Trends in Microbiology

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Review

Quorum-Sensing Systems as Targets for Antivirulence Therapy

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The development of novel therapies to control diseases caused by antibiotic-resistant pathogens is one of the major challenges we are currently facing. Many important plant, animal, and human pathogens regulate virulence by quorum sensing, bacterial cell-to-cell communication with small signal molecules. Consequently, a significant research effort is being undertaken to identify and use quorum-sensing-interfering agents in order to control diseases caused by these pathogens. In this review, an overview of our current knowledge of quorum-sensing systems of Gram-negative model pathogens is presented as well as the link with virulence of these pathogens, and recent advances and challenges in the development of quorum-sensing-interfering therapies are discussed.

Trends

Research over the past decades has shown that bacteria communicate with each other with small signal molecules in a process that has been termed quorum sensing, and the list of bacterial quorum-sensing molecules is still growing.

The virulence of many bacterial pathogens of plants, animals, and humans is controlled by quorum sensing, and quorum-sensing-interference is one of the most intensively studied strategies for controlling disease caused by antibiotic-resistant bacteria.

Various quorum-sensing-interfering agents have been described in recent years, including natural and synthetic compounds, enzymes, and antibodies, and these agents have been proven to attenuate bacterial disease in animal and plant models.

Targeting Virulence to Control Bacterial Infections

Antibiotics are still critically important for the treatment of bacterial diseases, both in human and veterinary medicine. The modes of action of all currently available antibiotics are variations on a single theme, bacterial eradication, and this implies that very strong selective pressures are imposed on bacterial communities that come into contact with these drugs [1]. Because antibiotic resistance confers a very strong selective advantage over susceptible competitors in the presence of antibiotics, resistance often spreads rapidly, and bacteria showing clinically relevant resistance to antibiotics consistently appear within as little as a few years after their first use [2]. Moreover, very few novel classes of antibiotics have been discovered in the past 50 years, and the pipeline of agents under development is very limited [3]. As a result of the development and spread of antibiotic resistance, diseases caused by antibiotic-resistant bacteria are currently a major cause of death worldwide, and this situation is predicted to become even more precarious in the near future if no adequate measures are undertaken [4].

Bacterial pathogens synthesise different compounds and structures that enable them to colonise and damage their host, that is, virulence factors (Box 1). As virulence factors are required for infection, preventing pathogens from producing them constitutes an important alternative strategy for the control of bacterial diseases, that is, antivirulence therapy. Rather than killing, antivirulence therapy aims at 'disarming' the pathogens, thereby preventing them from attacking their host [2,13,14].

The production of virulence factors is metabolically costly, and therefore, the expression of virulence genes is usually controlled by a complex regulatory network (Box 2). Inhibition of specific virulence factors, such as pili or secretion systems, is possible [21]. However, the alternative approach of interfering with virulence regulatory mechanisms in order to interfere with multiple virulence factors, has received more attention.

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Box 1. Virulence Factors in Bacterial Pathogens

Motility, Chemotaxis, and Adhesion

Flagella are rotating propulsion organelles, produced by a variety of bacteria, that act as helical propellers [5]. Flagellar motility is thought to enhance the initial interaction of a bacterium with a surface by enabling the cell to overcome negative electrostatic forces. All bacteria in which motility has been studied seem to perform chemotaxis, a process in which bacteria move either towards favourable or away from unfavourable environments [6]. This might enable bacteria to move towards target host tissues (e.g., chemotaxis for mucus components). Another group of structures involved in adhesion are pili, fiber-like structures composed of many pilin subunits packed tightly into a helical array [7]. The adhesins located at the tip of the pilus bind surface carbohydrates on host cells, and some pili (e.g., type IV pili) are able to retract through the bacterial cell wall while the tip remains firmly attached to the surface.

Production of Extracellular Polysaccharides and Biofilm Formation

Capsular polysaccharides are high-molecular-weight polysaccharides that form a dense coat surrounding the bacterial cells. This capsule is involved in attachment to host cells and plays an important role in immune evasion [8]. Another group of extracellular polysaccharides, the exopolysaccharides, form a loose slime outside the cell that is a major constituent of the intercellular matrix in biofilms. The biofilm matrix provides protection from detergents, antimicrobials, phagocytic cells, and drying [9].

Production of Lytic Enzymes

Lytic enzymes are produced by many pathogenic bacteria and often play a central role in pathogenesis [10]. These enzymes cause damage to host tissues, thereby allowing the pathogen to obtain nutrients and to spread through tissues. The most well-known lytic enzymes include haemolysins, proteases, and lipases.

Siderophores

The bioavailability of iron is limited during infection of a host, and in order to overcome this, many pathogenic bacteria can acquire iron by means of siderophores, secreted low-molecular-weight iron-binding compounds [11]. Many different siderophores (with a high variety in chemical structures) have been reported.

Secretion Systems

Nearly all bacterial virulence factors are located on the bacterial surface or are extracellular. Hence, the bacteria need specific systems to transport virulence factors out of the cells. In Gram-negative bacteria, a classification of the secretion pathways into types I–VI was based on the characteristics of the secretion mechanism [12].

Quorum-Sensing Systems and Their Impact on the Virulence of Bacterial Pathogens

A key regulatory hub for virulence is quorum sensing, bacterial cell-to-cell communication, and this is one of the most intensively studied targets for antivirulence therapy [22]. In the quorum-sensing process, bacteria coordinate the expression of certain genes in response to the presence of small signal molecules, and it was first discovered to control bioluminescence in the marine bacterium *Vibrio fischeri*. Since then, many other Gram-negative bacteria have been found to contain a similar quorum-sensing system, based on the production and detection of acylated homoserine lactones (AHLs; Figure 1). In its simplest form, such a system consists of a homolog of *Vibrio fischeri* LuxI that produces the AHL, and a homolog of *Vibrio fischeri* LuxR that detects the AHL and subsequently binds to the promoter of the quorum-sensing target genes, in this way affecting expression of these genes [22]. AHLs of different species differ in the acyl side chain, which usually contains between 4 and 18 carbons and can have an oxo or a hydroxyl substitution at the third position.

Research over the past 40 years has shown that the production of several virulence factors in bacterial pathogens of plants, animals, and humans is controlled by quorum sensing (Table 1). In addition to AHLs, many more signal molecules with different chemical structures have been

Box 2. Constituents of the Virulence Regulatory Network in Bacterial Pathogens

In addition to quorum sensing, several other regulatory mechanisms have been found to control the production of virulence factors in bacterial pathogens. These regulatory mechanisms are often interconnected, forming a complex regulatory network [15].

Regulatory RNAs

Regulatory RNAs are effective regulators that can influence protein expression and function in response to external cues such as temperature, pH, and metabolite levels [16]. Regulatory RNAs include 5′ untranslated regions (UTRs), 3′ UTRs, *cis*-acting antisense RNAs and *trans*-acting RNAs. The RNA chaperone protein Hfq is often required to facilitate a stable *trans*-acting sRNA–mRNA interaction, especially if the level of complementarity between the *trans*-acting sRNA and the target mRNA is low [17]. A major advantage of regulation by RNA structures is the speed at which it can occur (no translation needed).

Second Messengers

Second-messenger molecules are involved in relaying external signals from membrane receptors to one or more targets within the cell. The cyclic nucleotide cyclic AMP (cAMP) is generated from ATP by adenylyl cyclases, and phosphodiesterases catalyse its hydrolytic degradation [18]. In bacteria, transcription factors of the cAMP-receptor protein (CRP) family are activated by direct binding of cAMP. cAMP has central roles in regulating biofilm formation, type III secretion, carbon metabolism, and virulence gene regulation in many pathogens. Cyclic-di-GMP (c-di-GMP) is produced from two molecules of GTP by diguanylate cyclases and is broken down by specific phosphodiesterases [18]. In general, c-di-GMP stimulates biofilm formation and inhibits various forms of motility [19]. Diguanylate cyclases and phosphodiesterases respond, for example, to oxygen and redox conditions, light, starvation, and various extracellular substances [18].

Alternative Sigma Factors

Sigma factors are proteins that form essential subunits of prokaryotic RNA polymerase, thereby affecting gene expression [20]. They provide promoter recognition specificity to the RNA polymerase enzyme. The association of a certain sigma factor with core RNA polymerase enables the cells to express a particular set of genes under the appropriate conditions [20]. As the regulon of a single sigma factor can comprise hundreds of genes, sigma factors provide effective mechanisms for simultaneously regulating large numbers of genes. RpoS and RpoN are two examples of sigma factors that have been implied in the regulation of virulence.

discovered in Gram-negative pathogens, including autoinducer-2 (AI-2) [35], quinolones [36], indole [37], pyrones [38], and dialkylresorcinols [30]. Furthermore, it is becoming evident that bacteria usually do not rely on only one signal molecule, and different quorum-sensing-system architectures have been described (including both hierarchical and parallell configurations) [22,39]. In the following paragraphs, examples of such configurations are discussed as well as their impact on the virulence of model Gram-negative pathogens. We have previously hypothesised that the use of different signal molecules enables bacteria to rely on their quorum-sensing systems under various environmental conditions (with varying stabilities of the signal molecules) [31]. Indeed, different subunits of a complex quorum-sensing network can have a different impact on virulence in different hosts. In addition to this, Even-Tov *et al.* have shown that the presence of multiple quorum-sensing systems can evolve as a result of social exploitation as a strain that has acquired an additional quorum-sensing system can exploit its ancestor without this additional system [40].

