FEMS Microbiology Reviews, 2023, 47, 1–18
DOI: 10.1093/femsre/fuad031
Advance access publication date: 13 June 2023
Review Article

Quorum sensing architecture network in *Escherichia coli* virulence and pathogenesis

Celia Mayer [1,2], Anabela Borges [1,3], Saskia-Camille Flament-Simon [2], Manuel Simões [1,3,*]

[1]LEPABE—Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
[2]Laboratorio de Referencia de E. coli, FIDIS—Instituto de Investigación Sanitaria de Santiago de Compostela, Universidade de Santiago de Compostela, 15706 Santiago de Compostela, Spain
[3]ALiCE—Associate Laboratory in Chemical Engineering, Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal
*Corresponding author. Faculty of Engineering, University of Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal. E-mail: mvs@fe.up.pt
Editor: [Claudio Avignone Rossa]

Abstract

Escherichia coli is a Gram-negative commensal bacterium of the normal microbiota of humans and animals. However, several *E. coli* strains are opportunistic pathogens responsible for severe bacterial infections, including gastrointestinal and urinary tract infections. Due to the emergence of multidrug-resistant serotypes that can cause a wide spectrum of diseases, *E. coli* is considered one of the most troublesome human pathogens worldwide. Therefore, a more thorough understanding of its virulence control mechanisms is essential for the development of new anti-pathogenic strategies. Numerous bacteria rely on a cell density-dependent communication system known as quorum sensing (QS) to regulate several bacterial functions, including the expression of virulence factors. The QS systems described for *E. coli* include the orphan SdiA regulator, an autoinducer-2 (AI-2), an autoinducer-3 (AI-3) system, and indole, which allow *E. coli* to establish different communication processes to sense and respond to the surrounding environment. This review aims to summarise the current knowledge of the global QS network in *E. coli* and its influence on virulence and pathogenesis. This understanding will help to improve anti-virulence strategies with the *E. coli* QS network in focus.

Keywords: *Escherichia coli*, quorum sensing, acyl-homoserine lactone, SdiA, AI-2, AI-3, indole

Introduction

Escherichia coli is a Gram-negative, rod-shaped, and non-sporulating bacterium that belongs to the *Gammaproteobacteria* class and *Enterobacteriaceae* family (Gomes 2016). *E. coli* is the most abundant facultative anaerobic microorganism associated with the human and animal intestinal microbiome (Kaper et al. 2004), providing many advantages to their hosts as a symbiotic (Nicolas-Chanoine et al. 2014). Although *E. coli* includes commensal inhabitants of the normal microbiota of the digestive tract, several *E. coli* strains or serotypes are also opportunistic pathogens responsible for severe infections. For that, they constitute a major problem for the healthcare systems and animal industries worldwide (Denamur et al. 2021). Due to the phenotypic plasticity of *E. coli*, several adapted clones have acquired specific virulence determinants by mobilizing genetic elements, leading to the emergence of new virulence factor combinations that produce a wide spectrum of diseases (Kaper et al. 2004, Gomes et al. 2016). Although these most successful pathotypes are organised based on different criteria, they are further classified into intra and extraintestinal pathogenic *E. coli*—InPEC and ExPEC, respectively (Nicolas-Chanoine et al. 2014, Riley 2020). The InPEC group encompasses several toxin-producing *E. coli* strains associated with enteric syndromes and haemorrhages, and are the major cause of diarrhoea and haemolytic uraemic syndrome around the world (Kaper et al. 2004, Sperandio 2010, Sharma et al. 2016, Yang et al. 2017, Culler et al. 2018). The latest surveillance report of the European Centre for Disease Prevention and Control (2022) has estimated for 2021 an overall rate of 2.2 cases of shiga-toxin-producing *E. coli* infection per 100 000 population in Europe, representing a 37.5% increase compared with 2020 (ECDC 2022). The highest notification rate of confirmed cases was observed in children aged 0–4 years, with 12.7 cases per 100 000 population for males and 10.8 cases per 100 000 population for females (ECDC 2022). Worldwide, 18 million disability-adjusted life years were attributed to foodborne diarrhoeal disease agents, particularly non-typhoid *Salmonella* and enteropathogenic *E. coli*, or EPEC (WHO, 2015). InPEC can produce biofilms on abiotic and biotic surfaces, which promote their persistence in the gastrointestinal tract for longer periods (Culler et al. 2018). Moreover, they are responsible for relevant food and waterborne illness outbreaks, causing important economic losses to farmers, the animal industry, and clinical sectors worldwide (Yang et al. 2017).

As an extraintestinal pathogen, *E. coli* is the most frequent agent (~80%) of urinary tract infections (UTIs) in humans, with a total of 404.61 (95% UI: 359.43–446.55) million of cases reported in 2019 globally (Yang et al. 2022), and one of the most common causes of Gram-negative bacteraemia in hospitalized patients (Foxman 2002, Croxen 2010). They can also produce meningitis, neonatal sepsis, and animal infections such as mastitis (Sharma et al. 2016). It has been described that *E. coli* and other *Enterobacteriaceae* are the first colonizing microorganisms of the urinary tract in UTIs, as well as in catheter-acquired UTIs (CAUTIs) (Nicolle *et al.* 2012). Urinary pathogenic *E. coli* (UPEC) are responsible for many CAUTIs, particularly due to the ability to produce

Received 2 September 2022; **revised** 2 June 2023; **accepted** 12 June 2023

biofilms on different indwelling medical devices, such as urethral and intravascular catheters, causing several problems in healthcare facilities (Nicolle *et al.* 2005, Mobley et al. 2009, Nicolle *et al.* 2012, Reisner et al. 2014, Delcaru et al. 2016). UPEC are also capable of attaching uroepithelial cells, invading urinary tissues, and persisting by producing intracellular bacterial communities or biofilm-like pods within the bladder cells (Amalaradjou and Venkitanarayanan 2013, Sarkar et al. 2016). Recently, various *E. coli* strains have been described as oncogenic risk factors and seem to contribute to the initiation of colorectal cancer (Kim et al. 2020).

To complicate the situation, several *E. coli* pathogens, particularly those causing extraintestinal infections, have developed resistance to every class of antibiotics used for the treatment of human and animal infections (Nicolas-Chanoine et al. 2014). Extended-spectrum β-lactamases (ESBL)-producers and carbapenem-resistant *E. coli* strains have been included in the third position on the last global priority list of antibiotic-resistant bacteria for which alternative treatments are urgently needed (WHO, 2017). Aware of these facts, further research is needed to contribute to the development of new therapeutic alternatives in an attempt to control and prevent *E. coli* infectivity and virulence.

Numerous bacterial species coordinate important biological functions including virulence factors, antibiotic biosynthesis, plasmid transfer, luminescence, or biofilm formation, through a cell density-dependent gene regulation system known as quorum sensing (QS; Waters and Bassler 2005, Williams et al. 2007, Papenfort and Bassler 2016). Bacteria produce, release, and sense extracellular small molecules called autoinducers (AIs), which are accumulated in the environment, to monitor their own population. When the concentration of AIs reaches a threshold level, the bacteria detect it and alter gene expression, approaching the behaviour of a multicellular organism (Waters and Bassler 2005) (Fig. 1). This traditional definition of QS is progressively changing, since QS accepts more complicated and comprehensive roles in many bacterial processes (Hense and Schuster 2015, Papenfort and Bassler 2016, Mukherjee and Bassler 2017, Striednig and Hilbi 2022). QS signals are termed AIs as part of their function to induce their own synthesis via up-regulation of the genes responsible for QS signal production and the formation of a positive auto-inductive loop. Moreover, the cell-to-cell signal molecule definition is reserved for those small and diffusible AIs that play a role in cell-to-cell communication (Winzer et al. 2002). Therefore, careful investigation is required to identify new signals that truly are part of QS AIs and differentiate them from other signal response mechanisms (Winzer et al. 2002, Diggle et al. 2007). Different kinds of signal molecules have been described as well as diverse QS systems can appear to overlap in some bacteria. For instance, three parallel QS circuits of the marine bacterium *Vibrio harveyi* converge to control one set of genes that in turn produce bacterial phenomena such as bioluminescence (Henke and Bassler 2004). In the same manner, the QS systems described for *E. coli* include a LuxR homologue (the SdiA regulator), a LuxS (synthetase)/autoinducer-2 (AI-2), and an autoinducer-3 (AI-3) system (Kim et al. 2020) (Fig. 1). Indole has also been considered a QS signal (Wang et al. 2001), but there are still controversial issues, which we discuss below, relating to whether it is a relevant metabolic signal or a QS molecule that acts in cell-density-dependent communication processes. Since these QS systems seem to be involved in interspecific-, intra- and interspecific-, or inter-kingdom communication processes, respectively, *E. coli* can sense and adapt to the environment. Earlier reports have described the involvement of QS in *E. coli* virulence and other bacterial functions in this bacterium. Therefore, understanding of QS circuits allows the investigation of differentiated strategies based on the application of anti-virulence/anti-pathogenic mechanisms. The interference with these signalling mechanisms, or QS inhibition, has been proposed as a promising approach to counteract several bacterial infectious diseases (Bjarnsholt and Givskov 2007, Dong et al. 2007, Williams et al. 2007, LaSarre and Federle 2013). This study aims to review the current knowledge of the cell-to-cell signalling systems in *E. coli* as well as their influence on different biological functions related to virulence and pathogenesis.

SdiA-mediated QS system

Most of the Gram-negative bacteria present the typical LuxIR-type system that was first identified in the marine bioluminescent bacterium *V. fischeri*. This canonical QS system relies on the presence of two genes: the signal synthase LuxI and the signal receptor LuxR that detect *N*-acyl homoserine lactones (AHLs) (Ng and Bassler 2009) (Fig. 1). AHLs are the best-studied AIs and the primary QS signalling molecules used by Gram-negative bacterial species (Dong et al. 2001). These molecules are constituted by a conserved homoserine lactone ring (HSL) connected to a lateral acyl chain, which confers the specificity to the QS signal (Dong et al. 2001, Whitehead et al. 2001). Thus, they play a crucial role in the differential signalling recognition by LuxR-type receptors in diverse bacterial species (Fuqua and Greenberg 2002, Waters and Bassler 2005). LuxIR homologues have been found and identified in numerous Gram-negative bacteria and are already considered part of an intraspecific communication system. However, the QS system in *E. coli* is poorly characterized in comparison with other important pathogenic microorganisms.

E. coli does not produce AHLs due to the lack of a *luxI* gene, however, it possesses an orphan receptor known as SdiA (suppressor of cell division inhibitor), which can respond to AHLs released by other bacterial species (Dyszel et al. 2010, Kim et al. 2014) (Fig. 2A). SdiA was first described as a transcriptional activator of the *ftsQAZ* operon, which encodes essential proteins for cellular division (Wang et al. 1991, Kanamaru et al. 2000). The positive regulation of the *ftsQAZ* operon produces an increment in the frequency of septation events in cells and also suppresses the effects of several endogenous cell division inhibitors. Moreover, the effect of *sdiA* on the *ftsQAZ* operon is potentially increased in response to AHLs, confirming the role of the AHL-mediated QS system in the regulation of this operon (Sitnikov *et al.* 1996, Yamamoto et al. 2001).

