropharyngeal flora may enhance *P. aeruginosa*-induced lung injury in animal models by releasing AI-2 signals that upregulate *P. aeruginosa* virulence genes. Substantial quantities of AI-2 can be measured in the respiratory secretions in cystic fibrosis patients, suggesting that this may of clinical significance in some patients with *P. aeruginosa* pneumonia [19].

Quorum sensing systems in *E. coli* pathogenesis

Biofilm communal living is a common characteristic among *E. coli* infections along catheter surfaces and on mucous membranes along epithelial surfaces [1,26,49]. Bacterial communities residing on urinary catheter surfaces are relatively refractory to the lytic action of many antibiotics. The intracellular bacterial communities with *E. coli* residing within uroepithelial cells also shelter bacteria from many antimicrobial agents that are limited to the extracellular space [52]. The QS systems expressed by *E. coli* are instrumental to biofilm construction. Moreover, a number of QS networks regulate the synthesis of an array of *E. coli* virulence factors in enteric infections and extra-intestinal infections [41].

No *E. coli* gene for *luxI*-type autoinducer type 1 homoserine lactones has been described thus far, but a gene homologue for the LuxR receptor, known as *sidA*, is expressed in some stains of *E. coli*. This receptor could recognize AHL molecules produced by other bacterial genera, providing *E. coli* with a mechanism to detect competing bacterial populations [17].

Components of the AI-2 and AI-3 QS systems are found in *E. coli* strains and might play a role in microbial pathogenesis. The AI-2 system was initially thought to regulate the *E. coli* virulome, but current evidence ascribes a primary metabolic control function for AI-2 systems [17,35]. The epinephrine/norepinephrine AI-3 system regulates many virulence genes in *E. coli* strains, including the attaching and effacing colonic lesions essential to the pathogenesis of enterohemorrhagic *E. coli* infections [40,60]. Deletion of the AI-3 receptor attenuates enterohemorrhagic *E. coli* infection in animal models. This QS network might also regulate motility and promote adhesion expression for biofilm formation in the early phases of enteropathogenic *E. coli* infections [17].

Quorum sensing systems in the pathogenesis of *S. aureus* infections

QS-mediated virulence gene regulation is fine tuned in some strains of *S. aureus*, where the specific sets of virulence gene transcriptional programs are phased on or phased off in preset patterns over the course of an invasive infection [20]. *S. aureus* has at least two QS systems at its disposal plus a large number of other sensing systems and transcriptional and post-transcriptional control mechanisms. The cyclic peptide Agr system is the dominant QS regulator of genetic programs in *S. aureus.* This major human pathogen, and other staphylococcal species, might also, however, use LuxS signals to regulate virulence and to initiate detachment from biofilms by expression of phenol-soluble modulin peptides [25,38].

In the early phase of microbial invasion, the population density is low and most virulence genes are turned off in favor of surface adherence structures and the immunoglobulin inhibitory molecule, protein A. This is controlled by low *agr* expression and the *sarA* regulon of *S. aureus*. Once infection begins in earnest, the QS apparatus is activated and sequential gene activation rapidly proceeds with exotoxin, protease, and hemolysin production [20]. Simultaneously, adhesin molecule expression is turned off, thereby facilitating dissemination to other regions in the host. *In vivo* detection of *agr* expression indicates that a biphasic activation of the system occurs with early activation, secondary reduction, and then late reactivation of this QS system during infection [61]. Deletion mutations of the *agr* system in *S. aureus* experimental infection models generally, but not uniformly, support a functional role for this QS system in invasive staphylococcal disease [20,30].

Biofilm formation is actually inhibited by both the *agr* system and AI-2 signals in staphylococci. The utility of QS in staphylococcal biofilms may actually be the expression of solublizing enzymes and phenol-soluble modulin peptides that permit release and detachment of bacteria from mature biofilms [30,38]. This detachment process appears to be under QS control and facilitates dissemination of *S. aureus* to distant sites with establishment of metastatic foci of abscess formation. This process is characteristic of infection by this highly virulent, Gram-positive pathogen.

Interspecies and interkingdom signaling via quorum sensing systems

QS biosensors might provide other advantages for bacterial pathogens as environmental cues to the presence of nearly

Table 2

Selected quorum sensing inhibitors as potential therapeutic agents

Agent	References	Target	Proposed mechanism of action	Current status
Macrolide and aminoglycoside antibiotics	[24,66,71]	AHL signal generation	Inhibit C_{12}-homoserine lactone by *Pseudomonas aeruginosa* quorum sensing system	Experimental use of existing antibiotics
S-adenosyl-homosysteine	[70]	AHL signal generation	Inhibits generation of AHL by RhlI synthesis	*In vitro* testing
Antibody to AHL	[80]	C_{12}-homoserine lactone of *P. aeruginosa*	Antibodies to AHL block cell to cell signaling	*In vitro* and animal models
AiiA degrading enzymes	[70]	AHL lactone ring	Lactonolysis of AHL disrupts signaling potential	*In vitro* testing; might be useful as topical agent
PvdQ-type degrading enzymes	[70]	Fatty acid side chain of AHL	Aminoacylase releases fatty acid and destabilizes lactone ring	*In vitro* studies
Halogenated furanones, other natural or synthetic AHL analogues	[70,72,73,75]	AHL receptors and LuxR homologues	Competitive inhibitors for AHL receptor binding	*In vitro* and animal studies
RIP and similar RNAIII-inhibiting peptides	[47,76,78,79]	TRAP in *agr* peptide system	Inhibits phosphorylation of TRAP blocking RNAIII signaling	*In vitro* and animal studies
RAP inhibitors	[77]	RNAIII-activating protein of staphylococci	Bind RAP and block *agr* activation	*In vitro* and animal studies

agr, accessory gene regulator; AHL, *N*-acyl homoserine lactone; RAP, RNAIII-activating protein; RIP, RNAIII-inhibiting peptide; TRAP, target of RNAIII-activating protein.