Regulation of the Virulence of *Pseudomonas aeruginosa* by a Hierarchical Quorum-Sensing System

Pseudomonas aeruginosa is an opportunistic human pathogen capable of causing severe, often multiple antibiotic-resistant infections [41]. Quorum sensing plays a key role in the virulence of this bacterium, and given its importance as a human pathogen, *P. aeruginosa* has become one of the model organisms in quorum-sensing research [25]. The quorum-sensing system of

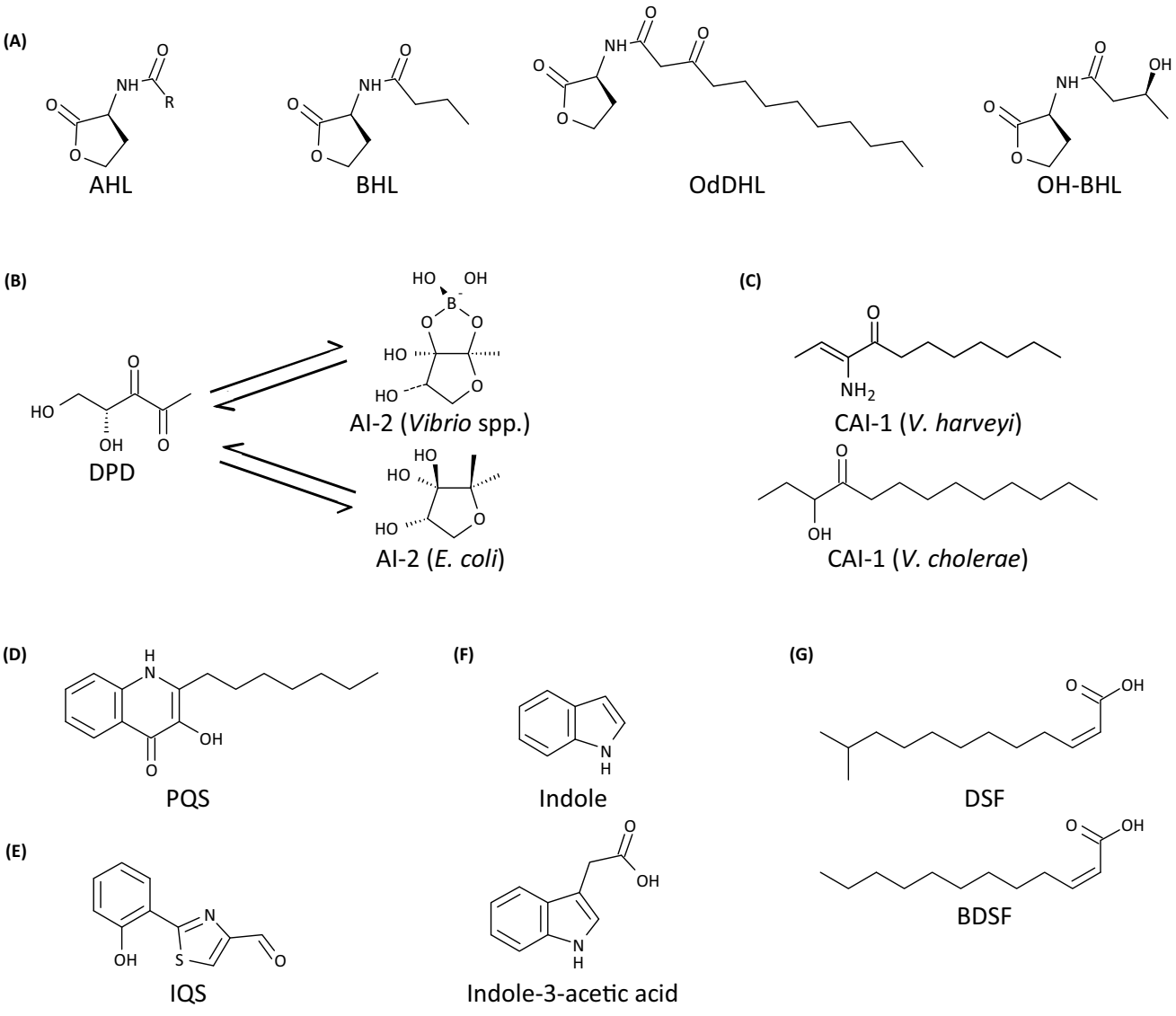

Figure 1. Chemical Structures of Selected Quorum-Sensing Signal Molecules. (A) Acylhomoserine lactones (AHLs). From left to right: general structure of an AHL; *N*-butanoyl-L-homoserine lactone (BHL) and *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL), the AHLs produced by *Pseudomonas aeruginosa*; *N*-(3-oxo-butanoyl)-L-homoserine lactone (OH-BHL), the AHL produced by *Vibrio harveyi*. (B) Autoinducer-2 (AI-2) produced by vibrios and enterobacteria, and their precursor 4,5-dihydroxy-2,3-pentanedione (DPD). (C) Cholerae autoinducer-1 (CAI-1) produced by *V. harveyi* and *Vibrio cholerae*. (D) *Pseudomonas* quinolone signal (PQS) produced by *P. aeruginosa*. (E) Integrated quorum-sensing signal (IQS) produced by *P. aeruginosa*. (F) Indole and indole-3-acetic acid. (G) Diffusible signal factor (DSF) and *Burkholderia* diffusible signal factor (BDSF).

P. aeruginosa consists of four subunits (LasI/LasR, RhlI/RhlR, pqs, and iqs) that each use a specific signal (*N*-oxododecanoyl-L-homoserine lactone (OdDHL), *N*-butanoyl-L-homoserine lactone (BHL), the *Pseudomonas* quinolone signal (PQS), and the integrated quorum sensing signal (IQS), respectively; Figure 1) and that are organised in a hierarchical manner, with the LasI/LasR system at the top of the hierarchy (Figure 2A, Key Figure).

Transcriptomic studies have revealed that the LasI/LasR and RhlI/RhlR systems control the expression of nearly 10% of the *P. aeruginosa* genome, with 254 genes (including several

Table 1. Examples of Quorum-Sensing-Regulated Virulence Factors in Bacterial Pathogens of Humans, Animals, and Plants[a]

Pathogen	Signal molecule	Selected phenotypes	Refs
Human pathogens			
Escherichia coli O157:H7	indole	Biofilm formation, motility	[23]
Helicobacter pylori	AI-2	Motility	[24]
Pseudomonas aeruginosa	OdDHL, BHL, PQS	Elastase, protease, hemolysin, rhamnolipids, virulence	[25]
	indole	Biofilm formation, motility	[26]
Vibrio cholerae	AI-2, CAI-1	Biofilm formation, protease	[27]
	indole	Biofilm formation, motility	[28]
Animal pathogens			
Aeromonas hydrophila	BHL	Protease, virulence	[29]
Photorhabdus luminescens	dialkylresorcinols	Virulence	[30]
Vibrio harveyi	OH-BHL, AI-2, CAI-1	Protease, type III secretion, siderophore, virulence	[31]
	indole	Biofilm formation, motility, virulence	[32]
Plant pathogens			
Pectobacterium carotovorum	HHL, OHHL, OOHL	Extracellular cell wall-degrading enzymes	[33]
Pseudomonas syringae	OHHL	Extracellular polysaccharides, motility, virulence	[33]
Xanthomonas campestris	DSF	Extracellular polysaccharides, biofilm formation, virulence	[34]

[a]Abbreviations: AI-2, autoinducer-2; OdDHL, *N*-(3-oxododecanoyl)-L-homoserine lactone; BHL, *N*-butanoyl-L-homoserine lactone; PQS, *Pseudomonas* quinolone signal; CAI-1, cholerae autoinducer-1; OH-BHL, *N*-(3-hydroxybutanoyl)-L-homoserine lactone; HHL, *N*-hexanoyl-L-homoserine lactone; OHHL, *N*-(3-oxohexanoyl)-L-homoserine lactone; OOHL, *N*-(3-oxo-octanoyl)-L-homoserine lactone; DSF, diffusible signal factor.

virulence genes) being induced by AHLs [42,43]. Some of the genes respond to both OdDHL and BHL, and some to only one of these signals. Virulence factors that are affected by the quorum-sensing signals of *P. aeruginosa* include the LasA protease (disrupting the epithelial barrier; OdDHL-controlled), the LasB elastase (degrading matrix proteins such as collagen; OdDHL- and BHL-controlled), alkaline protease (degrading host defense proteins; OdDHL-controlled), rhamnolipids (causing necrosis of immune cells; BHL-controlled), pyocyanin (involved in immune evasion; OdDHL-, BHL- and PQS-controlled), and LecA lectin (enhancing colonisation; PQS-controlled) [25]. The quorum-sensing system of *P. aeruginosa* is required for full virulence in various hosts, including zebrafish, fruitflies, nematodes, and mice (burn wound, pneumonia, and chronic lung infection models) [25,44]. Furthermore, both OdDHL and PQS have a direct effect on the host by modulating the immune response and apoptosis of different eukaryotic cell types, including epithelial cells and macrophages [45].