Previous observations have demonstrated that LuxR-type regulators need AHLs for stabilizing overall foldings and activating the transcription of QS-regulated genes (Miller and Bassler 2001, Nguyen et al. 2015). Normally, the AHL-LuxR complex dimerizes, and this is a requirement for LuxR family proteins to bind to DNA and activates transcription of QS-controlled genes. Although SdiA belongs to the LuxR family, analysis of the crystal structure revealed that SdiA is properly folded, forming a stable dimer in solution *in vitro* with or without AHLs (Kim et al. 2014, Nguyen et al. 2015). In addition, orphan or solo Lux regulators, including SdiA, have also shown some basal activity in the absence of AHLs (Dyszel et al. 2010, Hughes et al. 2010, Sperandio 2010). However, SdiA is able to bind a wide range of exogenous AHLs enhancing the stability of SdiA and DNA-binding affinity and improving the transcriptional regulation ability of SdiA (Bez et al. 2023). The promiscuity of SdiA together with these facts have opened up the possibility of the existence of more different ligands, complicating the QS circuit regulation (Kim et al. 2014, Bez et al. 2023). For instance, a LuxR homolog from *Photorhabdus* spp. can detect

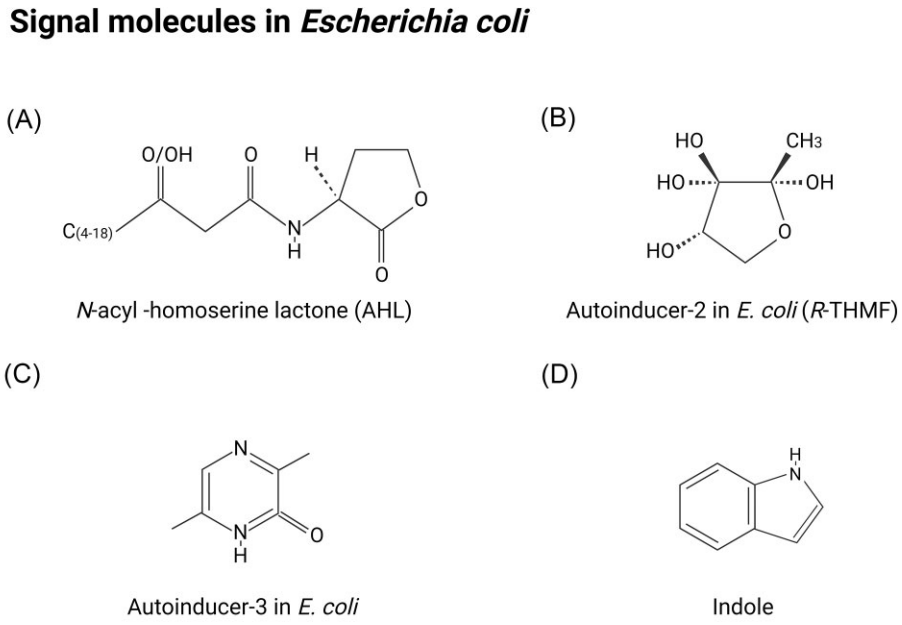

Figure 1. Chemical structure of different signal molecules involved in *E. coli*. **(A)** *N*-acyl homoserine lactones play a crucial role in Gram-negative bacteria. AHLs are constituted by a conserved homoserine lactone ring connected to a lateral acyl chain with varieties in length (from 4 to 18 carbon atoms) and in oxo- or hydroxyl-substitutions. They are produced and recognized by the LuxI/LuxR homologous. *E. coli* does not produce AHLs but is able to sense these molecules through the SdiA regulator, participating in the inter-specific signalling communication. **(B)** Auto-inducer 2 (AI-2) derives from SAM by the LuxS synthase, which produces 4,5-dihydroxy-2,3-pentanedione (DPD) resulting after further rearrangements in the known AI-2 molecules. *E. coli* AI-2 structure is the non-borated *R*-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (*R*-THMF) and is recognized by the LsrB binding protein. Gram-positive and Gram-negative bacteria share the AI-2, which is responsible for both inter and intraspecies bacterial communication, serving as a universal signalling molecule in the coordination of cell behaviour among prokaryotic species. **(C)** Autoinducer-3 (AI-3) molecules family is produced by Tdh from threnonine. AI-3 participates in the communication processes between prokaryotic and eukaryotic cells, establishing an inter-kingdom communication. **(D)** Indole is produced from the tryptophane degradation by tryptophanase (TnaA) and is recognized by the CpxR/A two-component system and the IsrR receptor in *E. coli*. Several authors have considered indole as a QS signal molecule. However, there is still controversy as to whether it is a mere signal involved in metabolism or whether it is really a QS signal involved in cell density-dependent communication processes.

non-AHLs signals such as dialkylresorcinols, cyclohexanediones, and α-pyrones (Kim and Park 2015). Apart from AHLs, the binding of SdiA to other ligands, including the ubiquitous molecule 1-octanoyl-rac-glycerol (OCL), has been hypothesized (Hughes et al. 2010, Sharma and Bearson 2013, Nguyen et al. 2015, Sabag-Daigle et al. 2015). Phylogenetic analysis has revealed a conserved distribution of SdiA in several genera from the *Enterobacteriaceae* family (Styles et al. 2020, Bez et al. 2023). Although SdiA is also present in *Salmonella, Enterobacteria, Citrobacter*, and *Klebsiella* spp., none of these genera presents a LuxI synthase in their genomes (Michael et al. 2001, Sabag-Daigle et al. 2015). This led to the establishment of a new model of QS system, a phenomenon also known as eavesdropping, in which the LuxI homologue is not present (Smith et al. 2011, Swearingen et al. 2013, Gorelik et al. 2019). It has already been suggested that Lux solos could establish interspecific communication, allowing these bacteria to sense other bacterial species and providing cues on the bacterial population structure and density by activating or repressing genes accordingly (Bez et al. 2023). *Salmonella* can detect other bacteria such as *Pseudomonas aeruginosa*, *Yersinia enterocolitica*, and *Aeromonas hydrophila* through the SdiA regulator (Soares and Ahmer 2011). Similarly, SdiA can respond to synthetic AHLs or those exclusively produced by other bacterial species (Van Houdt et al. 2006, Dyszel et al. 2010, Hughes et al. 2010, Kim et al. 2014) (Fig. 2). The fact that Lux orphans can detect and respond to signals produced by eukaryotic hosts, self, or other microbial species (Whiteley et al. 2017) may be a result of the great versatility of *E. coli* and its ability to inhabit different niches. Hence, *E. coli* establishes interspecific bacterial communication as an adaptive advantage in sensing the host environment and responding to QS signals produced by competing or cooperating bacteria, to adapt to complex environments, such as mammalian hosts (Lee et al. 2009, Sperandio 2010, Sharma and Bearson 2013, Prescott and Decho 2020, Bez et al. 2023). Indeed, LuxR solos, including SdiA, are a source of adaptation and development of 'crosstalk' with microorganism communities and regulate key bacterial traits for fitness in the environment or hosts (Prescott and Decho 2020, Bez et al. 2023).The latest analysis have revealed a relationship between SdiA receptors and their ecological role, since all the described SdiA orthologs have been detected in human- or terrestrial-isolated bacteria (Bez et al. 2023). Recently, another example of eavesdropping has been described between *E. coli* (EPEC) and *V. cholerae* through the AI-1. Both pathogens share the common infectious site, and using the *V. cholerae* AI known as CAI-1, *E. coli* enhances its virulence by regulating its own population in a *V. cholerae* density-dependent manner. However, this response has not been found in the close enterohaemorrhagic *E. coli* (EHEC) or *Salmonella* spp., indicating a more sensitive communication between competing pathogens, such as

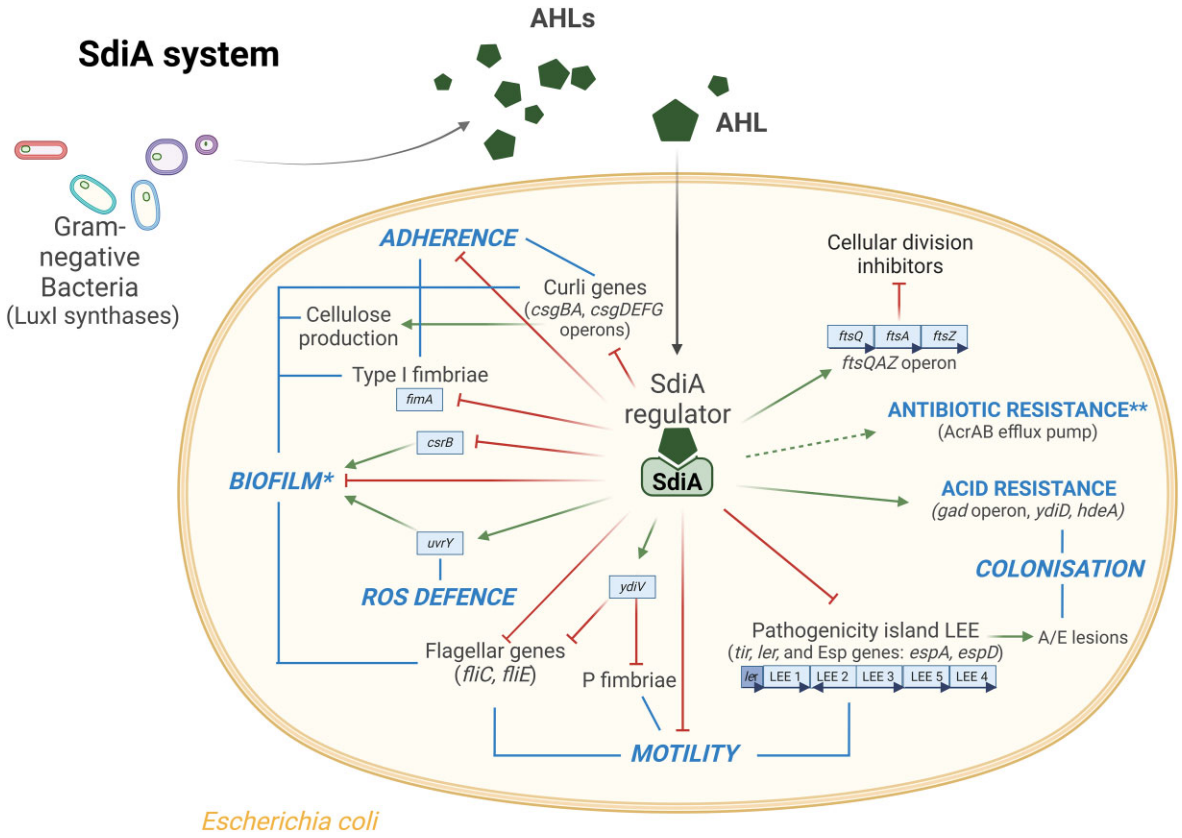

Figure 2. Schematic representation of the LuxR-type QS system in *E. coli*. Although *E. coli* does not present a synthase that produces AHLs (green hexagon), it can sense these QS signals produced by other bacterial species. Through the orphan SdiA regulator, *E. coli* can induce a signalling to regulate the expression of several genes, including the *ftsQAZ* operon, in a cellular density-dependent manner. Effects on *E. coli* virulence-associated genes and phenotypes mediated by the LuxR solo regulator SdiA are shown. Green arrows indicate positive regulation, red lines indicate gene or phenotype inhibition, and blue lines indicate a link between different phenotypes. ∗Biofilm regulation by SdiA is a controversial point in the literature as explained below. ∗∗Although SdiA plays a role in drug resistance regulation, the effect of AHLs on this phenotype (dotted line) needs to be confirmed. ROS: reactive oxygen species. Created with BioRender.com.

V. cholerae and EPEC, to increase the opportunities to successfully colonise their hosts (Gorelik et al. 2019).

Evidence suggests that SdiA is involved in the regulation of several genes that present a role in several cellular functions, including virulence, metabolism, motility, adherence, biofilm formation, survival, multi-drug efflux, and defence mechanisms, in a cell density-dependent manner (Kim et al. 2014) (Fig. 2).