bacterial species and potential competitors. Cross-talk communication signals from other bacteria may simply indicate undirected background chatter, or they might provide some form of general alert signal [12]. Bacteria might actually consume AI-2 signals produced by neighboring species and disrupt their QS-regulated gene activation [62]. Bacteria appear to be capable of usurping the signaling potential of some bacterial strains to their advantage, without expending the metabolic energy necessary to generate their own communication system. This form of eavesdropping or cheating may be quite common in complex bacterial communities [63,64].

Some QS signal molecules may have more direct survival value for the producer strains. The staphylococcal Agr system can inhibit the growth of the eukaryotic fungal pathogen *Candida albicans* [4]. This is a clear survival advantage for these bacterial strains competing with fungi for limited space within a common ecologic niche (that is, the respiratory mucosal surface in a mechanically ventilated patient). Eliminating microbial competition permits QS-bearing bacteria unfettered access to nutrients and favored staging areas within the host for microbial invasion [1].

More complex pathways of communication exist, including two-way signaling between human cells and QS among bacterial populations. That such a system exists is evidenced by recent experiments that identify QS-dependent alterations of multiple genetic programs, epithelial cells, and immune effector cells in patients [6]. AHL molecules freely diffuse into human cells and bind to intracellular signaling proteins. These proteins transcriptionally regulate human genes that mediate the host response to bacterial invasion such as chemokines and cytokines. AHL molecules of Gram-negative bacteria promote a shift from a T-helper type 1 cytokine response to a T-helper type 2 cytokine response, an environment more hospitable to bacterial growth [3]. The microorganism gains a distinct advantage in host:pathogen interactions if the human immune response can be regulated by the pathogen. QS molecules promote apoptosis of human neutrophils [56,65], stimulate mucin production in the airway epithelium [24,66], and can even promote vasodilatation in the microcirculation [67].

Interkingdom communication via bacterial QS networks can be bidirectional. Not only can bacteria signal human cells, but human cytokines can also signal bacteria using QS circuitry. Alverdy and colleagues uncovered the capacity of human stress molecules to be recognized by the QS systems of *P. aeruginosa* [68]. A specific receptor for human IFNγ exists on the outer membrane (OmpF) of some *P. aeruginosa* strains that can activate QS-regulated virulence genes. Excess levels of IFNγ signify a compromised and possibly vulnerable host. Activation of virulence genes and invasive phenotypes during times of host stress favors the infecting microorganism against a weakened host.

Quorum sensing systems as a new treatment strategy against bacterial pathogens

Inhibitors of these cell-to-cell bacterial communication systems may provide a much needed novel treatment modality against bacterial infections. Progressive emergence of resistance to standard antibiotics in many bacterial pathogens threatens the very future of the modern antimicrobial era [69]. New drugs that interfere with microbial virulence and biofilm formation would be a welcome addition to our therapeutic armamentarium against bacterial pathogens [62]. As QS is not essential to bacterial growth or viability, selection pressures to resist QS inhibitors should, at least theoretically, not be as major an impediment to QS inhibition drug development as it is with antibiotics [70]. Some standard antibiotics may have unintended benefits in the management of bacterial infections independent of any intrinsic bactericidal properties. As an example, the macrolide antibiotics can block C_{12}-homoserine lactone production by *P. aeruginosa* [24,66, 71]. This AHL promotes mucous production in patients with pulmonary infections [24]. Despite the lack of intrinsic activity of macrolides against this pathogen, limitation of excess mucin production may aid in the resolution of persistent airways infection with *P. aeruginosa* [71].

Natural and synthetic molecular mimics of autoinducer type 1 and AI-2 signaling structures are under study as inhibitors of QS [72-76]. A short peptide known as RNAIII-inhibiting peptide inhibits the initiation of *agr*-mediated biofilm formation by staphylococci in experimental studies of vascular graft infection [47,77]. This peptide and similar QS inhibitory strategies have been proposed as locally applied or systemically administered agents to prevent vascular graft infections [77-79]. It may even be possible to vaccinate susceptible hosts against *P. aeruginosa* autoinducer type 1 signal molecules and generate protective antibodies to disrupt QS [80].

The current status of these QS inhibitors as potential therapeutic agents is summarized in Table 2. A number of these agents appear promising *in vitro* and in animal models but, other than the experimental use of existing macrolide antibiotics as QS inhibitors, none have reached clinical trials in patients. Human trials have been delayed until toxicity, drug penetration, potency, and feasibility issues with QS inhibitors are resolved. Hopefully, some of these antibodies and small-molecule inhibitors will ultimately provide new treatment options in the clinical management of bacterial infections.

Summary and conclusions

QS systems serve many functions for bacterial pathogens. QS provides a system to coordinate the expression of virulence based upon cell densities by many common and medically important bacteria. The molecular details of these signaling networks are now being defined in both Gram-positive and Gram-negative bacterial pathogens. Cross-talk via conserved QS signals between different bacterial species and even between prokaryotes and eukaryotes (including humans) appears feasible and may be commonplace. Understanding these signaling pathways might provide new treatment options to disarm potential pathogens and improve the outcome in septic patients.

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Competing interests

The authors declare that they have no competing interests.

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