Remarkably, clinical isolates of *P. aeruginosa* from chronic infections often have mutations in *lasR*, and although based on research with laboratory strains, it was hypothesised that such mutants would have a quorum-sensing-nonresponsive phenotype, recent research revealed that some of these mutant isolates retained LasR activity, whereas others had uncoupled the RhlI/RhlR system from the LasI/LasR system [46]. Furthermore, environmental factors, such as phosphate and iron limitation, have a major impact on the hierarchy of the system as the RhlI/RhlR and the iqs system drive virulence factor production under phosphate or iron limitation [47,48]. Finally, Mukherjee *et al.* recently demonstrated that RhlR is able to control virulence

Key Figure

Quorum-Sensing Systems of Model Pathogens

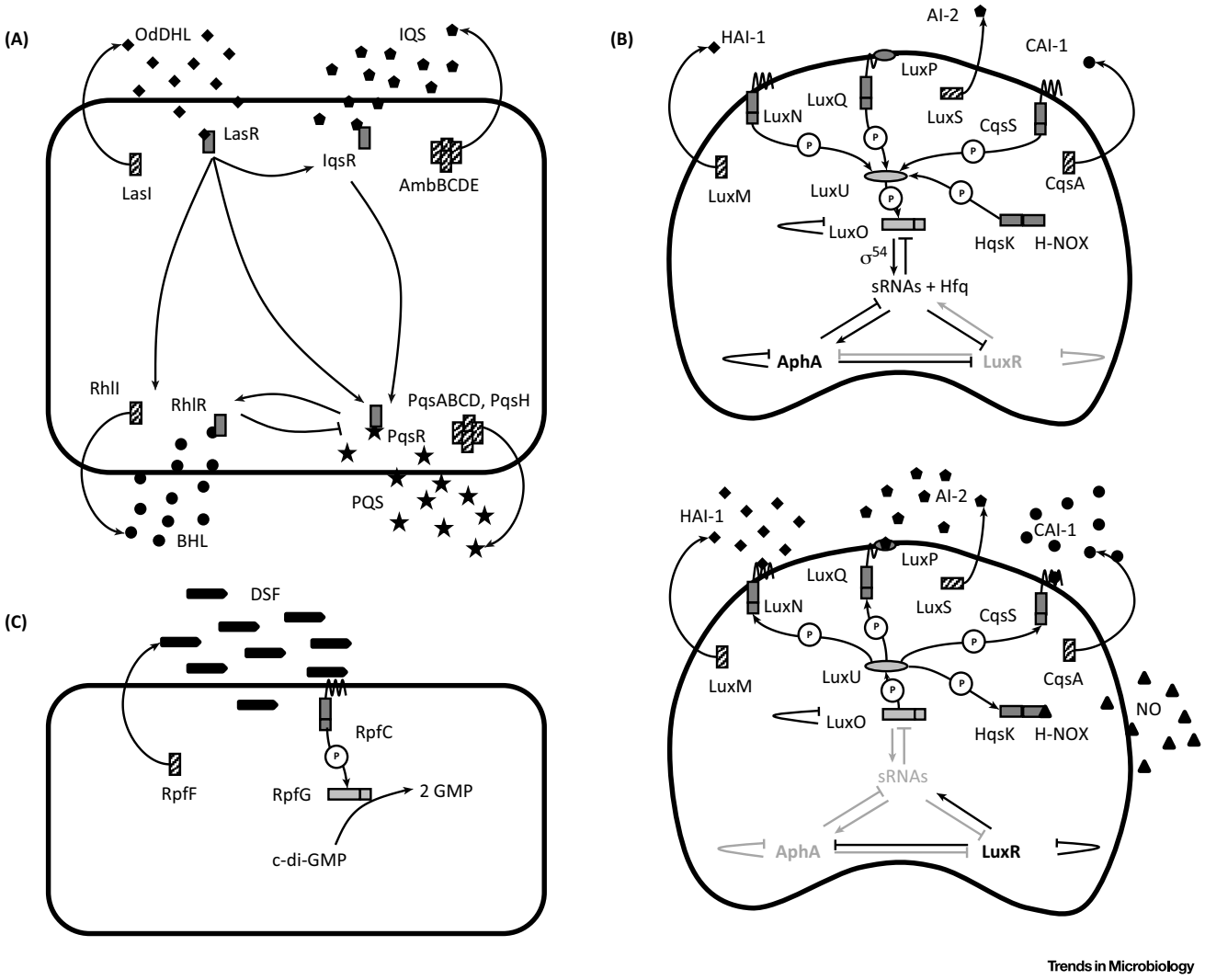

Figure 2. (A) The hierarchical quorum-sensing system of *Pseudomonas aeruginosa.* The signal molecules *N*-(3-oxo-dodecanoyl)-L-homoserine lactone (OdDHL), *N*-butanoyl-L-homoserine lactone (BHL), *Pseudomonas* quinolone signal (PQS), and integrated quorum-sensing signal (IQS) are produced by LasI, RhlI, PqsABCD, and AmbBCDE, respectively. The corresponding receptors are LasR, RhlR, PqsR, and IqsR, respectively. LasR-OdDHL activates the three other systems through activating the expression of *rhlR*, *rhlI, pqsR, pqsH*, and *ambBCDE*. PQS is able to enhance transcription of *rhlI*, whereas RhlR-BHL inhibits expression of *pqsR* and *pqsABCD*. Finally, IQS controls the PQS and RhlI/RhlR systems through a yet unknown mechanism. (B) The three-channel quorum-sensing system of *Vibrio harveyi*. LuxM, LuxS and CqsA are the synthases of the signal molecules HAI-1, AI-2, and CAI-1, respectively. These signal molecules are detected at the cell surface by the receptors LuxN, LuxPQ, and CqsS, respectively. In the absence of signal molecules (top), the receptors autophosphorylate and transfer phosphate to LuxO via LuxU. Phosphorylated LuxO is active, and together with σ^{54} it activates the production of five small regulatory RNAs (sRNAs). The sRNAs promote and inhibit translation of the master regulators AphA and LuxR, respectively. In the presence of high concentrations of the signal molecules (bottom), the receptor proteins switch from kinases to phosphatases, resulting in dephosphorylation of LuxO. Dephosphorylated LuxO is inactive and, therefore, the sRNAs are not formed, AphA is not translated, and LuxR is translated. In addition to the three signal molecules, nitric oxide (NO) is also able to dephosphorylate LuxU. (C) Diffusible signal factor (DSF) signaling in *Xanthomonas campestris*. The DSF signal molecule is produced by the RpfF protein and sensed by the two-component receptor protein RpfC. In the presence of high levels of DSF, RpfC transfers phosphate to RpfG, leading to activation of its c-di-GMP phosphodiesterase activity. See Figure 1 for the chemical structures of the signal molecules.

factors independently of BHL, probably by responding to another (thus far unknown) ligand [49]. Hence, a quorum-sensing system consisting of several subunits with a flexible hierarchy might enable *P. aeruginosa* to maintain quorum-sensing control under varying environmental conditions. Indeed, mathematical modeling revealed that the use of multiple signal molecules with distinct half-lives enables *P. aeruginosa* to effectively express secreted factors under the appropriate conditions [50]. As a consequence, when studying the impact of quorum sensing on the virulence of a pathogen, it is highly important to include clinical strains in the study and to investigate quorum-sensing regulation of virulence under different environmental conditions (preferably also including *in vivo* studies with a relevant host).

Regulation of the Virulence of *Vibrio harveyi* by a Multichannel Quorum-Sensing System

Vibrio harveyi is a major pathogen of aquatic animals, causing significant losses in the aquaculture industry worldwide [51,52]. *V. harveyi* BB120 (= ATCC BAA-1116) is a quorum-sensing model organism [27]. *V. harveyi* contains a three-channel quorum-sensing system, with three different types of signal molecule (harveyi autoinducer-1 (HAI-1), autoinducer-2 (AI-2), and cholerae autoinducer-1 (CAI-1); Figure 1) feeding a shared signal transduction cascade that controls the production of the master regulators LuxR and AphA (Figure 2B). Similar multichannel quorum-sensing systems are found in other vibrios, including the human pathogen *Vibrio cholerae* [53,54]. In addition to the three signal molecules mentioned above, nitric oxide (NO) is also able to dephosphorylate LuxO through an NO-responsive channel that also feeds into the quorum-sensing system at LuxU [55]. This channel is composed of a cytoplasmic H-NOX type NO receptor and an H-NOX-associated kinase (HqsK). It has been hypothesised that NO might enable *V. harveyi* to sense the host environment [55], and this is consistent with our previous observations that the bacterium showed a 200-fold higher maximal quorum-sensing-regulated bioluminescence when associated with a host than when free-living, and that the expression of the type III secretion system is >1000-fold higher in shrimp-associated vibrios than in vibrios grown in the absence of a host [56,57].

Microarray analyses have revealed that the quorum-sensing master regulators AphA and LuxR regulate 167 and 625 genes, respectively, and they coregulate 77 genes [58]. In addition to bioluminescence, the three-channel quorum-sensing system of *V. harveyi* controls the expression of different virulence-related phenotypes, including a type III secretion system [57], siderophore [59], chitinase [60], phospholipases [61], *vhp* metalloprotease [62], and flagellar motility [63]. The system is required for full virulence towards different hosts [64], and activity of the quorum-sensing system during infection of a host is proportional to the virulence to that host [56]. Interestingly, although the information provided by the different signal molecules is transferred through one shared signal transduction cascade, *V. harveyi* seems to be able to distinguish between them. Indeed, the different signal molecules have a different impact on virulence of the pathogen in different host organisms [64]. This might be related to a different stability of the signal molecules in different (host) environments, and to asymmetric regulation of the production of receptors, leading to higher sensitivity of the system to one of the signal molecules, as shown for the HAI-1 receptor in *V. harveyi* [65] and recently also for the CAI-1 receptor in *V. cholerae* [66]. Furthermore, Lorenz *et al.* recently reported that the signal molecule receptors show differences in copy numbers per cell and in cellular localisation [67], which might also contribute to different impacts of the different signal molecules. Finally, additional signal transduction cascades might exist that are only controlled by one of the signal molecules, as we observed AI-2-specific regulation of the *V. harveyi vhh* hemolysin gene that was independent of LuxO [62].