Impact of SdiA on acid resistance

E. coli presents different acid-resistance (AR) systems that enable its ability to deal with the acidic environment of the human and animal digestive tract. In particular, the glutamate-dependent acid resistance system or AR2, which is required for EHEC survival within the acidic stomachs of mammalians, is the most effective AR system to withstand extremely acidic stress (Ma et al. 2020). The AR2 system encompasses three genes (*gadA*, *gadB*, and *gadC*) that are found in the acid fitness island, which also comprises several regulatory genes related to the AR2 system (Dyszel et al. 2010, Hughes et al. 2010, Ma et al. 2020). SdiA has been proposed as a key factor for *E. coli* to survive and colonize the gastrointestinal tract of cattle, constituting a natural reservoir of *E. coli*, due to its ability of resistance to acidic pHs (Hughes et al. 2010) (Fig. 2). In agreement, insertion and deletion *sdiA* mutants in EHEC were defective for colonization of cattle compared to the wild-type strains, indicating a crucial role of this gene in the colonization of bovine intestine and persistence (Hughes et al. 2010, Sharma and Bearson 2013, Sabag-Daigle et al. 2015). Moreover, SdiA increases the transcription (activated upon AHLs) of several genes involved in the mentioned AR2 system in *E. coli*, including *gadA*, *gadW*, *gadE*, *yhiD*, and *hdeA*, to withstand with low pH environment (Dyszel et al. 2010, Sperandio 2010, Smith et al. 2011) (Fig. 2). Recently, the enhancement of the acid tolerance ability and the positive regulation of *gadW* and *gadY* genes, the key components of the AR2 system, by SdiA have been confirmed (Fig. 2) (Ma et al. 2020). It was also demonstrated that OC6-HSL improves acid resistance in *E. coli* K12 (Mayer et al. 2015), and C4, C6, OC6, OC8, and OC12-HSL significantly increase the expression of *gad* genes (Van Houdt et al. 2006, Dyszel et al. 2010, Hughes et al. 2010, Sheng et al. 2013, Ma et al. 2020). However, the *gad* system can be activated by SdiA even in the absence of QS signals, supporting that SdiA-AHL could need a second factor to activate the transcription of the *gad* system (Dyszel et al. 2010, Hughes et al. 2010). In a recent work using β-galactosidase reporter assays, it was observed that the regulation of the *gadW* and *gadY* promoters occurs through SdiA, and the presence of AHLs (C4 and OC12-HSL) enhances the *gadW* regulation (Ma et al. 2020). However, further studies are required to confirm the specific interaction between SdiA and DNA binding sites from the *gad* system (Ma et al. 2020). Interestingly, this acid resistance regulation by the SdiA-QS system was not observed in avian pathogenic *E. coli* (APEC), probably because these strains did not need to resist the low pH environments (Yang et al. 2021). Given that lifestyles differ considerably between APEC and

EPEC or EHEC, these strains could present a different regulation, depending on their dissimilar infection routes (Yang et al. 2021).

Impact of SdiA on *E. coli* colonization and survival in the gastrointestinal tract

Ruminants, the most notable are cattle, are the primary hosts for *E. coli* EHEC, which they colonize asymptomatically using the LEE (locus for enterocyte effacement)-encoded type III secretion system. The LEE pathogenicity island encodes all the known virulence factors involved in attaching and effacing (A/E) lesions on host intestinal epithelia (Sharma and Bearson 2013). Among them, intimin (an outer membrane protein required for adhesion), Tir (the intimin receptor), and the three secreted proteins EspA, EspB, and EspD are the major virulence determinants of EHEC and EPEC (Kanamaru et al. 2000). Moreover, LEE island is constituted by several operons LEE1–LEE5 (Elliott et al. 2000), and their expression is regulated by the LEE-encoded positive regulator Ler (Sharma et al. 2010). The LEE-encoded genes, together with the *gad* system, are necessary for EHEC colonization of cattle. SdiA regulates various virulence factors from LEE in EHEC and EPEC (Fig. 2). SdiA is a negative regulator of the expression of EspD and intimin in EHEC, since the overexpression of SdiA leads to lower mRNA levels of both (Kanamaru et al. 2000). The addition of AHLs (unmodified HSL, OC6, and OC8-HSL) also inhibits the expression of *espA*, *espD*, and intimin genes, as well as represses the transcription of the LEE operon via binding to the *ler* promoter region, which is required for the expression of all LEE genes (Kanamaru et al. 2000, Hughes et al. 2010, Sharma and Bearson 2013) (Fig. 2).

Diverse studies have proposed an explicative and currently accepted model of the SdiA role in *E. coli* colonization of the bovine rumen (Hughes et al. 2010, Sperandio 2010, Styles et al. 2020). According to that, the regulation of the QS-related genes by SdiA is dependent on the different concentrations of QS signals along the gastrointestinal tract, as explained below. *E. coli* is consumed by ruminants and reaches their rumen, where commensal and/or pathogenic Gram-negative microorganisms produce QS signals. Different AHLs were detected in the rumen (C6 and C8-HSL) (Hughes et al. 2010). Also, AHLs-producers, such as *P. aeruginosa* and *A. hydrophyla*, were recently isolated from cattle rumen and swine intestines (Elliot *et al.* 2000, Yang et al. 2018), a controversial point of this model due to the previous lack of evidence (Swearingen et al. 2013). Once in the rumen, QS signals detected by SdiA activate genes from the *gad* system that allow *E. coli* survival in cow stomach as well as represses the LEE operon through the *ler* promoter region. This implies that SdiA regulates *E. coli* passage through the bovine gastrointestinal tract (Dyszel et al. 2010, Hughes et al. 2010, Sperandio 2010, Sheng et al. 2013, Conolly et al. 2015, Nguyen et al. 2015). Then, LEE expression is necessary for colonization of the recto-anal junction, where AHLs are absent or in a low concentration (Hughes et al. 2010, Nguyen and Sperandio 2012, Styles et al. 2020). Until now, QS signals have not been detected in the bovine intestines probably because they are hydrolytically unstable in alkaline environments (Yates et al. 2002, Hughes et al. 2010, Swearingen et al. 2013), the presence of AHL-degrading enzymes (Nguyen and Sperandio 2013; Swearingen et al. 2013), or due to a lack of AHLs-producer microorganisms. Due to the low QS signals concentration, or failure of detection, gene regulation by SdiA is not active in the intestine and, consequently, *E. coli* reactivates the virulence factors encoded by LEE to attach to the host cell membrane (Kanamaru et al. 2000). While A/E lesion formation produces disease in humans through LEE activation, resulting in bloody diarrhoea and other health problems (Kaper et al. 2004), A/E lesions do not seem to affect cattle (Sperandio 2010). These injuries could also promote the colonization of other hosts by releasing *E. coli* into the environment (Styles et al. 2020). It should be noted that the kinetics of AHLs along the intestinal tract should be analysed using new detection methods to confirm the presence or absence of AHL in the mammalian intestine and improve the understanding of this model.

Impact of SdiA on drug resistance

The involvement of SdiA in multidrug resistance has been reported in *E. coli* (Fig. 2). Resistance to fluoroquinolones is frequently related to multidrug resistance phenotypes in *E. coli* and other bacterial strains that produce ESBLs. Overexpression of *sdiA* positively regulates the AcrAB efflux pump, which exudes different quinolone antibiotics (Fig. 2) (Rahmati et al. 2002, Tavío et al. 2010). Indeed, when *sdiA* is expressed from a plasmid, it could increase the resistance to several antibiotics, such as fluoroquinolones, chloramphenicol, as well as kanamycin and tetracycline (Dyszel et al. 2010). On the other hand, an insertion *sdiA* mutant was more sensitive to various antibiotics, including the fluoroquinolones class, and presented decreased levels of AcrB in the mutant strain (Rahmati et al. 2002). The decrease in AcrB in the mutant strain may also contribute to fluoroquinolone hypersensitivity (Rahmati et al. 2002). Supporting this hypothesis, it has also been proposed that SdiA might activate efflux pumps, through increasing *acr* gene expression, to enhance *E. coli* resistance, after observing that the overexpression of *sdiA* also increases resistance to mitomycin C (Wei et al. 2001). Surprisingly, Dyszel et al. (2010) could not find a regulation of AcrAB by SdiA, when it is expressed from its natural position in the chromosome or when AHLs are present, suggesting that SdiA regulation may change depending on the environmental or metabolic conditions (Dyszel et al. 2010). In other pathogens, including *P. aeruginosa* or *Acinetobacter baumannii*, efflux pumps have already been linked to AHL-mediated QS (Minagawa et al. 2012, Mayer et al. 2020). Similarly, previous studies have hypothesized that AcrAB might be involved in the export of specific QS signals (Rahmati et al. 2002), proposing a potential relationship between efflux pumps and QS in *E. coli*. However, these authors also found that the null *sdiA* mutant was not significantly more sensitive to other different antibiotics, and the *sdiA* overexpression in an *acrAB* mutant did not decrease fluoroquinolone resistance (Rahmati et al. 2002). This indicated that SdiA might regulate the expression of another efflux pump or affect another pathway related to drug resistance (Rahmati et al. 2002). Although the SdiA function on drug resistance has been investigated by the effect of the *sdiA* mutation and SdiA complementation, showing an effect of this QS system in *E. coli* virulence, further investigation is required to confirm the influence of the AHL addition for the drug-resistance phenotype.

Impact of SdiA on *E. coli* motility

Motility is a virulence trait that allows both InPEC and ExPEC to disperse, and reach new surfaces, as well as helps pathogenic *E. coli* strains to escape from host immune responses, inducing the spread of the infection. In *E. coli*, active motility is dependent on the flagellum, which is required to swim in a liquid environment and overcome the hydrodynamic forces (Beloin et al. 2008). Flagellum-mediated motility and chemotaxis have already been proposed to contribute to UPEC virulence, since they are utilized to ascend the urinary tract, and, in turn, enhance the pathogenesis of UTIs (Lane et al. 2005, Spurbeck et al. 2013). Several authors have reported the repression of *E. coli* motility by the

SdiA-mediated QS system (Fig. 2). Overexpression of *sdiA* inhibits the transcription of *fliC*, which encodes the major flagellar protein known as flagellin, and, as a consequence, reduces motility in *E. coli* EHEC (Fig. 2) (Kanamaru et al. 2000). On the other hand, *fliC* transcription was significantly increased in an *sdiA* mutant, and insertion and deletion *sdiA* mutants were more motile compared to the wild-strains in EHEC and atypical EPEC (aEPEC), supporting the negative effect of SdiA on *E. coli* motility (Lee et al. 2007, Sharma et al. 2010, Culler et al. 2018). Consistent with this repressor role, SdiA was described as a positive regulator of the *ydiV* expression in *E. coli* K12 (Zhou et al. 2008), a gene encoding a protein that represses flagellar motility and P fimbrial expression in UPEC (Spurbeck et al. 2013) (Fig. 2). AHL (OC6-HSL) or AI-1 from *V. fischeri* also promoted *ydiV* expression in an SdiA-dependent manner (Zhou et al. 2008), supporting the role of the AHL-mediated QS system in *E. coli* motility.

Impact of SdiA on *E. coli* adherence

AHL-mediated QS has also been related to adherence phenotype in *E. coli* (Fig. 2). Adherence to epithelial cells allows *E. coli* to colonize different tissues and withstand the shear force of host fluids, such as urine (Spurbeck et al. 2013). Apart from the LEE genes, the expression of flagellar and fimbriae (type I fimbriae, curli, and conjugative pili), is also involved in the adherence phenotype (Beloin et al. 2008, Sharma et al. 2010). Flagellar genes are repressed by SdiA in *E. coli* K12 and EHEC, since *fliC* and *fliE* expression were down-regulated by SdiA (Fig. 2) (Kanamaru et al. 2000, Dyszel et al. 2010). In *E. coli*, curli fimbriae are encoded by two operons: the *csgBA* and the *csgDEFG* (Beloin et al. 2008). CsgD, the activator and transcription regulator of *csgBA* operon, also controls cellulose production, which is related to biofilm matrix production (Laverty et al. 2014). SdiA downregulates curli expression through repression of both *csgBA* and *csgDEFG* operons in *E. coli* K12, according to a whole-transcriptome analysis (Fig. 2) (Lee et al. 2009). SdiA also showed a negative effect on curli fimbriae since an *sdiA* deletion produced an up-regulation of *csgA* gene expression in EHEC, which encodes the major structural component of curli fimbriae (Sharma et al. 2010). In agreement, several authors have demonstrated that SdiA is a repressor of adherence in EHEC, aEPEC, by using deletion *sdiA* mutants and *sdiA* overexpression (Kanamaru et al. 2000, Sharma et al. 2010, Sharma and Bearson 2013, Culler et al. 2018). Furthermore, when AHLs (OC6 and OC8-HSL) were added, the transcription of *csgA*, *csgD*, and *fimA* (type I fimbria) genes was reduced in wild-type aEPEC strains, supporting the curli repression by SdiA (Culler et al. 2018).