It is often assumed that activation of the quorum-sensing system results in a collective response in all members of the population [39]. However, using *V. harveyi* quorum sensing as a model, Anetzberger *et al.* demonstrated that the response in fact is not homogeneous as bioluminescence and other quorum-sensing-regulated genes are heterogeneously expressed in populations of wild-type *V. harveyi* [68]. High-level induction of both the luminescence gene *luxC* and the *vhp* metalloprotease gene was very rare since only 0.5% of the cells activated both genes at the same time. A similar phenomenon has been observed for various other species of bacteria (including *Pseudomonas* and *Xanthomonas* [69]), indicating that we need to reconsider our view of how cells in a population of bacteria respond to quorum-sensing signals.

Regulation of the Virulence of *Xanthomonas campestris* by DSF Signaling

Pathovars of *Xanthomonas campestris* cause diseases of agronomic importance in various crops throughout the world [70]. A particular type of quorum-sensing molecule, the diffusible signal factor (DSF; Figure 1), was originally identified in *X. campestris* pv. *campestris* [71], and DSF is perceived by the bacterium through a two-component system (Figure 2C). The presence of DSF is translated into phenotypic changes via the second messenger c-di-GMP (Box 2). A natural DSF turnover mechanism has recently been identified in *X. campestris* which enables efficient termination of DSF signaling [72]. In addition to *Xanthomonas* species, DSF-type signals are also produced by other bacteria, such as *Burkholderia* and *Pseudomonas* species [73]. Comparison of the expression profiles of wild-type *X. campestris* and a deletion mutant of the DSF synthase RpfF revealed 165 DSF-dependent genes, among which >80% are activated by DSF [74]. DSF signaling has been associated with the regulation of virulence factors such as motility, biofilm formation, iron uptake, extracellular polysaccharide and extracellular enzyme production, and virulence to plant hosts [34]. Recently, Deng *et al.* reported that *X. campestris* RpfF is responsible for the production of six different, but structurally related, DSF-type signals, and that deletion of RpfF decreases the competitive fitness of the bacterium against *Bacillus thuringiensis* by interfering with cell division and sporulation [75]. These findings indicate that quorum-sensing systems can be important for the interaction between different species of bacteria.

Virulence Regulation by the Interspecies Signals Autoinducer-2 and Indole

Many quorum-sensing signal molecules (such as AHLs) are produced only by a particular species (or a narrow range of closely related species), whereas others are produced by multiple species [22]. The best known example is AI-2, which in fact refers to a group of molecules that are in equilibrium with each other and with their precursor, 4,5-dihydroxy-2,3-pentanedione (DPD) (Figure 1). DPD is produced by the LuxS enzyme, and AI-2 and/or LuxS have been documented in many different bacteria (both Gram-negative and Gram-positive), leading scientists to hypothesise that AI-2 might serve as an interspecies signal [76]. Three types of AI-2 receptors have been identified thus far: LuxP in vibrios, LsrB in enteric bacteria, and RbsB in *Haemophilus influenzae* [35]. AI-2 regulates the production of virulence factors and virulence in host models of various pathogens, including enterohemorrhagic *Escherichia coli, H. influenzae, Helicobacter pylori, Streptococcus pneumoniae*, and *V. cholerae* [35]. This suggests that virulence inhibitors targeting AI-2 signaling might have a relatively broad spectrum. On the other hand, the fact that LuxS also has a metabolic function in the activated methyl cycle often confounds the interpretation of results, and there still is no consensus as to whether AI-2 can really be considered as a signal molecule in all bacteria that produce it, or whether it is rather a metabolic by-product that only in some cases (e.g., vibrios) serves as a true signal molecule [77]. Interestingly, Ismail *et al.* recently reported that mammalian epithelial cells produce a mimic of AI-2 in response to a secreted bacterial component and tight-junction disruption [78]. The mimic was able to stimulate AI-2-regulated phenotypes in both *V. harveyi* and *Salmonella*

enterica serovar Typhimurium. This suggests that, on the one hand, a host can steer AI-2-controlled behaviours in its associated microbiota, and on the other hand that the host-associated microbiota can force the host to induce AI-2-controlled phenotypes by producing the mimic.

Indole is another molecule that has recently gained more attention as an interspecies signaling molecule. Indole has been known for quite some time to be synthesised from tryptophan by tryptophanase (TnaA) in many different bacteria, both Gram-negative and Gram-positive [79], and enteric bacteria can produce copious amounts of indole (up to mM levels) in the mammalian gut [37]. However, the appreciation of its role as a signal molecule is of relatively recent origin. Indole has been reported to control various virulence-related phenotypes (most notably biofilm formation and motility) and virulence in human, animal, and plant pathogens [37]. Despite the fact that indole seems to have a signaling function in several bacteria, thus far, an indole receptor has not been definitively identified for any bacterium [80]. In *V. harveyi*, indole signaling decreases the activity of the three-channel quorum-sensing system [32]. Furthermore, indole signaling and the stress sigma factor RpoS are connected in vibrios, and a transcriptomic analysis indicated that indole might serve as a starvation signal in these bacteria [32,81]. The use of indole thus might increase the fitness of the bacteria in stress conditions by enabling them to sense and respond to ecological competition [82]. Finally, in addition to its impact on bacteria, indole also has a direct effect on the host as, for example, it increases the resistance of tight-junctions in epithelial cells [83].

Advances and Challenges in the Development of Quorum-Sensing-Interfering Therapies

Types of Quorum-Sensing-Interfering Agent

Because of the importance of quorum sensing in pathogenesis, there has been much investigation into interference with quorum sensing, and many potential agents have been put forth. For a comprehensive overview of reported quorum-sensing-interfering agents, I refer the reader to the excellent reviews on this topic that have recently been published [22,84–89]. Depending on the type of regulation (i.e., whether quorum sensing induces or represses virulence), the agents will need to either inhibit or stimulate quorum sensing-regulated gene expression. The latter is the case, for instance, for the human pathogen *V. cholerae*, in which quorum sensing represses biofilm formation and virulence factor production [27]. Quorum-sensing-interfering agents can be either natural or synthetic compounds acting as inhibitors or agonists of signal molecule biosynthesis, signal molecule detection, or signal transduction, or enzymes that inactivate the signal molecules, or antibodies that sequester signal molecules and induce an immune response (Table 2).

In addition to the development of clinical applications, quorum-sensing inhibitors are also valuable as research tools as they can lead to insights with respect to the functioning of quorum-sensing systems that are unnoticed in a classical genetic (gene knockout) approach, where the inactivation of a gene within a complex network does not just inactivate the specific target but can also affect other components of the network. In this respect, Welsh *et al.* observed an unexpected effect of compounds that interfere with RhlR on pyocyanin production in *P. aeruginosa*, and this was related to suppression of the pqs system by RhlR agonism, which is abolished by knockout of RhlR (thus leading to different results when using RhlR deletion mutants versus RhlR antagonists) [107]. Furthermore, the use of inhibitors enables us to screen the impact of quorum sensing on the virulence of multiple strains (laboratory strains as well as clinical isolates), which will result in a broader and more relevant picture of the impact of quorum-sensing-interference as a new strategy to control disease than we could obtain by

Table 2. Examples of Quorum-Sensing-Interfering Agents

Agent	Target bacterium	Molecular target	Activity	Refs
Natural compounds				
Ajoene	*Pseudomonas aeruginosa, Staphylococcus aureus*	Small regulatory RNAs	Inhibitory	[90,91]
Citrus limonoids	*Vibrio harveyi*	Signal transduction	Inhibitory	[92]
Flavonoids	*Pseudomonas aeruginosa*	AHL receptor	Inhibitory	[93]
Indole-3-acetic acid	*Vibrio harveyi*	Indole signaling	Agonistic	[32]
Naringenin	*Pseudomonas aeruginosa*	AHL production and detection	Inhibitory	[94]
Five compounds identified via high- throughput screening	*Pseudomonas aeruginosa*	AHL receptor	Inhibitory	[95]
Synthetic compounds				
Brominated thiophenones	*Vibrio harveyi*	Signal transduction	Inhibitory	[96]
Five compounds identified via high- throughput screening	*Burkholderia mallei, Yersinia pestis*	AHL biosynthesis	Inhibitory	[97]
Thiazolidinediones and dioxazaborocanes	*Vibrio harveyi*	AI-2 receptor	Inhibitory	[98]
3-acylpyrroles	*Vibrio cholerae*	CAI-1 receptor	Agonistic	[99]
Enzymes				
Achromobacter xylosoxidans strain Q19	*Pseudomonas aeruginosa*	PQS signal molecules	Degradation	[100]
Bacillus sp. strain NFMI-C	*Vibrio harveyi*	AHL signal molecules	Degradation	[101]
Five bacterial strains from plants	*Xanthomonas campestris*	DSF signal molecules	Degradation	[102]
Four bacterial strains from plants	*Xanthomonas citri*	DSF signal molecules	Degradation	[103]
Lactonase from *Bacillus* sp. strain QSI-1	*Aeromonas hydrophila*	AHL signal molecules	Degradation	[104]
Modified acylase PvdQ	*Burkholderia cenocepacia*	AHL signal molecules	Degradation	[105]
Antibodies				
MAb HSL-2 and HSL-4	*Pseudomonas aeruginosa*	AHL signal molecules	Immune activation	[106]

genetic studies involving only one (laboratory) strain. Indeed, in addition to decreased virulence factor production, many of the papers on quorum sensing-interfering agents also documented that these agents are capable of attenuating disease in various plant and animal models [32,42,96,101–105,108]. Together, these studies can be considered as a proof-of-concept, demonstrating the effectiveness of using quorum-sensing-interfering agents to control bacterial diseases in humans and animals as well as for crop protection.