Impact of SdiA on biofilm formation by *E. coli*

E. coli is responsible for producing several infections and plays a key role in bacterial persistence due to its ability to produce biofilms (Sharma et al. 2016). Among the biofilm-related determinants in *E. coli*, curli, fimbriae, type I fimbriae, motility capability, and polysaccharide production are included (Beloin et al. 2008, Culler et al. 2018). SdiA seems to be a repressor of biofilm, since insertion and deletion mutants of *sdiA* resulted in an increase in biofilm formation by *E. coli* K12 and EHEC, as several authors have reported (Lee et al. 2007, Sharma et al. 2010, Culler et al. 2018) (Fig. 2). Supporting this hypothesis, various studies have verified that the addition of exogenous AHLs (C4, C6, C8, OC8, C10, C12, and OC12-HSL) reduced biofilm formation by *E. coli* (Moons et al. 2006, Lee et al. 2007, 2009). In agreement, a different role for SdiA has been suggested between planktonic and biofilm cells. Thus, a reduction of early biofilm formation was observed through the repression of curli genes mediated by SdiA, whereas the expression of these curli fimbriae was induced in planktonic *E. coli* cells (Lee et al. 2009). However, controversial results regarding SdiA and biofilm regulation were found in the literature, since a few authors observed no effect of insertion and deletion *sdiA* mutants on biofilm production or just observed the opposite effect of SdiA (Suzuki et al. 2002, Sabag-Daigle et al. 2012, Spurbeck et al. 2013). Regarding UPEC strains, another work has also supported the positive regulation of biofilm by SdiA (Vinothkannan et al. 2018). In this case, biofilm inhibition was reported after the addition of a QS inhibitor, presumably due to a competition with the putative native AHL of the SdiA regulator (Vinothkannan et al. 2018). The biofilm reduction by *sdiA* deletion was associated with a decreased expression of *uvrY* and *csrB* genes, involved together with the CsrA, and CsrC in the carbon storage regulatory or Csr system (Suzuki et al. 2002). CsrA has been shown to suppress biofilm formation, leading to their dispersal. Besides, its activity is antagonized through CsrB and CsrC by sequestering CsrA (Wei et al. 2001, Beloin et al. 2008) (Fig. 2). Diverse authors have proposed that the UvrY protein, from the BarA/UvrY two-component system, is also necessary for the development of *E. coli* biofilm (Suzuki et al. 2002, Beloin et al. 2008). These authors have suggested a biofilm formation regulation mediated by SdiA, which would antagonize the CsrA effect through the CsrB and UvrY activation, and in turn, would activate biofilm formation (Suzuki et al. 2002, Beloin et al. 2008). As UvrY participates in defence mechanisms against reactive oxygen species (ROS) (Fig. 2), the regulation of SdiA by other signals apart from AHLs has also been proposed, such as the oxidation state of the cell. Indeed, although the binding of SdiA to the *uvrY* promoter has already been confirmed, the binding affinity was reduced in the presence of oxidizing agents (Kim et al. 2014). These contradictory results related to biofilm regulation by SdiA, could be attributed to differences in biofilm models, experimental conditions, or strains. Furthermore, most laboratory *E. coli* strains are poor biofilm formers; hence, the biofilm-forming ability of this microorganism may be underestimated, complicating the assessment of the real effects of the AHL-mediated QS system.

In conclusion, the SdiA-QS system plays a crucial role in several virulence-associated phenotypes in *E. coli*, including acid resistance, colonization, and survival in the gut, drug resistance, motility, adherence, and biofilm production. As a solo LuxR receptor, SdiA senses AHLs produced by other microorganisms to activate or repress virulence genes accordingly. In addition, several studies have demonstrated that AHLs are involved in the regulation of these phenotypes by binding SdiA.

AI-2-mediated QS system

Apart from the AHL-mediated QS system, *E. coli* presents a second QS circuit, which is constituted by a two-component regulatory system and is mediated by the autoinducer-2 (AI-2) (Fig. 1). Numerous Gram-positive and Gram-negative bacteria have been found to produce and release this QS signalling molecule that is widely distributed among bacterial kingdoms and controls different bacterial functions (Xavier and Bassler 2005, Zuo et al. 2019). These features have led to the hypothesis that AI-2 is responsible for both inter and intraspecies bacterial communication, serving as a universal signalling molecule in the coordination of cell behaviour among prokaryotic species (Sharma et al. 2016, Zhang et al. 2020). AI-2 derives from S-adenosyl methionine (SAM) by the S-ribosyl homocysteine lyase or LuxS synthase, which plays a major role in its production, and by the S-adenosyl homocysteine nucleosidase or Pfs (Sharma et al. 2016, Zuo et al.

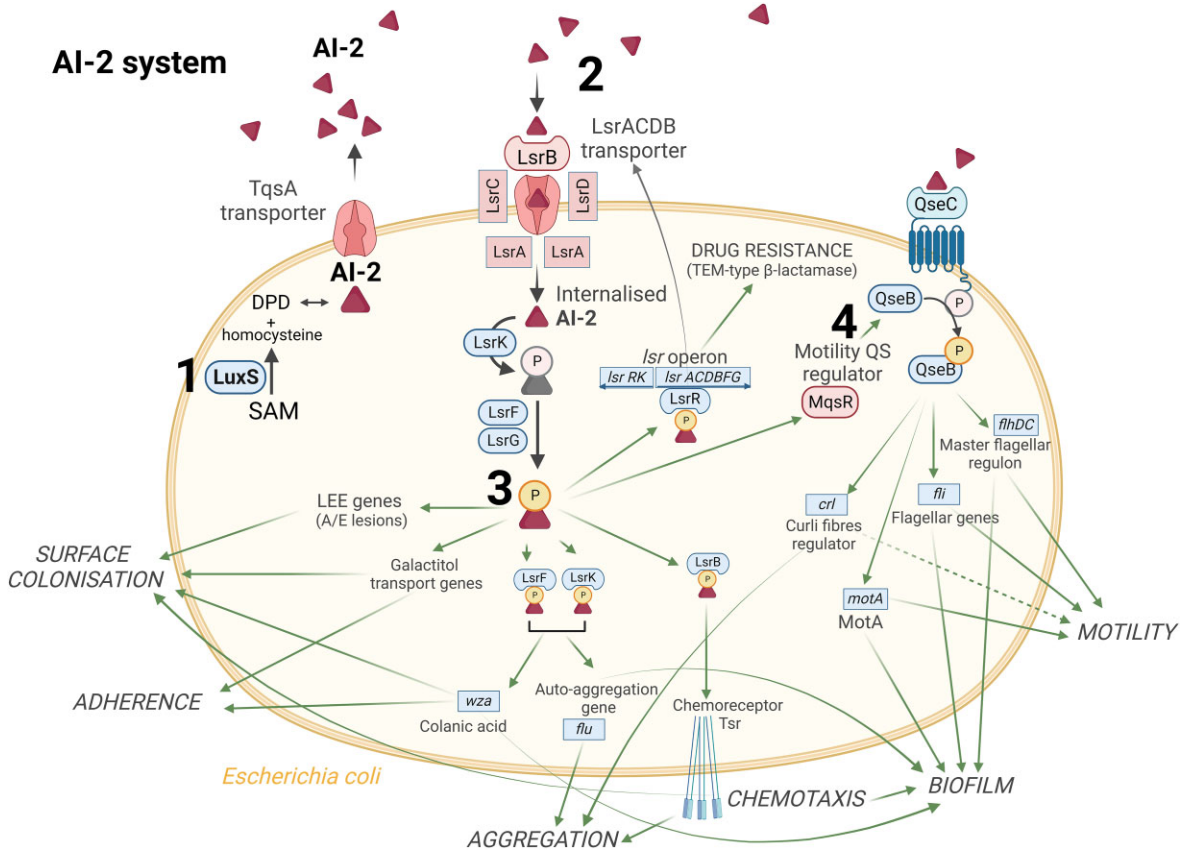

Figure 3. AI-2 QS system in *E. coli*. (1) LuxS catalyses the reaction to produce 4,5-dihydroxy-2,3-pentanedione (DPD) and homocysteine from S-adenosyl-methionine (SAM). DPD spontaneously cyclizes to form in *E. coli* the DPD derivative S-THMF-borate, or AI-2 (dark red triangle) that is transported by the TqsA protein and is accumulated extracellularly. (2) When the threshold concentration is reached, AI-2 is internalized by the Lsr ABC-type transporter, and then, AI-2 is phosphorylated by the LsrK kinase into an activated molecule that promotes its own uptake by binding to the LsrR. LsrF and LsrG are required for the further process of phosphorylated AI-2. (3) Phospho-AI-2 is the inducer of a signalling pathway to activates the transcription of several genes, including the *lsr* operon. By binding and inactivating LsrR, the repression of the *lsr* operon is blocked, then, *lsr* genes are transcribed, and the AI-2 uptake is increased. The AI-2-LsrR binding also enhances drug resistance by TEM-type β-lactamase. Moreover, AI-2 promotes surface colonization and adherence through positive regulation of LEE gene expression and galactitol transport genes. By binding to the global regulators LsrF and LsrK, AI-2 also promotes aggregation, biofilm formation, adherence, and colonization through up-regulation of colanic acid gene (*wza*) and auto-aggregation gene (*flu*). By binding the receptor protein LsrB, AI-2 promotes chemotactic rescponse via Tsr chemoreceptor, which in turn increase aggregation and biofilm formation. (4) Through the motility QS regulator (MqsR), AI-2 activates QseB/QseC two-component system, which also participates in the regulation of the flagellar genes (*fli* genes) and the master regulon *flhDC*, and the proton exchange conductor for flagellum movement (MotA), all involved in *E. coli* motility and biofilm formation. QseB/C also increases curli expression via curli fibres regulator (*crl*), which promotes aggregation and possibly (dotted arrow) stimulates motility. Created with BioRender.com.

2019, Hu et al. 2022) (Fig. 3). In brief, LuxS produces homocysteine and 4,5-dihydroxy-2,3-pentanedione (DPD), which cyclizes spontaneously and undergoes further rearrangements to form the generally called AI-2 (Xavier and Bassler 2005). AI-2 molecules from several bacterial species may differ in their structure; however, all of them are derived from the DPD. Until now, two AI-2 structures bound to the corresponding bacterial receptors have been described, the boron-containing DPD derivative S-2–methyl-2,3,3,4-tetrahydroxytetrahydrofuran-borate (S-THMF-borate) recognized by LuxP and present only in *Vibrio* spp., and the non-borated R-2-methyl-2,3,3,4-tetrahydroxytetrahydrofuran (R-THMF) recognized by LsrB and found in enteric bacteria, and members of several other families (Chen et al. 2002, Zhang et al. 2020, Khera et al. 2022) (Fig. 1). However, both AI-2 structures are present in some bacterial species that can use one or the other due to the rapid interconversion between the two AI-2 forms (Xavier and Bassler 2005, Zhang et al. 2020). Similar to other QS systems, when AI-2 reaches a threshold concentration in the environment, a signal transduction cascade is triggered resulting in a coordinated genetic expression in the bacterial population. Several Gram-negative bacteria, including *Salmonella* Typhimurium, *Vibrio* spp., *Enterococcus faecalis*, and *E. coli*, have shown identical biosynthetic pathways and biochemical intermediates in AI-2 synthesis. After its synthesis, AI-2 is transported outside the cell by the transporter QS protein TqsA (YdgG), and AI-2 is accumulated in the media (Herzberg et al. 2006, Zhou et al. 2008, Khera et al. 2022). Extracellular AI-2 peaks are observed during the mid- to late-exponential phase and with a quick decrease during the entry into the stationary phase in *E. coli* (Li et al. 2007). This occurs because the extracellular AI-2 can be taken up inside the cell through the *luxS*-regulated (Lsr) transporter, an ATP-binding cassette (ABC) transporter (Xavier and Bassler 2005, Li et al. 2007, Sharma et al. 2016) (Fig. 3). Transporter proteins (LsrABCD) for AI-2 uptake are encoded by the *lsr* operon, which is regulated by cyclic AMP (cAMP)/cyclic AMP receptor protein (CRP) and proteins transcribed from the *lsrRK* operon. The *lsrRK* operon is located immediately upstream of *lsr* and divergently transcribed (Wang et al. 2005, Li et al. 2007, Zhou et al. 2008) (Fig. 3). Once inside the cell, AI-2 is phosphorylated by the cytoplasmic kinase LsrK into an activated molecule that promotes its own uptake by binding to the

Lsr repressor (LsrR) (Zhou et al. 2008, Sharma et al. 2016, Witsø et al. 2016, Stephens et al. 2019). Phosphorylated AI-2 inactivates LsrR, resulting in the transcription of *lsr*-regulated genes and in the increase in the uptake of AI-2 (Sharma et al. 2016, Witsø et al. 2016) (Fig. 3). LsrF and LsrG are needed for further process of phosphorylated AI-2, degrading AI-2 into Co-A and dihydroxyacetone phosphate (Zhou et al. 2008, Jani et al. 2017). Deletion of *lsrF* and *lsrG* genes together with overexpression of LsrK and LsrACDB can be combined to engineer *E. coli* cells with increased AI-2 uptake and AI-2 sensitivity, leading to activation of protein expression at lower AI-2 levels, as a recent study has shown (Stephens et al. 2019).