Reliable Identification of Quorum-Sensing Inhibitors

Candidate quorum-sensing inhibitors are usually identified based on their impact on signal-molecule reporter strains that have a particular phenotype (such as green fluorescent protein, luminescence, or β-galactosidase activity) in response to quorum-sensing molecules. An important limitation of the use of these reporter strains is that such a phenotype is often codependent on other factors and/or the metabolic activity of the cells. As a consequence, compounds claimed to be quorum-sensing inhibitors based solely on this kind of experiment, might end up as false positives after further study [109,110]. Hence, adequate control experiments are needed to further substantiate true quorum-sensing inhibition by candidate compounds identified on the basis of their impact on signal molecule reporter strains. The most straightforward control experiment involves verifying the impact of a putative inhibitor on the

reporter phenotype independent of quorum sensing (i.e., under the control of a constitutive or inducible promoter). We recently proposed a new parameter, A_{QSI} (specific quorum sensing-disrupting activity), to determine the specificity of putative quorum-sensing inhibitors using this kind of reporter assay [96]. At a given concentration of a putative inhibitor, A_{QSI} is defined as the ratio of the percentage inhibition of the reporter phenotype when controlled by quorum sensing relative to the percentage inhibition of the same phenotype when it is independent of quorum sensing. A_{QSI} values need to be interpreted together with the actual impact on the quorum-sensing reporter, and the best candidates for drug development should show both high inhibition of the quorum-sensing reporter and a high A_{QSI} value, implying that they are both strong and specific inhibitors [111].

Other approaches to further substantiate quorum-sensing-inhibition by candidate inhibitors include the assessment of the impact on other quorum-sensing-regulated phenotypes in addition to the reporter phenotype [32,42,48,90], transcriptomic and/or proteomic analyses [90,95], identification of the molecular target of the compound [91–93,98,112,113], and sensitive toxicity tests [94,109]. It needs to be stressed that toxicity tests need to be very sensitive in order not to miss subtle toxic effects that have a significant impact on the expression of the reporter phenotype without affecting growth [110].

Can Pathogens Evolve Resistance to Quorum-Sensing-Interference?

One of the attractive aspects of quorum-sensing-interference is that it does not aim to kill the pathogens, and therefore it has been thought unlikely to cause harsh selective pressures, thereby minimizing the risk of resistance development [114]. It is clear that (point) mutations can arise that confer resistance to quorum-sensing inhibitors [115]. However, whether or not these mutants would become dominant in a population (and thus whether resistance would spread) will depend on whether they would have a fitness advantage under quorum-sensing inhibition. We have argued that the assumption that it is unlikely that resistance will spread might be too optimistic because it was based on experiments in an environment in which quorum sensing is not essential for growth (i.e., nutrient-rich growth media) [115]. We also argued that some antibiotic-resistance mechanisms might confer cross-resistance to quorum-sensing inhibitors. Only 2 years later, both of these hypotheses were proven correct as Maeda *et al.* demonstrated that *P. aeruginosa* can become resistant to the model quorum-sensing inhibitor furanone C-30, and that clinical antibiotic-resistant isolates showing an increased expression of a multidrug-resistant efflux pump were also resistant to the furanone [116].

The quorum-sensing-inhibitor-resistant mutants documented by Maeda *et al.* were obtained in a medium containing adenosine as the sole carbon source [116]. Growth on adenosine is dependent on the quorum-sensing-regulated intracellular nucleoside hydrolase enzyme in *P. aeruginosa*. However, Mellbye and Schuster showed that the spread of resistance will depend on whether quorum sensing affects fitness predominantly via extracellular (public) or via intracellular (private) products [117]. Indeed, if the impact is mainly on public goods (e.g., extracellular protease that determines growth on casein as sole carbon source in *P. aeruginosa*) rather than private goods (such as nucleoside hydrolase in *P. aeruginosa*), then quorum-sensing-inhibitor-sensitive mutants will take advantage of the quorum-sensing-regulated public goods produced by resistant mutants (i.e., they will behave as cheats) in the presence of an inhibitor and, as a consequence, the resistance does not spread [117]. This situation is similar to cheating by quorum-sensing-nonresponsive mutants on public goods produced by quorum-sensing-proficient wild types in the absence of an inhibitor. Social cheating by quorum-sensing-nonresponsive mutants has mainly been studied in *P. aeruginosa* [15], although such mutants have recently also been documented to spread as social cheaters

in *V. cholerae* [118]. Dandekar *et al.* reported that the presence of a substrate for quorum-sensing-regulated private goods (adenosine) can suppress cheating on quorum-sensing-regulated public goods (extracellular protease) [119]. However, Schuster *et al.* very recently argued that this observation might have been biased by other adaptations to the specific growth environment rather than pleiotropic control of quorum-sensing behavior [120]. Interestingly, given the fact that quorum-sensing-proficient *P. aeruginosa* is less susceptible to cyanide than quorum-sensing-deficient mutants, the bacterium is capable of restricting the spread of cheaters via the quorum-sensing-regulated production of toxic cyanide [121]. In contrast to *P. aeruginosa* and *V. cholerae*, quorum sensing provides resistance against invasion by a quorum-sensing-nonresponsive mutant in *V. harveyi* when cocultured in a medium where growth depends on quorum-sensing-controlled public goods (extracellular protease) [122]. Hence, in addition to environmental conditions that determine the impact of public and private goods on fitness, the capability of quorum-sensing-nonresponsive mutants to spread as cheaters also seems to depend on species-specific characteristics.

Because most of the known quorum-sensing-regulated phenotypes are extracellular, quorum sensing is often considered to mainly control the production of public goods (including various virulence factors, e.g., lytic enzymes and toxins) [120]. However, several private goods that can significantly affect the fitness of bacteria have recently been reported to be controlled by quorum sensing. These include flagellar motility in *V. harveyi* [63], resistance to oxidative stress in *P. aeruginosa* [123], resistance to osmotic stress in *V. harveyi* [124], resistance to phages in *P. aeruginosa* [125,126], *Vibrio anguillarum* [127], and *V. cholerae* [128], and resistance to predation by protozoa in *P. aeruginosa* [129]. Interestingly, the bacterial adaptive immune system CRISPR-Cas is also controlled by quorum sensing in *P. aeruginosa*, resulting in maximal CRISPR-Cas function at high cell density, that is, when the risk of phage infection is highest [130]. Hence, the question still remains as to the net effect of quorum-sensing-regulated expression of both public and private goods in a host environment. Rather than using *in vitro* systems with defined media containing different ratios of substrates for private and public goods, a better approach would be to investigate the evolution of resistance in a host model [131].

Concluding Remarks and Further Perspectives

During recent decades we have started to appreciate that the production of several virulence factors in bacterial pathogens of plants, animals, and humans is controlled by a still growing list of quorum-sensing systems. Because antibiotic resistance is becoming more and more problematic in human and veterinary medicine, the development of alternative therapies is one of the major societal challenges we are facing at this moment, and antivirulence therapy by using agents that interfere with quorum sensing in bacterial pathogens currently is an intensively studied strategy. Many quorum-sensing-interfering agents have been identified, and they have been shown to be capable of attenuating disease in various plant and animal models in the laboratory. Thus far, these agents have mainly been tested against pure cultures of pathogens. However, there are some indications that other members of mixed communities can affect the outcome of quorum-sensing-interference [132]. Moreover, quorum sensing can have an impact on the competitive fitness of bacteria in mixed communities [75]. Finally, in real infections, different species can cooperate to enhance virulence and increase tolerance to the immune defense or to antimicrobials [133]. Hence, it will be of significant interest to investigate the impact of candidate quorum-sensing inhibitors on virulence of (clinical isolates of) target pathogens in mixed communities in clinical or field trials (see Outstanding Questions).

Outstanding Questions

Are quorum-sensing-interfering agents able to control disease in real-life (i.e., clinical or field) situations?

Does quorum-sensing-interference have curative properties in addition to preventive properties?

What are the most appropriate routes for delivery of quorum-sensing-interfering agents for practical applications?

What is the impact of quorum-sensing-interfering agents on beneficial activities of the host-associated microbiota?

What is the impact of quorum sensing on the fitness of pathogens in the host and in the environment?

How quickly do pathogens evolve resistance to quorum-sensing-interfering agents?

Despite the promising results obtained with various quorum-sensing inhibitors in laboratory studies, it will probably take many more years before these agents will be used in the clinic. The reason for this is that many regulatory hurdles need to be overcome before a new drug can be released on the market [89]. Although many compounds have been claimed as quorum-sensing inhibitors, at this moment, only for a few of them has the molecular target been identified [91–93,98,112,113]. Furthermore, this kind of agent has usually been tested in preventive set-ups (i.e., by adding them before an infection was established), and consequently, it still is not clear to what extent quorum-sensing-interference would also have curative properties. Moreover, many of the virulence factors that are controlled by quorum sensing (e.g., motility, biofilm formation, type III secretion) probably have the strongest impact during early stages of infection. Therapeutic use of quorum-sensing-interfering agents as a single treatment in human medicine therefore seems less conceivable than utilisation in combination with other drugs (e.g., antibiotics) [87]. They might, however, be valuable in order to prevent infections from spreading in animal and plant production.

An approach that will save significant amounts of time and money is the use of already available drugs that were originally developed and used in the clinic for other purposes, that is, drug repurposing [134]. Some compounds have already been proven to interfere with quorum sensing in this respect, including the anticancer drug 5-fluorouracil [135], the anthelmintic drug niclosamide [136], and the antimycotic drug flucytosine [137]. Of these, 5-fluorouracil has also been shown to prevent biofilm formation on catheters in large-scale clinical trials [138].