AI-2 participates in the virulence of numerous bacteria (Waters and Bassler 2005, Rutherford and Bassler 2012), including *E. coli*, where it also has an effect on several phenotypes, such as motility, biofilm production, and persistence (Laganenka and Sourjik 2018, Stephens et al. 2019, Khera et al. 2022). AI-2 has been found in the gastrointestinal tract since it is produced by many gut-associated bacteria and is recognized by several bacterial species as an important signal in this complex environment (Walters and Sperandio 2006b, Bansal et al. 2008, Stephens et al. 2019). AI-2 was able to alter the composition of the gut microbiome after antibiotic treatment in a mouse model (Thompson et al. 2015). Additionally, AI-2 influences the survival of *E. coli*, up-regulating the expression of several genes involved in *E. coli* virulence (Bansal et al. 2008, Soni et al. 2008) (Fig. 3). Furthermore, the presence of AI-2 can affect the expression of mammalian cell genes in the gut, regulating the immune response (Zargar et al. 2015). This study has reported that AI-2 up-regulated the expression of the inflammatory interleukin IL-8 and then significantly down-regulated it, indicating a modulation of the immune response by AI-2 (Zargar et al. 2015). Communication processes between eukaryotes and prokaryotes occur in both directions since eukaryotic cells also use cell-to-cell signalling based on several different hormones. The mammalian epithelium can produce an AI-2 mimic, which stimulates AI-2-dependent phenotypes in *V. harveyi* and *Salmonella* Typhimurium (Ismail et al. 2016). This evidence suggests that hosts can trigger QS-controlled behaviours in their associated flora, and at the same time, the host-associated bacteria can force the host to induce QS-controlled phenotypes by producing the mimic (Defoirdt 2018). QS circuits can also be mimicked using different molecules as AIs to regulate specific bacterial behaviours in a cell-density dependent manner. For instance, a bacterial population has been programmed by a successfully designed QS circuit, using sulphide as an AI (Liu et al. 2019).

By using hormones as messengers for eukaryotic cellular communication, bacteria can cause opportunistic infections in their hosts depending on hormone concentrations (Yang et al. 2014). Thus, the ability of *E. coli* to exploit host-produced signals and use them to regulate its virulence genes accordingly is not surprising (Walters and Sperandio 2006a, 2006b, Karavolos et al. 2013). This capacity provides an advantage to bacteria since they would not need to reach higher cell densities to produce their own AIs (Karavolos et al. 2013). Pathogenic *E. coli* strains present a significantly low infectious dose compared to other enteric bacterial pathogens, estimated to be as few as 10–100 microorganisms for EHEC (Walters et al. 2006a). These low densities do not reach the quorum needed for their own QS activation. Therefore, this unusually low number could be explained by the ability of pathogenic *E. coli* to detect AI signals produced by other species present in the environment (Sperandio et al. 2002b).

AI-2 role in surface colonization and adherence

Due to the presence of AI-2 in the gastrointestinal tract together with the increment of EHEC genes involved in colonization, AI-2 has been related to *E. coli* colonization, a process in which adherence is a prerequisite (Kaper et al. 2004). Genes associated with surface colonization in *E. coli*, such as colanic acid (*wza*) and galactitol transport genes, together with adherence to epithelial cells, were shown to be increased by the presence of AI-2 (Bansal et al. 2008) (Fig. 3). Furthermore, increased adherence of pathogenic *E. coli* to epithelial cells has been reported to be induced by AI-2 signalling (Sircili et al. 2004). Both EPEC and EHEC possess a LuxS/AI-2 system that regulates the expression of their LEE and intimin genes, as studies with *luxS* deletion and complementation have shown (Sperandio et al. 1999, Han et al. 2013). LEE genes are up-regulated upon exposure to AI-2 in EHEC, supporting the importance of AI-2 as a key signal in gastrointestinal infections (Kendall et al. 2007, Bansal et al. 2008) (Fig. 3). However, this QS system does not activate cell division genes as SdiA (Kanamaru et al. 2000). The LuxS/AI-2 system activates the expression of the three operons LEE 1, 2, and 3 that encode type III secretion components but not the LEE 4, which encodes *esp* genes (Sperandio et al. 1999, Kanamaru et al. 2000). In contrast, other authors observed that AI-2 did not activate LEE 1 genes transcription or *ler* expression (Sperandio et al. 2003, Walters and Sperandio 2006b).

Interactions of AIs and stress hormones with bacterial and host adrenergic receptors seem to contribute to the maintenance of microbial endocrine homeostasis and avoidance of dysbiosis (Karavolos et al. 2013). For instance, it was shown that increased AI-2 production by engineering *E. coli* promotes Firmicutes over Bacteroidetes gut colonization (Bivar 2018). Since Firmicutes are relevant for many gut functions, this effect regulates the balance between the microbiota as well as the gut homeostasis recovery (Bivar 2018). Moreover, significant AI-2 production by a gut commensal bacterium can also limit *V. cholerae* infections, repressing several QS-dependent colonization factors (Hsiao et al. 2014).

Expression of *E. coli* virulence factors could also be controlled by the SdiA and AI-2-mediated QS systems depending on the stages of growth, as a few authors have observed. At the mid-exponential growth phase, AI-2 could activate different virulence determinants to allow EHEC colonization. When the stationary phase is reached, AI-2 has already been broken down, and virulence factors might be regulated by the AHL-mediated QS system that would have been acting during this stage (Kanamaru et al. 2000). Additionally, the temporal regulation of the AI-2 uptake and signalling (Bansal et al. 2008) could complicate the understanding of the results. The interpretation of the results is often hard since it is still unknown if AI-2 could be detected by all bacteria to use it as a signal, or a cue, or whether it is produced as waste or metabolite that only in some cases serves as a true signal molecule (Walters and Sperandio 2006b, Whiteley et al. 2017, Defoirdt 2018). Given the pleiotropic effects that *luxS* produces in EHEC, the *luxS* mutation produces an effect not only on QS genes but on other genes involved in metabolic pathways, complicating the current understanding of the AI-2 role in the *E. coli* QS system. As a result, not all the reported phenotypes for *luxS* mutants are a consequence of the loss of AI-2 production (Karavolos et al. 2013).

AI-2 participates in *E. coli* chemoreception and aggregation

The AI-2 receptor protein LsrB bound to AI-2 has been proposed to interact with the periplasmic sensory domain of the chemoreceptor Tsr to drive chemotactic responses in *E. coli* (Hegde et al.

2011) (Fig. 3). A *luxS* deletion was not able to synthesize AI-2 and was also defective in aggregation (Hegde et al. 2011), as well as deletions of *lsrR* and *lsrK* genes lead to a defect in *E. coli* auto-aggregation (Li et al. 2007). Thus, chemotaxis towards the self-produced AI-2 can mediate auto-aggregation of *E. coli*, serving as a chemoattractant that recruits planktonic cells to grow cell aggregates through this LsrB binding (Laganenka et al. 2016, Jani et al. 2017). Indeed, *E. coli* cells lacking *tsr* and *lsrB* genes are completely defective in AI-2 chemotaxis (Hegde et al. 2011, Witsø et al. 2016, Jani et al. 2017). Notably, chemotaxis, as well as motility, also enhances the capacity of auto-aggregation in *E. coli* at physiological cell densities, a bacterial behaviour that allows communal cell behaviours such as gene exchange, antibiotic degradation, or QS processes (Park et al. 2003, Laganenka et al. 2016). High densities within aggregates promote AI-2 signalling compared to non-aggregating cells. Hence, *E. coli* can use auto-aggregation to reach a minimal density required for QS activation (Laganenka et al. 2016). A recent work has revealed that CheY protein, which is involved in chemotactic response, and AI-2 signalling (through *lsrB*) promote *E. coli* colonization of the murine gut, supporting the AI-2 role in these virulence-associated phenotypes (Laganenka et al. 2023).

AI-2 role in *E. coli* biofilm and motility

Most of the bacteria are found as aggregates or surface-attached communities of microorganisms, being very uncommon to find individual bacterial cells in the environment. AI-2 has been shown to be linked to biofilm formation by *E. coli* (González Barrios *et al.* 2006, Niu et al. 2013). Auto-aggregation and chemotaxis through AI-2 contribute to producing surface-attached biofilms (Laganenka et al. 2016, 2023). Indeed, auto-aggregation and co-aggregation were reported at low densities in the early stages of the formation of mixed biofilms by *E. coli* and *E. faecalis*, both pathogens from mammalian hosts (Laganenka and Sourjik 2018). AI-2 produced by *E. faecalis* enhanced *E. coli* auto-aggregation and resulted in coaggregation between both species, indicating a key role of AI-2 in mixed biofilms (Laganenka and Sourjik 2018). However, chemotaxis mediated by AI-2 is necessary but does not seem essential to completely induce the density-dependent behaviours required for biofilm production in this bacterium (Jani et al. 2017). On the other hand, insertion and deletion mutants of *luxS* have produced defects in biofilm formation by *E. coli* (Niu et al. 2013, Jani et al. 2017), confirming a link between this QS system and biofilm production (Fig. 3). LsrR and LsrK proteins act as global regulators of gene expression, sensing the internalized AI-2 signal, and causing an effect on the expression of downstream genes that participate in aggregation, attachment, and biofilm production (Li et al. 2007, Laverty et al. 2014) (Fig. 3). Indeed, it has been observed that insertion *lsrR* and *lsrK* mutations affected biofilm architecture, reducing the biofilm thickness and biomass by the regulation of biofilm-associated genes, including *wza* (responsible for colanic acid) (Fig. 3) and the auto-aggregation gene *flu* (Li et al. 2007). Likewise, insertion and deletion mutants of *lsrR, lsrK, lsrC,* and *lsrG* genes that encode components required for sensing AI-2 were also defective in biofilm formation (Jani et al. 2017).

On the other hand, deletion of *luxS*, but not *lsrB*, produced a reduction in motility by *E. coli* (Sperandio et al. 2001, Han et al. 2013, Laganenka et al. 2016) (Fig. 3). Given that AI-2 presence increases motility and chemotactic recognition in EHEC, it has been suggested that AI-2 could participate in its migration to host epithelial cells (Bansal et al. 2008), supporting the link between AI-2 and *E. coli* virulence. Moreover, it was observed that an APEC mutant strain with the deleted *lsr* operon was also defective in motility and AI-2 uptake (Zuo et al. 2019). Further, a deletion *lsr* mutant down-regulates the expression of genes involved in APEC pathogenicity, supporting an association between *lsr* operon and *E. coli* virulence (Zuo et al. 2019).

Although the relationship between the AHL-QS system and the AI-2-QS system in *E. coli* remains unknown, the *ydiV* gene and cAMP are involved in the interaction between both QS systems (Zhou et al. 2008). Although a *luxS* or *lsrR* deletion mutants did not affect *sdiA* gene expression, it seems that the SdiA-AHL complex activates *ydiV*, repressing flagellar motility and P fimbrial expression and regulating the AI-2 QS system through the control of intracellular cAMP concentration (Zhou et al. 2008). A double-deletion *sdiA-ydiV* mutant inhibits the AI-2 QS system in *E. coli* by producing a significant decrease in intracellular cAMP concentration (Zhou et al. 2008). Consistent with that, SdiA, together with YdiV participates in the activation of gene expression of the cyclic AMP receptor protein (CAP) and Cya, the enzyme that synthesizes cAMP (Zhou et al. 2008, Spurbeck et al. 2013). Environmental cues, such as glucose, also participate in these signalling pathways, since *sdiA* and *ydiV* expression decreases when glucose is added to the culture (Zhou et al. 2008). Recently, an inhibitory effect of lactic acid on EHEC biofilm was also observed through the suppression of QS genes expression, such as *sdiA* and *luxS* genes (Liu et al. 2021).

In addition, a link between biofilm and motility regulation has been observed in *E. coli*. AI-2 significantly enhances biofilm mass through the motility QS regulator gene MqsR, which in turn regulates flagellar movement and motility in *E. coli* through the QS regulators QseB and QseC (Sperandio et al. 2002b, González Barrios *et al.* 2006, Sharma et al. 2016) (Fig. 3). This QseBC two-component system participates in the control of the master flagellar regulon (*flhDC*) transcription. Moreover, QseBC regulates the transcription of genes involved in flagellar expression, including genes such as *fliA, fliC*, or *motA*, that encode the sigma factor, flagellin, and the proton exchange conductor for flagellum movement, respectively. MqsR stimulates QseB, which activates *flhDC* expression binding itself to the *flhDC* promoter (Clarke and Sperandio 2005), promoting *E. coli* motility and improving flagellar motion and motility by MotA (Fig. 3). Through the regulation of MotA, AI-2 increases flagellar motion, motility, as well as biofilm production. Therefore, MqsR can be considered a mediator between AI-2 and motility (González Barrios *et al.* 2006, Sharma et al. 2016). In addition, MqsR induces curli expression through the *crl* gene, a transcriptional regulator for curli fibres, and possibly stimulates motility through *csrA* (González Barrios *et al.* 2006, Li et al. 2007) (Fig. 3). The *luxS* deletion showed in general reduced motility than a wild-type strain, and consistent with that, the down-regulation of *flhD*, *fliA*, and *fliC* has been noticed in an insertion *luxS* mutant (Sperandio et al. 2001). MotA transcription is also down-regulated in the *luxS* deletion, and its expression is restored by complementation or AI-2 addition, supporting the role of AI-2 in *E. coli* motility (Sperandio et al. 2001). Finally, protein TqsA, which exports AI-2 and is induced in *E. coli* biofilms (Fig. 3), was also found to negatively regulate the expression of flagellum and motility genes, type I fimbriae, and curli production (Herzberg et al. 2006, Zhou et al. 2008, Sharma et al. 2016).

AI-2 involvement in stress conditions, phage defence, and *E. coli* drug resistance

AI-2 has been related to osmotic stress tolerance in *E. coli* since a *luxS* mutant improved specific stress resistance of EHEC (Park et

al. 2017). However, this difference *in vitro* was not observed *in vivo*, indicating that AI-2 may experience some interference by other compounds or metabolites from the commensal microbiota (Park et al. 2017). Auto-aggregation through AI-2 also improves physical protection against oxidative stress (Laganenka et al. 2016). A recent study has observed that transcription levels of *lsrR* significantly decreased under hydrogen peroxide (H_2O_2) stress conditions (Wang et al. 2021). Moreover, the survival rate of a deletion *lsrR* mutant was significantly higher compared to the wild-type in the presence of H_2O_2. These results have suggested an adaptation of *E. coli* to oxidative stress conditions by reducing the *lsrR* expression (Wang et al. 2021). However, further research is needed to understand how *E. coli* could down-regulate the *lsrR* expression under these stress conditions (Wang et al. 2021). Catabolite repression is also important to manage the environmental stress in *E. coli* and is controlled by the alternative subunit of RNA polymerase (σ^S) (RpoS) (Laverty et al. 2014). RpoS has already been associated with QS in other bacteria, such as *P. aeruginosa* or *Vibrio* spp. (Laverty et al. 2014, Defoirdt 2018). In *E. coli,* catabolite repression can negatively regulate AI-2 uptake through the RpoS regulator (Wang et al. 2005).

Since AI-2 is also involved in metabolic pathways, a recent study has demonstrated that AI-2 promotes host defence against phages in *E. coli* (Ding et al. 2021). These authors have also described that the AI-2 receptor LsrB plays a role in maintaining the balance of cellular metabolism, indicating that it is essential for the phage defence mechanisms through AI-2 and also suggesting a link between phage susceptibility and QS (Ding et al. 2021).

The ESBL is one of the most common known mechanisms that confers *E. coli* resistance to several drugs. It has been suggested a link between antibiotic resistance and AI-2, since exogenous AI-2 enhanced the antibiotic resistance of *E. coli* in an LsrR-dependent manner (Xue et al. 2016). That is, in the presence of β-lactam antibiotics, AI-2 up-regulates the transcription of TEM-type β-lactamase through LsrR (Xue et al. 2016). Moreover, in the presence of drugs, exogenous AI-2 addition increased bacterial cell densities together with the cell growth rate, and the antibiotic resistance in the drug-resistant *E. coli* was enhanced in response to AI-2. This also suggests that long-term exposure to drugs may drive *E. coli* to develop an improved survival strategy (Xue et al. 2016). Supporting the role of AI-2 in antibiotic resistance of *E. coli*, another study has described that the presence of imidazole, a furan carbocyclic analogue of AI-2, can interfere with this regulatory effect on bacterial drug resistance (Yu et al. 2018). In the presence of ampicillin, exogenous imidazole inhibited the regulatory effect of AI-2 on TEM-type β-lactamase, and even decreased the *E. coli* resistance to ampicillin of a clinical *E. coli* strain resistant to β-lactam antibiotics, resulting in a lower survival rate (Yu et al. 2018). A recent work has observed a relationship between the Pfs protein, involved in AI-2 synthesis, and the increase of β-lactam resistance in an APEC strain (Hu et al. 2022). However, AI-2 does not participate in this resistance effect (Hu et al. 2022), indicating that new studies are needed to elucidate this process.

In conclusion, the AI-2-QS system participates in the regulation of several virulence-associated phenotypes in *E. coli*, including surface colonization, adherence and biofilm formation, stress and drug resistance, phage defence, chemoreception, and motility.

AI-3-mediated QS system

Besides the AHL and AI-2 QS systems, the autoinducer 3 (AI-3) has been identified in *E. coli*, which is known to control different virulence traits in this bacterium (Witsø et al. 2016, Gorelik et al. 2019). In contrast to the AHL and AI-2 systems involved in interspecies communication, it seems that the AI-3 system participates in the communication between prokaryotic and eukaryotic cells (Sperandio et al. 2003, Walters et al. 2006), establishing an inter-kingdom communication (Clarke et al. 2006, Sperandio 2010, Lustri et al. 2017). As in the previous QS systems, when AI-3 reaches a critical concentration threshold, bacteria sense and respond to this signal by changing their gene expression patterns in a coordinated manner (Fig. 4). Different biosynthesis pathways have already been proposed to elucidate the AI-3 formation (Hernandez and Sintim 2020). Recently, threonine dehydrogenase (Tdh) and essential tRNA synthetases have been shown as the responsible for producing substrates, which react spontaneously to form the AI-3 molecules family (Hernandez and Sintim 2020, Kim et al. 2020) (Fig. 1). As with other QS signals, AI-3 is produced by several commensal and pathogenic *E. coli*, including EPEC and EHEC. In addition, analogues of AI-3 are also distributed in Gram-negative and Gram-positive bacteria, including other enteric bacteria such as *Klebsiella pneumoniae, Shigella* spp., *Salmonella* spp., or *Enterobacter cloacae* (Walters and Sperandio 2006b, Kim et al. 2020). Recently, it has been observed that AI-3 can act as markers of cellular stress, producing diverse immunological effects on primary human tissues (Kim et al. 2020).

AI-3 was first described in EHEC, where it is involved in the control of its pathogenesis (Sperandio et al. 2003, Lustri et al. 2017) (Fig. 4). A linkage between AI-2 and AI-3 QS systems has also been observed. Nevertheless, the *luxS* deletion causes a higher effect on metabolism compared to AI-3 (Walters et al. 2006). AI-3 is produced by the human gastrointestinal microbiota, as revealed by a study with anaerobic faeces culture in a simulated intestinal ecosystem, and cross signals with the host hormones epinephrine and norepinephrine (Sperandio et al. 2003). Both epinephrine and norepinephrine belong to the class of hormones called catecholamines. EHEC can sense these hormones, as well as AI-3, by the bacterial regulator QseC from the QseBC two-component system (Fig. 4). In response to these signals, QseC increases its autophosphorylation and regulates its own transcription (Clarke et al. 2006). This regulator acts as a bacterial adrenergic receptor and triggers the activation of several virulence genes in a coordinated manner (Witsø et al. 2016, Kim et al. 2020) (Fig. 4).

Epinephrine and norepinephrine hormones have also been shown to promote growth and virulence in *E. coli* (Freestone et al. 1999), as well as to improve motility and adherence to HeLa cells in EHEC (Clarke et al. 2006, Bansal et al. 2007). A *qseC* mutant was defective in motility phenotype (Yang et al. 2014), and similarly, AI-3 and epinephrine positively regulate virulence and flagellar gene expression (Sperandio et al. 2003). QseBC also activates transcription of the flagellar regulon responsible for swimming motility in EHEC (Clarke and Sperandio 2005) (Fig. 4). Indeed, QseC is considered a global regulator involved in the expression of several genes and pathogenesis *in vivo*, since insertion and deletion *qseC* mutants have shown a more attenuated infection in animal models (Clarke et al. 2006, Rasko et al. 2008, Machado *et al.* 2019). Although transcription of EHEC virulence genes is induced by AI-3 and epinephrine, neither of the two had any effect on promoting virulence in a deletion *qseC* mutant, supporting the involvement of AI-3/QseC in *E. coli* virulence (Rasko et al. 2008). QseC is also an important sensor kinase for the passage through the gastrointestinal tract, and it seems to participate in *E. coli* infection. The blocking of QseC through a QseC autophosphorylation inhibitor (*N*-phenyl-4-{[(phenylamino) thioxomethyl] amino}—benzenesulfonamide or LED209) in an enteroaggregative *E. coli* (EAEC) strain led to a lower colonization efficiency in a

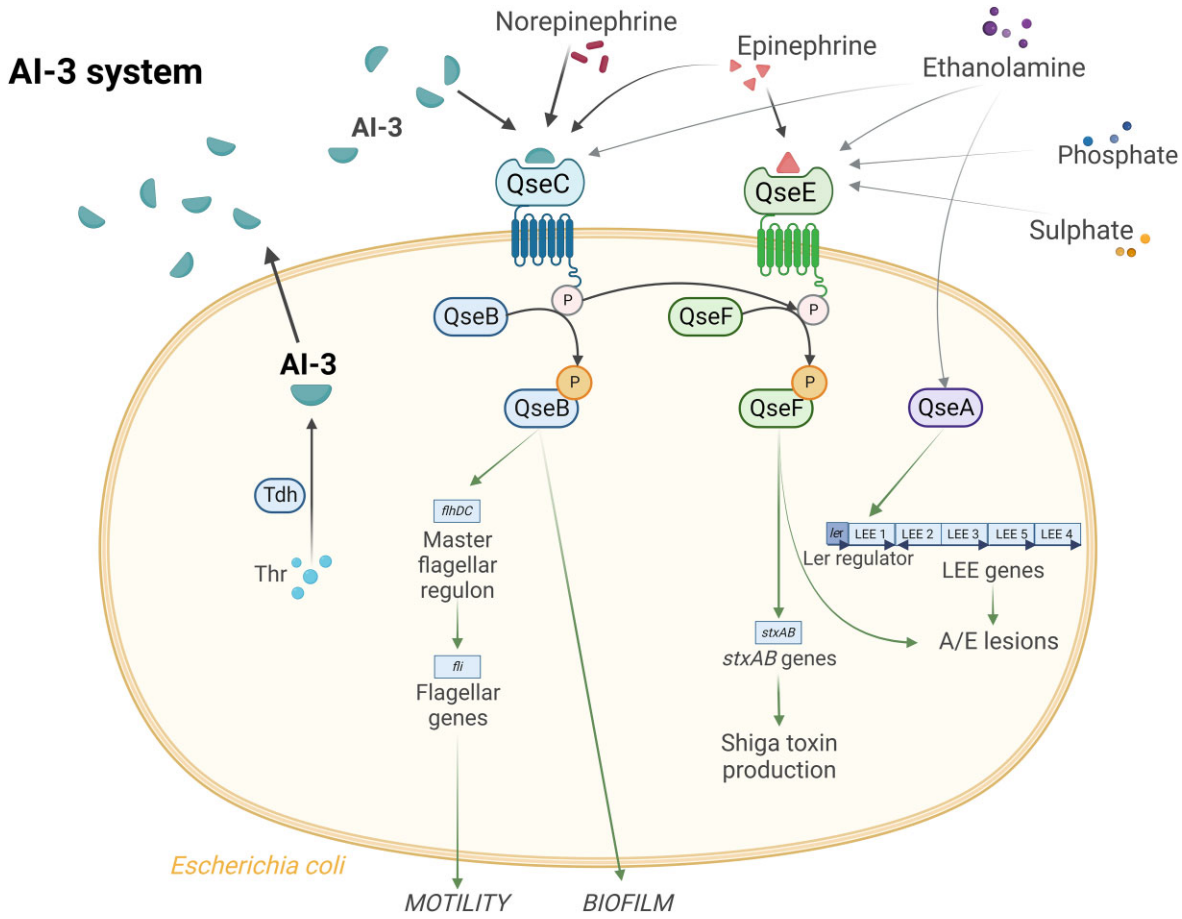

Figure 4. Schematic representation of the AI-3/epinephrine/norepinephrine system that participates in inter-kingdom communication in *E. coli*. A biosynthetic proposal for the formation of AI-3 is shown. AI-3 is produced from threonine (Thr) by Tdh resulting in AI-3. AI-3, hormones such as norepinephrine or epinephrine, and other metabolites such as phosphate, sulphate, or ethanolamine are sensed by the two-component systems QseBC and/or QseEF, which initiate a complex signalling cascade resulting in the control of several virulence phenotypes in *E. coli*. Then, A/E lesions on intestinal epithelial cells through LEE genes are produced, motility through flagellar regulon is improved, biofilm production increases, and the Shiga toxin secretion is activated. The QseA regulator also participates in the AI-3 system in *E. coli*, which activates the expression of LEE genes resulting in A/E lesions formation. Created with BioRender.com.