One of the major obstacles with respect to practical applications of quorum-sensing-interfering agents will be to find adequate formulations and routes for delivery. Delivery via nanocarriers has been routinely used in other fields of medicine, and this might also be an effective method for the delivery of quorum-sensing-interfering agents [139]. This has been demonstrated, for instance, for delivery of niclosamide in *in vitro* experiments [140] and for CAI-1, the major quorum-sensing signal molecule in *V. cholerae*, in a mouse model [141].

Quorum-sensing-interfering agents have long been considered to have fewer side effects towards nontarget organisms than conventional antibiotics, and to include a low risk for resistance development. However, thus far, any definitive proof of these assumptions is still lacking. Hence, further research is needed to investigate the impact of quorum-sensing-interfering agents on beneficial activities of nontarget bacteria in the host microbiome. The interactions between a host and its associated microbiota are fairly complex and are only beginning to be understood (mainly in mammals). The major beneficial effects of the microbiota include contributions to nutrition and metabolism, and immunomodulation [142]. Quorum-sensing-interfering agents might affect these activities as well as the colonisation of the host by beneficial bacteria. This is especially relevant with respect to compounds targeting systems that are present in a broad range of bacteria. This is the case, for instance, for the AI-2 synthase LuxS, which has a significant impact on growth and biofilm formation of beneficial bacteria such as *Lactobacillus* spp. and *Bifidobacterium* spp. [143,144], and as a consequence, broad-spectrum LuxS inhibitors might have a negative impact on the fitness of these bacteria in the intestinal tract.

References

1. Cegelski, L. *et al.* (2008) The biology and future prospects of antivirulence therapies. *Nat. Rev. Microbiol.* 6, 17–27
2. Clatworthy, A.E. *et al.* (2007) Targeting virulence: a new paradigm for antimicrobial therapy. *Nat. Chem. Biol.* 3, 541–548
3. Hancock, R.E.W. (2014) Collateral damage. *Nat. Biotechnol.* 32, 66–68
4. WHO (2014) *Antimicrobial Resistance: Global Report on Surveillance,* World Health Organization

5. McCarter, L.L. (2004) Dual flagellar systems enable motility under different circumstances. *J. Mol. Microbiol. Biotechnol.* 7, 18–29
6. Wadhams, G.H. and Armitage, J.P. (2004) Making sense of it all: bacterial chemotaxis. *Nat. Rev. Mol. Cell Biol.* 5, 1024–1037
7. Maier, B. and Wong, G.C.L. (2015) How bacteria use type IV pili machinery on surfaces. *Trends Microbiol.* 23, 775–788
8. Chen, Y. *et al.* (2010) Genetic analysis of the capsule polysaccharide (K antigen) and exopolysaccharide genes in pandemic *Vibrio parahaemolyticus* O3:K6. *BMC Microbiol.* 10, 274
9. Donlan, R.M. and Costerton, J.W. (2002) Biofilms: survival mechanisms of clinically relevant microorganisms. *Clin. Microbiol. Rev.* 15, 167–193
10. Finlay, B.B. and Falkow, S. (1997) Common themes in microbial pathogenicity revisited. *Microbiol. Mol. Biol. Rev.* 61, 136–169
11. Skaar, E.P. (2010) The battle for iron between bacterial pathogens and their vertebrate hosts. *PLoS Pathog.* 6, e1000949
12. Gerlach, R.G. and Hensel, M. (2007) Protein secretion systems and adhesins: the molecular armory of Gram-negative pathogens. *Int. J. Med. Microbiol.* 297, 401–415
13. Rasko, D.A. and Sperandio, V. (2010) Anti-virulence strategies to combat bacteria-mediated disease. *Nat. Rev. Drug Discov.* 9, 117–128
14. Defoirdt, T. (2013) Antivirulence therapy for animal production: filling an arsenal with novel weapons for sustainable disease control. *PLoS Pathog.* 9, e1003603
15. Asfahl, K.L. and Schuster, M. (2017) Social interactions in bacterial cell-cell signaling. *FEMS Microbiol. Rev.* 41, 92–107
16. Gripenland, J. *et al.* (2010) RNAs: regulators of bacterial virulence. *Nat. Rev. Microbiol.* 8, 857–866
17. Waters, L.S. and Storz, G. (2009) Regulatory RNAs in bacteria. *Cell* 136, 615–628
18. Kalia, D. *et al.* (2013) Nucleotide, c-di-GMP, c-di-AMP, cGMP, cAMP, (p)ppGpp signaling in bacteria and implications in pathogenesis. *Chem. Soc. Rev.* 42, 305–341
19. Hengge, R. (2009) Principles of c-di-GMP signalling in bacteria. *Nat. Rev. Microbiol.* 7, 263–273
20. Kazmierczak, M.J. *et al.* (2005) Alternative sigma factors and their roles in bacterial virulence. *Microbiol. Mol. Biol. Rev.* 69, 527–543
21. Ruer, S. *et al.* (2015) Virulence-targeted antibacterials: concept, promise, and susceptibility to resistance mechanisms. *Chem. Biol. Drug Des.* 86, 379–399
22. LaSarre, B. and Federle, M.J. (2013) Exploiting quorum sensing to confuse bacterial pathogens. *Microbiol. Mol. Biol. Rev.* 77, 73–111
23. Bansal, T. *et al.* (2007) Differential effects of epinephrine, norepinephrine and indole on *Escherichia coli* O157:H7 chemotaxis, colonization, and gene expression. *Infect. Immun.* 75, 4597–4607
24. Rader, B.A. *et al.* (2007) The quorum-sensing molecule autoinducer 2 regulates motility and flagellar morphogenesis in *Helicobacter pylori*. *J. Bacteriol.* 189, 6109–6117
25. Lee, J. and Zhang, L. (2015) The hierarchy quorum sensing network in *Pseudomonas aeruginosa*. *Protein Cell* 6, 26–41
26. Lee, J. *et al.* (2009) Indole and 7-hydroxyindole diminish *Pseudomonas aeruginosa* virulence. *Microb. Biotechnol.* 2, 75–90
27. Ng, W.L. and Bassler, B.L. (2009) Bacterial quorum-sensing network architectures. *Annu. Rev. Genet.* 43, 197–222
28. Mueller, R.S. *et al.* (2009) Indole acts as an extracellular cue regulating gene expression in *Vibrio cholerae*. *J. Bacteriol.* 191, 3504–3516
29. Natrah, F.M.I. *et al.* (2012) The impact of quorum sensing on the virulence of *Aeromonas hydrophila* and *Aeromonas salmonicida* towards burbot (*Lota lota* L.) larvae. *Vet. Microbiol.* 159, 77–82
30. Brameyer, S. *et al.* (2015) Dialkylresorcinols as bacterial signaling molecules. *Proc. Natl. Acad. Sci. U. S. A.* 112, 572–577
31. Defoirdt, T. *et al.* (2008) Quorum sensing and quorum quenching in *Vibrio harveyi*: lessons learned from *in vivo* work. *ISME J.* 2, 19–26
32. Yang, Q. *et al.* (2017) Indole signalling and (micro)algal auxins decrease the virulence of *Vibrio campbellii*, a major pathogen of aquatic organisms. *Environ. Microbiol.* 19, 1987–2004
33. Helman, Y. and Chernin, L. (2015) Silencing the mob: disrupting quorum sensing as a means to fight plant disease. *Mol. Plant Pathol.* 16, 316–329
34. Zhou, L. *et al.* (2017) The DSF family of quorum sensing signals: diversity, biosynthesis, and turnover. *Trends Microbiol.* 25, 293–303
35. Pereira, C.S. *et al.* (2013) AI-2-mediated signalling in bacteria. *FEMS Microbiol. Rev.* 37, 156–181
36. Diggle, S.P. *et al.* (2007) 4-quinolone signalling in *Pseudomonas aeruginosa*: old molecules, new perspectives. *Int. J. Med. Microbiol.* 296, 83–91
37. Lee, J.H. *et al.* (2015) Roles of indole as an interspecies and interkingdom signaling molecule. *Trends Microbiol.* 23, 707–717
38. Brachman, A.O. *et al.* (2013) Pyrones as bacterial signaling molecules. *Nat. Chem. Biol.* 9, 573–578
39. Papenfort, K. and Bassler, B.L. (2016) Quorum sensing signal-response systems in Gram-negative bacteria. *Nat. Rev. Microbiol.* 14, 576–588
40. Even-Tov, E. *et al.* (2016) Social evolution selects for redundancy in bacterial quorum sensing. *PLoS Biol.* 14, e1002386
41. Lister, P.D. *et al.* (2009) Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. *Clin. Microbiol. Rev.* 22, 582–610
42. Hentzer, M. *et al.* (2003) Attenuation of *Pseudomonas aeruginosa* virulence by quorum sensing inhibitors. *EMBO J.* 22, 3803–3815
43. Schuster, M. and Greenberg, E.P. (2006) A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. *Int. J. Med. Microbiol.* 17, 189–195
44. Smith, R.S. and Iglewski, B.H. (2003) *P. aeruginosa* quorum-sensing systems and virulence. *Curr. Opin. Microbiol.* 6, 56–60
45. Liu, Y.C. *et al.* (2015) Modulation of host biology by *Pseudomonas aeruginosa* quorum sensing signal molecules: messengers or traitors. *Front. Microbiol.* 6, 1226
46. Feltner, J.B. *et al.* (2016) LasR variant cystic fibrosis isolates reveal an adaptable quorum-sensing hierarchy in *Pseudomonas aeruginosa*. *mBio* 7, e01513–16
47. Lee, J. *et al.* (2013) A cell-cell communication signal integrates quorum sensing and stress response. *Nat. Chem. Biol.* 9, 339–343
48. Welsh, M.A. and Blackwell, H.E. (2016) Chemical genetics reveals environment-specific roles for quorum sensing circuits in *Pseudomonas aeruginosa*. *Cell Chem. Biol.* 23, 361–369
49. Mukherjee, S. *et al.* (2017) The RhlR quorum-sensing receptor controls *Pseudomonas aeruginosa* pathogenesis and biofilm development independently of its canonical homoserine lactone autoinducer. *PLoS Pathog.* 13, e1006504
50. Cornforth, D.M. *et al.* (2014) Combinatorial quorum sensing allows bacteria to resolve their social and physical environment. *Proc. Natl. Acad. Sci. U. S. A.* 111, 4280–4284
51. Defoirdt, T. *et al.* (2007) Alternatives to antibiotics to control bacterial infections: luminescent vibriosis in aquaculture as an example. *Trends Biotechnol.* 25, 472–479
52. Ruwandeepika, H.A.D. *et al.* (2012) Pathogenesis, virulence factors and virulence regulation of vibrios belonging to the *Harveyi* clade. *Rev. Aquac.* 4, 59–74
53. Milton, D.L. (2006) Quorum sensing in vibrios: complexity for diversification. *Int. J. Med. Microbiol.* 296, 61–71
54. Ball, A.S. *et al.* (2017) Quorum sensing gene regulation by LuxR/HapR master regulators in vibrios. *J. Bacteriol.* 199, e00105–17