murine model (Machado *et al.* 2019). In agreement, an insertion *qseC* mutant presented similar results to the wild-type strain after the addition of this QseC inhibitor (Machado *et al.* 2019). Moreover, this inhibitor showed potential effects to control the biofilm formation by UPEC and EAEC (Curtis et al. 2014), indicating that the QseBC system is also relevant to biofilm formation. Indeed, the addition of epinephrine/norepinephrine increased biofilm thickness changing the biofilm architecture from scattered colonies to a biofilm of flatter aggregates in wild-type *E. coli* (Yang et al. 2014). However, the addition of these hormones to the deletion *qseC* mutant, which lacks the ability to sense them, was not able to stimulate biofilm production (Yang et al. 2014). To further complicate this signalling cascade, QseB can modulate the activation of flagellar regulon (*flhDC*) expression, given that QseB binds to different binding sites in the target promoters according to its phosphorylation state (Hughes et al. 2010) (Fig. 4). QseB regulates the expression of flagella, but only when there is enough concentration of QseB and is in the phosphorylated state, where the flagellar regulon is activated completely (Hughes et al. 2010, Lustri et al. 2017).

Downstream of QseBC, there is another sensor kinase known as QseE from the two-component system QseEF that upon sensing epinephrine initiates a complex signalling cascade resulting in a coordinated expression of virulence genes (Hughes et al. 2010) (Fig. 4). QseE can be phosphorylated by QseC and also increases its autophosphorylation after sensing epinephrine, phosphate, and sulphate (Reading et al. 2009, Hughes et al. 2010). Although QseE does not respond to AI-3 and is not involved in motility or flagella regulation, QseE presents a key role in the expression of genes involved in A/E lesions and the expression of Shiga toxin through the *stxAB* genes (Reading et al. 2009, Hughes et al. 2010) (Fig. 4). The QseBC system was described in EHEC, EPEC, *E. coli* K12, and also in UPEC (Hadjifrangiskou et al. 2011). AI-3 and QseC homologs are also found in diverse bacteria, many of them animal and plant pathogens (Clarke et al. 2006, Rasko et al. 2008, Curtis et al. 2014, Lustri et al. 2017, Kim et al. 2020), suggesting a relevant role in inter-kingdom signalling. Nevertheless, QseE homologues could only be found in enteric bacteria (Hughes et al. 2010). This fact reveals the importance of sensing and responding to signalling molecules between prokaryotes and eukaryotes, as they have co-existed for millions of years (Karavolos et al. 2013). Indeed, a synergistic effect between AI-3 and the epinephrine/norepinephrine hormones was suggested, indicating that both combined signals could allow a more efficient infection (Walters and Sperandio 2006a).

In the AI-3/epinephrine/norepinephrine regulatory cascade, another regulator is involved. QseA belongs to the LysR family of

transcription factors and is present in EHEC, EPEC, and K12. This regulator activates transcription of the *ler* regulator and consequently all the LEE genes (Sperandio et al. 2002a) (Fig. 4). Although insertion *qseA* mutants were still capable of producing A/E lesions, a significantly reduced secretion of Tir, EspA, and EspB was observed in an insertion *qseA* mutant compared to the wild-type strain, leading to a remarkable reduction in the type III secretion system. This effect could be restored with *qseA* complementation in EPEC and EHEC (Sperandio et al. 2002a). QseA activates the transcription of LEE1 by its binding directly upstream to one of its promoter regions (Sperandio et al. 2002a). However, further research is needed to know if QseA is directly activating *ler* transcription or if it is acting via another regulator. Recently, ethanolamine has been shown to be involved in this QS circuit, since QseC, QseE, and QseA, and LEE genes, were up-regulated by ethanolamine addition to *E. coli* cultures (Kendall et al. 2012) (Fig. 4). Other bacteria, such as *V. cholerae*, *Salmonella* spp., or *Enterococcus* spp., present different mechanisms to sense ethanolamine to control their gene expression, improving their fitness inside the host (Watve et al. 2020). Although in *E. coli* the specific mechanism of this gene regulation is not completely understood, it seems that EHEC not only uses this component as a carbon and/or nitrogen source, but also as a signalling molecule to activate virulence gene expression, conferring a competitive advantage for colonization regarding the commensal flora (Kendall et al. 2012).

To conclude, the AI-3-dependent QS system has recently been described to influence the control of different phenotypes associated with *E. coli* virulence. These include colonization, motility, and biofilm formation.

Impact of indole in *E. coli* virulence

Indole has been considered a QS signal in *E. coli*, since Wang et al. (2001) described that indole activated the QS loci *astCADBE, tnaAB,* and *gabDTP* involved in the uptake, synthesis, and catabolism of amino acids to generate pyruvate or succinate, providing energy to starving cells, and hypothesized that indole might be important for energy production in the stationary phase (Wang et al. 2001). However, if it is a real QS signal remains unclear because of contradictory results found in the literature, which will be explained in detail below (Sabag-Daigle et al. 2012, Kim and Park 2015, Zarkan et al. 2020). Indole is produced from tryptophan degradation by the enzyme tryptophanase (TnaA). Previous studies have reported an interaction between indole and SdiA in controlling biofilm production, proposing that SdiA could act in both intra- and inter-species communication (Martino et al. 2003, Lee et al. 2009, Chu et al. 2012). It has been observed that indole repressed biofilm formation in *E. coli*. Indeed, a study has suggested that *E. coli* uses SdiA to monitor indole and AHLs to control biofilms without confirming whether indole binds itself to SdiA (Lee et al. 2007). Since overexpression of *sdiA* led to a significant decrease in *tnaA* expression together with reduced indole production, a link between SdiA and indole was proposed (Lee et al. 2009). On the contrary, it has been described that indole promotes biofilm formation in other pathogens such as *P. aeruginosa* or *V. cholerae*, proposing a different regulation depending on the bacterial species. Then, the influence of indole on *E. coli* via SdiA was discarded, since the enhancement of *E. coli* growth in competition assays between *E. coli* and *P. aeruginosa* through indole has been attributed to the direct action of indole on *P. aeruginosa* (Chu et al. 2012). In agreement, the *sdiA* deletion itself showed a significant effect on biofilm formation by *E. coli*. Although high indole concentrations can inhibit AHL activity in *E. coli*, the biofilm regulation by indole is not SdiA-dependent, since SdiA is not able to respond to indole (Sabag-Daigle et al. 2012). Indole has also been related to the AI-2 system in *E. coli*, and it has been proposed as an important signal for EHEC infections (Bansal et al. 2007). YliH (BssR) and YceP (BssS) are proteins that repress *E. coli* motility, biofilm, and surface coverage by *E. coli* (Domka et al. 2006). These proteins have also been proposed to participate in the regulation of indole as well as in the uptake and export of AI-2 through a cAMP-dependent pathway (Domka et al. 2006). The latest findings indicate that the presence of indole inhibits the folding of some proteins related to QS, including LuxR regulators, and promotes protein degradation, reducing the stability of LuxR receptors (Kim and Park 2015). Hence, the binding of AHLs to LuxR regulators, such as SdiA, can prevent the effects of indole (Kim and Park 2015), supporting the role of AHLs in SdiA stability as discussed above.

Recent articles using *Citrobacter rodentium* and EHEC have described that exogenous indole is sensed by the CpxAR two-component system, constituted by the membrane-bound histidine-sensor-kinase CpxA and the response regulator CpxR (Kumar and Sperandio 2019, Kumar et al. 2022) (Fig. 5). Previously, the CpxR/CpxA two-component system was related to *E. coli* resistance to antimicrobial peptides, including protamine (Weatherspoon-Griffin et al. 2014). CpxA autophosphorylates itself and phosphorylates the CpxR that activates LEE genes expression by binding a regulatory region of ler regulator (Kumar et al. 2022) (Fig. 5). At physiological concentrations found in the gastrointestinal tract, indole has been reported to inhibit the CpxA/CpxR function. That is, CpxA acts as a phosphatase by sensing indole, dephosphorylating itself and the regulator CpxR, which results in blocking LEE genes expression, which is required to form A/E lesions on enterocytes (Kumar and Sperandio 2019). In agreement, indole at high concentrations (1.5 mM) represses LEE transcription genes compared to low concentrations (50 μM) that promote LEE genes transcription (Platenkam and Mellies 2018). This study has also reported a novel indole sensing regulator (*isrR*), previously known as *ygeV*, which is a predicted orphan regulator that may act independently of CpxA (Kumar et al. 2022). In the absence of endogenous indole, IsrR activates LEE genes together with the characteristic pedestal-like structure formation by actin accumulation that are involved in A/E lesions (Kumar et al. 2022). However, IsrR-dependent LEE induction is inhibited in the presence of endogenous indole (Kumar et al. 2022) (Fig. 5).

Although it has been described that the efflux of indole from *E. coli* is mediated by the AcrEF pump and that the main pathway for indole transport into the cell is via the Mtr permease (Wang et al. 2001), Kumar et al. (2022) have hypothesized another working model of indole regulation in which exogenous indole diffuses through the outer membrane and is sensed by CpxA. Exogenous indole can reach the cytoplasm through diffusion and directly interact with IsrR. The indole produced by EHEC and the presence of a pool of endogenous indole inhibit IsrR activation of virulence genes. In the absence of indole, IsrR acts as an activator of LEE genes allowing IsrR to fine-tune the expression of virulence genes under variable indole conditions. Indole treatment also promoted *tnaA* up-regulation (Kumar et al. 2022). Moreover, the CpxA/CpxR two-component system is also stimulated by a number of signals, including serotonin, high osmolarity, ethanol, *n*-butanol, copper, alkaline pH, amino-glycoside antibiotics, membrane/periplasmic protein, and phospholipid disruptions, among others (Platenkam and Mellies 2018, Kumar et al. 2022). The search for an indole receptor was one of the crucial points for several authors to consider indole as a QS signal. Although it was recently discovered, there are still several doubts in the literature to consider indole not only as an important signal involved in cellular metabolism but as a