55. Henares, B.M. *et al.* (2012) Discovery of a nitric oxide responsive quorum sensing circuit in *Vibrio harveyi*. *ACS Chem. Biol.* 7, 1331–1336
56. Defoirdt, T. and Sorgeloos, P. (2012) Monitoring of *Vibrio harveyi* quorum sensing activity in real time during infection of brine shrimp larvae. *ISME J.* 6, 2314–2319
57. Ruwandeepika, H.A.D. *et al.* (2015) Expression and quorum sensing regulation of type III secretion system genes of *Vibrio harveyi* during infection of gnotobiotic brine shrimp. *PLoS One* 10, e0143935
58. van Kessel, J.C. *et al.* (2013) Individual and combined roles of the master regulators AphA and LuxR in control of the *Vibrio harveyi* quorum-sensing regulon. *J. Bacteriol.* 195, 436–443
59. Lilley, B.N. and Bassler, B.L. (2000) Regulation of quorum sensing in *Vibrio harveyi* by LuxO and σ54. *Mol. Microbiol.* 36, 940–954
60. Defoirdt, T. *et al.* (2010) Quorum sensing negatively regulates chitinase in *Vibrio harveyi*. *Environ. Microbiol. Rep.* 2, 44–49
61. Natrah, F.M.I. *et al.* (2011) Regulation of virulence factors by quorum sensing in *Vibrio harveyi*. *Vet. Microbiol.* 154, 124–129
62. Ruwandeepika, H.A.D. *et al.* (2011) Quorum sensing regulation of virulence gene expression in *Vibrio harveyi in vitro* and *in vivo* during infection of gnotobiotic brine shrimp larvae. *Environ. Microbiol. Rep.* 3, 597–602
63. Yang, Q. and Defoirdt, T. (2015) Quorum sensing positively regulates flagellar motility in pathogenic *Vibrio harveyi*. *Environ. Microbiol.* 17, 960–968
64. Pande, G.S.J. *et al.* (2013) The *Vibrio campbellii* quorum sensing signals have a different impact on virulence of the bacterium towards different crustacean hosts. *Vet. Microbiol.* 167, 540–545
65. Teng, S.W. *et al.* (2011) Active regulation of receptor ratios controls intergration of quorum-sensing signals in *Vibrio harveyi*. *Mol. Syst. Biol.* 7, 1–14
66. Hurley, A. and Bassler, B.L. (2017) Asymmetric regulation of quorum-sensing receptors drives autoinducer-specific gene expression programs in *Vibrio cholerae*. *PLoS Genet.* 13, e1006826
67. Lorenz, N. *et al.* (2017) Activity, abundance, and localization of quorum sensing receptors in *Vibrio harveyi*. *Front. Microbiol.* 8, 634
68. Anetzberger, C. *et al.* (2012) Single cell analysis of *Vibrio harveyi* uncovers functional heterogeneity in response to quorum sensing signals. *BMC Microbiol.* 12, 209
69. Pradhan, B.B. and Chatterjee, S. (2014) Reversible non-genetic phenotypic heterogeneity in bacterial quorum sensing. *Mol. Microbiol.* 92, 557–569
70. Mansfield, J. *et al.* (2012) Top 10 plant pathogenic bacteria in molecular plant pathology. *Mol. Plant Pathol.* 13, 614–629
71. He, Y.W. and Zhang, L.H. (2008) Quorum sensing and virulence regulation in *Xanthomonas campestris*. *FEMS Microbiol. Rev.* 32, 842–857
72. Zhou, L. *et al.* (2015) Identification and characterization of naturally occurring DSF-family quorum sensing turnover system in the phytopathogen *Xanthomonas*. *Environ. Microbiol.* 17, 4646–4658
73. Deng, Y. *et al.* (2011) Listening to a new language: DSF-based quorum sensing in Gram-negative bacteria. *Chem. Rev.* 111, 160–173
74. He, Y.W. *et al.* (2006) Genome scale analysis of diffusible signal factor regulon in *Xanthomonas campestris* pv. *campestris*: identification of novel cell-cell communication-dependent genes and functions. *Mol. Microbiol.* 59, 610–622
75. Deng, Y. *et al.* (2016) Diffusible signal factor family signals provide a fitness advantage to *Xanthomonas campestris* pv. *campestris* in interspecies competition. *Environ. Microbiol.* 18, 1534–1545
76. Miller, M.B. and Bassler, B.L. (2001) Quorum sensing in bacteria. *Annu. Rev. Microbiol.* 55, 165–199
77. Vendeville, A. *et al.* (2005) Making 'sense' of metabolism: autoinducer-2, LuxS and pathogenic bacteria. *Nat. Rev. Microbiol.* 3, 383–396
78. Ismail, A.S. *et al.* (2016) A host-produced autoinducer-2 mimic activates bacterial quorum sensing. *Cell Host Microbe* 19, 470–480
79. Lee, J.H. and Lee, J. (2010) Indole as an intercellular signal in microbial communities. *FEMS Microbiol. Rev.* 34, 426–444
80. Melander, R.J. *et al.* (2014) Controlling bacterial behavior with indole-containing natural products and derivatives. *Tetrahedron* 70, 6363–6372
81. Li, X. *et al.* (2014) RpoS and indole signaling control the virulence of *Vibrio anguillarum* towards gnotobiotic sea bass (*Dicentrarchus labrax*) larvae. *PLoS One* 9, e111801
82. Cornforth, D.M. and Foster, K.R. (2013) Competition sensing: the social side of bacterial stress responses. *Nat. Rev. Microbiol.* 11, 285–293
83. Bansal, T. *et al.* (2009) The bacterial signal indole increases epithelial-cell tight-junction resistance and attenuates indicators of inflammation. *Proc. Natl. Acad. Sci. U. S. A.* 107, 228–233
84. Roy, V. *et al.* (2011) Developing next generation antimicrobials by intercepting AI-2 mediated quorum sensing. *Enzyme Microb. Technol.* 49, 113–123
85. Kalia, V.C. (2013) Quorum sensing inhibitors: an overview. *Biotechnol. Adv.* 31, 224–245
86. Tang, K. and Zhang, X.H. (2014) Quorum quenching agents: resources for antivirulence therapy. *Mar. Drugs* 12, 3245–3282
87. Grandclément, C. *et al.* (2016) Quorum quenching: role in nature and applied developments. *FEMS Microbiol. Rev.* 40, 86–116
88. Silva, L.N. *et al.* (2016) Plant natural products targeting bacterial virulence factors. *Chem. Rev.* 116, 9162–9236
89. Rampioni, G. *et al.* (2014) The art of antibacterial warfare: deception through interference with quorum sensing-mediated communication. *Bioorg. Chem.* 55, 60–68
90. Jakobsen, T.H. *et al.* (2012) Ajoene, a sulfur-rich molecule from garlic, inhibits genes controlled by quorum sensing. *Antimicrob. Agents Chemother.* 56, 2314–2325
91. Jakobsen, T.H. *et al.* (2017) A broad range quorum sensing inhibitor working through sRNA inhibition. *Sci. Rep.* 7, 9857
92. Vikram, A. *et al.* (2011) Citrus limonoids interfere with *Vibrio harveyi* cell-cell signalling and biofilm formation by modulating the response regulator LuxO. *Microbiology* 157, 99–110
93. Paczkowski, J.E. *et al.* (2017) Flavonoids suppress *Pseudomonas aeruginosa* virulence through allosteric inhibition of quorum-sensing receptors. *J. Biol. Chem.* 292, 4064–4076
94. Vandeputte, O.M. *et al.* (2011) The flavanone naringenin reduces the production of quorum sensing-controlled virulence factors in *Pseudomonas aeruginosa* PAO1. *Microbiology* 157, 2120–2132
95. Tan, S.Y.Y. *et al.* (2013) Identification of five structurally unrelated quorum-sensing inhibitors of *Pseudomonas aeruginosa* from a natural-derivative database. *Antimicrob. Agents Chemother.* 57, 5629–5641
96. Yang, Q. *et al.* (2015) Specific quorum sensing-disrupting activity (A_{QSI}) of thiophenones and their therapeutic potential. *Sci. Rep.* 5, 18033
97. Christensen, Q.H. *et al.* (2013) A high-throughput screen for quorum-sensing inhibitors that target acyl-homoserine lactone synthases. *Proc. Natl. Acad. Sci. U. S. A.* 110, 13815–13820
98. Brackman, G. *et al.* (2013) Synthesis and evaluation of thiazolidinedione and dioxazaborocane analogues as inhibitors of AI-2 quorum sensing in *Vibrio harveyi*. *Bioorg. Med. Chem.* 21, 660–667
99. Perez, L.J. *et al.* (2014) Highly potent, chemically stable quorum sensing agonists for *Vibrio cholerae*. *Chem. Sci.* 5, 151–155
100. Soh, E.Y.C. *et al.* (2015) Biotic inactivation of the *Pseudomonas aeruginosa* quinolone signal molecule. *Environ. Microbiol.* 17, 4352–4365

101. Pande, G.S.J. *et al.* (2015) Isolation of AHL-degrading bacteria from micro-algal cultures and their impact on algal growth and on virulence of *Vibrio campbellii* to prawn larvae. *Appl. Microbiol. Biotechnol.* 99, 10805–10813

102. Newman, K.L. *et al.* (2008) Virulence of plant pathogenic bacteria attenuated by degrdation of fatty acid cell-to-cell signaling factors. *Mol. Plant Microbe Interact.* 21, 326–334

103. Caicedo, J.C. *et al.* (2016) Bacteria from the citrus phylloplane can disrupt cell-cell signalling in *Xanthomonas citri* and reduce citrus canker disease severity. *Plant Pathol.* 65, 782–791

104. Chu, W. *et al.* (2014) Quorum quenching bacteria *Bacillus* sp. QSI-1 protect zebrafish (*Danio rerio*) from *Aeromonas hydrophila* infection. *Sci. Rep.* 4, 5446

105. Koch, G. *et al.* (2014) Reducing virulence of the human pathogen *Burkholderia* by altering the substrate specificity of the quorum-quenching acylase PvdQ. *Proc. Natl. Acad. Sci. U. S. A.* 111, 1568–1573

106. Palliyil, S. *et al.* (2014) High-sensitivity monoclonal antibodies specific for homoserine lactones protect mice from lethal *Pseudomonas aeruginosa* infections. *Appl. Environ. Microbiol.* 80, 462–469

107. Welsh, M.A. *et al.* (2015) Small molecule disruption of quorum sensing cross-regulation in *Pseudomonas aeruginosa* causes major and unexpected alterations to virulence phenotypes. *J. Am. Chem. Soc.* 137, 1510–1519

108. Defoirdt, T. *et al.* (2006) Quorum sensing-disrupting brominated furanones protect the gnotobiotic brine shrimp *Artemia franciscana* from pathogenic *Vibrio harveyi, Vibrio campbellii* and *Vibrio parahaemolyticus* isolates. *Appl. Environ. Microbiol.* 72, 6419–6423

109. Defoirdt, T. *et al.* (2013) The apparent quorum-sensing inhibitory activity of pyrogallol is a side effect of peroxide production. *Antimicrob. Agents Chemother.* 57, 2870–2873

110. Defoirdt, T. *et al.* (2013) Quorum sensing inhibitors: how strong is the evidence? *Trends Microbiol.* 21, 619–624

111. Defoirdt, T. (2016) Specific antivirulence activity, a new concept for reliable screening of virulence inhibitors. *Trends Biotechnol.* 34, 527–529

112. Defoirdt, T. *et al.* (2007) The natural furanone (5*Z*)-4-bromo-5-(bromomethylene)-3-butyl-2(5*H*)-furanone disrupts quorum sensing-regulated gene expression in *Vibrio harveyi* by decreasing the DNA-binding activity of the transcriptional regulator protein LuxR. *Environ. Microbiol.* 9, 2486–2495

113. Suneby, E.G. *et al.* (2017) *Pseudomonas aeruginosa* LasR-DNA binding is directly inhibited by quorum sensing antagonists. *ACS Infect. Dis.* 3, 183–189

114. Von Bodman, S.B. *et al.* (2008) Cell–cell communication in bacteria: united we stand. *J. Bacteriol.* 190, 4377–4391

115. Defoirdt, T. *et al.* (2010) Can bacteria evolve resistance to quorum sensing disruption? *PLoS Pathog.* 6, e1000989

116. Maeda, T. *et al.* (2012) Quorum quenching quandary: resistance to antivirulence compounds. *ISME J.* 6, 493–501

117. Mellbye, B. and Schuster, M. (2011) The sociomicrobiology of antivirulence drug resistance: a proof of concept. *mBio* 2, e00131–11

118. Katzianer, D.S. *et al.* (2015) 'Quorum non-sensing': social cheating and deception in *Vibrio cholerae*. *Appl. Environ. Microbiol.* 81, 3856–3862

119. Dandekar, A.A. *et al.* (2012) Bacterial quorum sensing and metabolic incentives to cooperate. *Science* 338, 264–266

120. Schuster, M. *et al.* (2017) Why quorum sensing controls private goods. *Front. Microbiol.* 8, 885

121. Wang, M. *et al.* (2015) Quorum sensing and policing of *Pseudomonas aeruginosa* social cheaters. *Proc. Natl. Acad. Sci. U. S. A.* 112, 2187–2191

122. Bruger, E.L. and Waters, C.M. (2016) Bacterial quorum sensing stabilizes cooperation by optimizing growth strategies. *Appl. Environ. Microbiol.* 82, 6498–6506

123. García-Contreras, R. *et al.* (2015) Quorum sensing enhancement of the stress response promotes resistance to quorum quenching and prevents social cheating. *ISME J.* 9, 115–125

124. van Kessel, J.C. *et al.* (2015) Quorum sensing regulates the osmotic stress response in *Vibrio harveyi*. *J. Bacteriol.* 197, 73–80

125. Saucedo-Mora *et al.* (2017) Selection of functional quorum sensing systems by lysogenic bacteriophages in *Pseudomonas aeruginosa*. *Front. Microbiol.* 8, 1669

126. Moreau, P. *et al.* (2017) Bacterial cell-to-cell signaling promotes the evolution of resistance to parasitic bacteriophages. *Ecol. Evol.* 7, 1936–1941

127. Tan, D. *et al.* (2015) Quorum sensing determines the choice of antiphage defense strategy in *Vibrio anguillarum*. *mBio* 6, e00627-15

128. Hoque, M.M. *et al.* (2016) Quorum regulated resistance of *Vibrio cholerae* against environmental bacteriophages. *Sci. Rep.* 6, 37956

129. Friman, V.P. *et al.* (2013) Protist predation can favour cooperation within bacterial species. *Biol. Lett.* 9, 2013548

130. Høyland-Kroghsbo, N.M. *et al.* (2017) Quorum sensing controls the *Pseudomonas aeruginosa* CRISPR-Cas adaptive immune system. *Proc. Natl. Acad. Sci. U. S. A.* 114, 131–135

131. García-Contreras, R. *et al.* (2016) Can resistance against quorum-sensing interference be selected? *ISME J.* 10, 4–10

132. Zhang, Y. *et al.* (2017) Pitfalls associated with evaluating enzymatic quorum quenching activity: the case of MomL and its effect on *Pseudomonas aeruginosa* and *Acinetobacter baumanii* biofilms. *PeerJ* 5, e3251

133. Tan, C.H. *et al.* (2017) All together now: experimental multispecies biofilm systems. *Environ. Microbiol.* 19, 42–53

134. Soo, V.W.C. *et al.* (2017) Repurposing of anticancer drugs for the treatment of bacterial infections. *Curr. Top. Med. Chem.* 17, 1–20

135. Ueda, A. *et al.* (2009) Uracil influences quorum sensing and biofilm formation in *Pseudomonas aeruginosa* and fluorouracil is an antagonist. *Microb. Biotechnol.* 2, 62–74

136. Imperi, F. *et al.* (2013) New life for an old drug: the antihelminthic drug niclosamide inhibits *Pseudomonas aeruginosa* quorum sensing. *Antimicrob. Agents Chemother.* 57, 996–1005

137. Imperi, F. *et al.* (2013) Repurposing the antimycotic drug flucytosine for suppression of *Pseudomonas aeruginosa* pathogenicity. *Proc. Natl. Acad. Sci. U. S. A.* 110, 7458–7463

138. Walz, J.M. *et al.* (2010) Anti-infective external coating of central venous catheders: a randomized, noninferiority trial comparing 5-fluorouracil with chlorhexidine/silver sulfadiazine in preventing catheter colonization. *Crit. Care Med.* 38, 2095–2102

139. Singh, B.N. *et al.* (2017) Bactericidal, quorum quenching and anti-biofilm nanofactories: a new niche for nanotechnologists. *Crit. Rev. Biotechnol.* 37, 525–540

140. Costabile, G. *et al.* (2015) Toward repositioning niclosamide for antivirulence therapy of *Pseudomonas aeruginosa* lung infections: development of inhalable formulations through nanosuspension technology. *Mol. Pharm.* 12, 2604–2617

141. Lu, H.D. *et al.* (2015) Modulating *Vibrio cholerae* quorum-sensing-controlled communication using autoinducer-loaded nanoparticles. *Nano Lett.* 15, 2235–2241

142. Sekirov, I. *et al.* (2010) Gut microbiota in health and disease. *Physiol. Rev.* 90, 859–904

143. Lebeer, S. *et al.* (2007) Fubctional analysis of *luxS* in the probiotic strain *Lactobacillus rhamnosus* GG reveals a central metabolic role important for growth and biofilm formation. *J. Bacteriol.* 189, 860–871

144. Christiaen, S.E.A. *et al.* (2014) Autoinducer-2 plays a crucial role in gut colonization and probiotic functionality of *Bifidobacterium breve* UCC2003. *PLoS One* 9, e98111