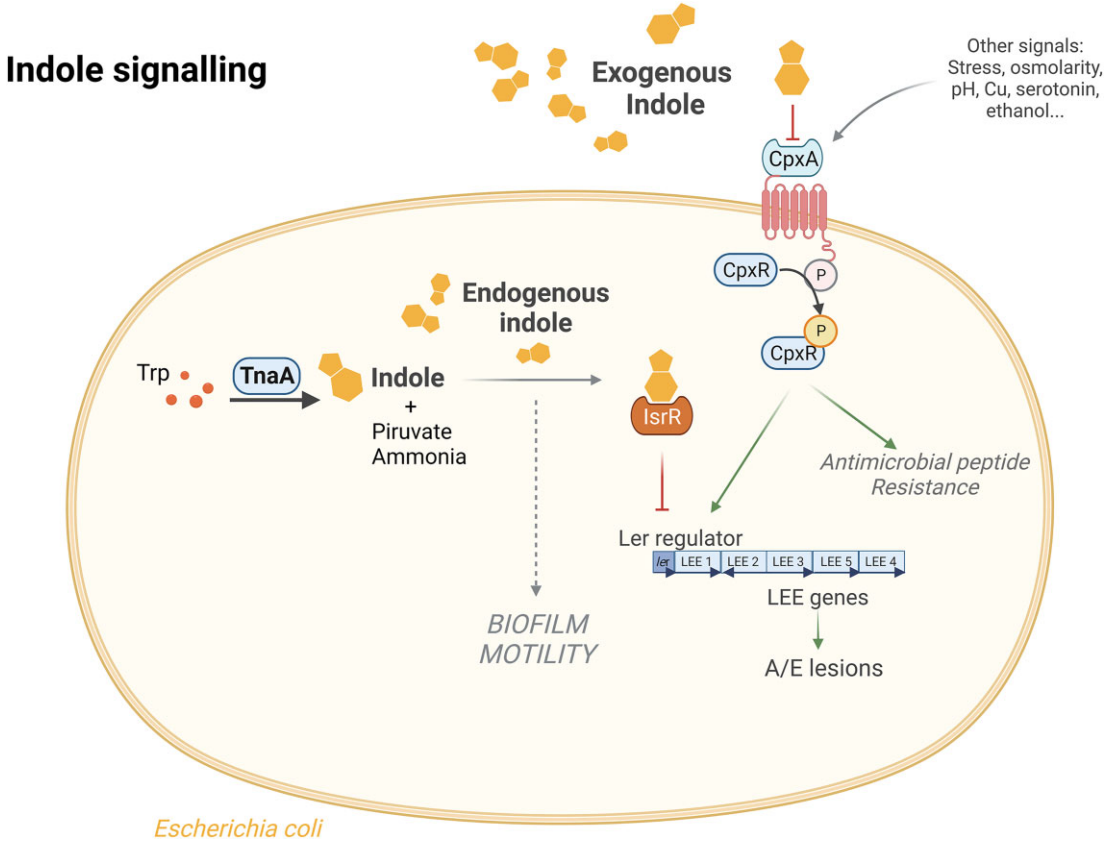

Figure 5. Schematic representation of the indole system that participates in inter-kingdom communication processes in *E. coli*. Indole is produced by the tryptophanase enzyme (TnaA) from tryptophane. Exogenous indole, as well as several signals, is sensed by the CpxA/CpxR two-component system. CpxA autophosphorylates itself and phosphorylates the CpxR activating LEE genes expression by binding a regulatory region of *ler* regulator. At physiological concentrations found in the gastrointestinal tract, indole has been reported to inhibit the CpxA/CpxR function, blocking LEE expression. On the other hand, endogenous indole is sensed by the novel indole sensing regulator IsrR, an orphan regulator that may act independently of CpxA. In the absence of endogenous indole, IsrR activates LEE genes and A/E lesions production. However, IsrR-dependent LEE induction is blocked in the presence of endogenous indole. Indole also plays a role in other *E. coli* phenotypes including biofilm and motility; however, it is not known if this effect is mediated by the CpxA/R two-component system or via the IsrR regulator. Created with BioRender.com.

true QS signal. For instance, IsrR only responds to endogenous indole (Kumar *et al.* 2019). According to that, the accumulation of indole in the external medium is less relevant, while the intracellular concentration of indole is crucial for *E. coli* viability as revealed a pulse signalling mechanism of indole (Gaimster et al. 2014). In *E. coli*, a *tnaA* mutant that has lost the ability to produce indole presented reduced survival and the addition of 1 mM indole was not sufficient to restore long-term survival (Gaimster et al. 2014). That study revealed that during the transition from exponential to stationary phase, cells transiently experienced a very high concentration (>50 mM) of indole, and that this pulse is required for long-term stationary phase viability in *E. coli*. But, the accumulation of indole is caused by enhanced production rather than a simple increase of cell numbers (Gaimster et al. 2014). Hence, this concentration is sufficient to inhibit growth and division by an ionophore-based mechanism and cause the cells to enter stationary phase before resources are exhausted (Gaimster et al. 2014). Indole signalling presents two action modes: A long-lasting but low-level signal (persistent) or a transient and high-level signal (pulse) (Zarkan et al. 2020). Given that pulse signalling is independent of cell density, indole seems to fail again to meet the criteria for either QS signal (Winzer et al. 2002, Diggle et al. 2007, Zarkan et al. 2020). A few authors have also proposed that indole could allow *E. coli* to adapt to environments with poor nutrients and where the breakdown of amino acids serves as an energy source, thus, it might act as a starvation signal in bacterial species (Speranzio et al. 2001, Wang et al. 2001, Diggle et al. 2007, Defoirdt 2018). Moreover, indole is accumulated at a much higher concentration compared to other signals that mostly work at the micromolar range for their physiological effect, questioning its role as a QS signal (Kim and Park 2015). In addition, up to our knowledge, the function of this compound in coordinated behaviour of a microbial population has not been described (which are requirements for a true QS signalling molecule). Wang et al. (2001) hypothesized that it may act as an extra QS system involved in the stationary phase. Nevertheless, these findings suggest that the functions of indole as either a QS signal dependent on coordinated behaviours or a metabolic signal still require further clarification.

To conclude, although indole plays a role in various bacterial functions, such as biofilm formation, sporulation, plasmid stability, and antibiotic resistance (King and Park 2015), further research is also required to enlighten if the role of indole in different phenotypes, such as motility or biofilm formation is mediated via IsrR or CpxA/CpxR system and to provide more knowledge about the role of exogenous and endogenous indole regulation on *E. coli* virulence.

Concluding remarks

QS enables bacteria to orchestrate collective behaviours, being a key component of the global gene regulatory networks. In this review, we have summarized the involvement of the different QS

systems in *E. coli* pathogenesis and virulence. However, the interconnection among all the QS pathways and the specific role of each circuit in the global QS network remains unclear. A completely different regulation of *E. coli* virulence has been observed depending on AHLs, AI-2, AI-3 systems, or indole. Bacteria had to develop several strategies to persist in environments inhabited by many different organisms; therefore, inter-kingdom communication would appear as a necessity to perceive these environments, adjusting their physiology accordingly. The presence of more than one QS circuit may be the result of a long evolution that results in the control of these delicately balanced interactions. Using multiple QS circuits that converge to control several bacterial functions would provide a fine-tuning, multiplying the possible QS input responses, and improving the monitoring of the environmental conditions. Although the use of several signals would seem functionally redundant, these specific circuit arrangements could improve the robustness of the QS system regulation, preventing signal perturbation. In *E. coli*, the different QS systems are interconnected, possibly due to the necessity of a tight control of gene expression mediated by each QS system. However, the function of each QS circuit is hard to interpret since it is still unknown if AI or signals could be detected by all bacteria to use them as a signal, or a cue, or if they are produced as a metabolite which takes part in several metabolic pathways and only in a few cases act as true QS signals. Thus, it is also complicated to determine which genes changed their expression level as a result of a true communication signal and which genes responded to metabolite concentrations produced by other microorganisms. On the other hand, the high emergence of antibiotic resistance requires the development of new targets to reinforce novel therapeutic strategies to inhibit microbial pathogenicity. Due to the involvement of QS in virulence-associated phenotypes of *E. coli*, the interference of QS circuits has been proposed as an attractive target for developing anti-pathogenic strategies to control *E. coli* pathogenic strains. Several strategies can be developed to interfere with QS circuits, including (i) interference of signal synthases, (ii) interception or degradation of the signals by enzymes or chemical degradation, and (iii) chemical inhibitors or antagonists that block or reduce receptor activity, which can or cannot be used synergistically with other anti-virulence agents as an approach to control and prevent *E. coli* virulence. Here, we report the impact of cell-to-cell signalling systems in *E. coli* and the current knowledge of their role on different biological functions related to virulence and pathogenesis. Although several QS inhibitors were reported as drug candidates, the knowledge of the broad QS network in *E. coli* and all the components involved, which we encompass in this review, will help to address new drug strategies. QS signal synthases, autoinducers, regulators, and other genes that participate in the AHL/SdiA, AI-2, AI-3, and indole circuits appear to be attractive target candidates to investigate new anti-virulence strategies. However, further research should take into account the relationship among the different QS regulators in the same bacterial species to elucidate the specific impact of each one and to complete the QS map in *E. coli*. In addition, several questions should be addressed, including (1) when bacteria use one or another QS circuit; (2) how bacteria can switch on their QS systems, taking into account the temporal regulation of the AI signalling and the different regulation of phenotypes depending on the QS signal; (3) why do bacteria prioritize one circuit or another one to activate or inhibit target phenotypes?; (4) how the circuits function—do they act in a concerted manner or do they work independently?; (5) or what is the exact role of each QS circuit in the global QS regulatory network—are one of them regulated by another one? Hence, investigation along these lines will further advance our understanding of the complicated QS regulatory mechanisms in relevant pathogens such as *E. coli* and will enable the knowledge of the sophisticated relationship between bacteria and their hosts. Research is needed to understand these complex communication processes, and it would help to develop new anti-pathogenic strategies to target this intricate QS network and control the virulence of this pathogen.

Conflict of interest statement. The authors declare no commercial or financial relationships that could be construed as a potential conflict of interest.

Funding

This work was financially supported by: Base Funding [LA/P/0045/2020 (ALiCE), UIDB/00 511/2020 and UIDP/00 511/2020 (LEPABE)], funded by national funds through [FCT/MCTES (PIDDAC)]; Projects Germirrad [POCI-01–0247-FEDER-072237] funded by FEDER funds through COMPETE2020–Programa Operacional Competitividade e Internacionalização (POCI) and by national funds (PIDDAC) through FCT/MCTES; and HealthyWaters—Identification, Elimination, Social Awareness and Education of Water Chemical and Biological Micropollutants with Health and Environmental Implications [NORTE-01–0145-FEDER-000069], supported by Norte Portugal Regional Operational Programme (NORTE 2020), under the PORTUGAL 2020 Partnership Agreement, through the European Regional Development Fund (ERDF). CM was supported by a post-doctoral fellowship from Xunta de Galicia [IN606B-2019/010]. AB thanks the Portuguese Foundation for Science and Technology (FCT; Lisbon, Portugal) for the financial support of her work contract through the Scientific Employment Stimulus—Individual Call—[CEECIND/00 823/2021].

References

Amalaradjou MA, Venkitanarayanan K. Role of bacterial biofilms in catheter-associated urinary tract infections (CAUTI) and strategies for their control. In: Nelius T (ed.), *Recent Advances in the Field of Urinary Tract Infections*. London: IntechOpen, 2013. https://doi.org/10.5772/55200.

Bansal T, Englert D, Lee J *et al.* Differential effects of epinephrine, norepinephrine, and indole on *Escherichia coli* O157:H7 chemotaxis, colonization, and gene expression. *Infect Immun* 2007;**75**:4597–607.

Bansal T, Jesudhasan P, Pillai S *et al.* Temporal regulation of enterohemorrhagic *Escherichia coli* virulence mediated by autoinducer-2. *Appl Microbiol Biotechnol* 2008;**78**:811–9.

Beloin C, Roux A, Ghigo JM. *Escherichia coli* biofilms. *Curr Top Microbiol Immunol* 2008;**322**:249–89.

Bez C, Geller AM, Levy A *et al.* Cell–cell signaling proteobacterial LuxR solos: a treasure trove of subgroups having different origins, ligands, and ecological roles. *Msystems* 2023;**8**:e0103922.

Bivar Xavier K. Bacterial interspecies quorum sensing in the mammalian gut microbiota. *CR Biol* 2018;**341**:297–9.

Bjarnsholt T, Givskov M. Quorum-sensing blockade as a strategy for enhancing host defences against bacterial pathogens. *Phil Trans R Soc B* 2007;**362**:1213–22.

Chen X, Schauder S, Potier N *et al.* Structural identification of a bacterial quorum-sensing signal containing boron. *Nature* 2002;**415**:545–9.

Chu W, Zere TR, Weber MM *et al.* Indole production promotes *Escherichia coli* mixed-culture growth with *Pseudomonas aeruginosa* by inhibiting quorum signaling. *Appl Environ Microbiol* 2012;**78**:411–9.

Clarke MB, Hughes DT, Zhu C *et al.* The QseC sensor kinase: a bacterial adrenergic receptor. *Proc Natl Acad Sci USA* 2006;**103:** 10420–5.
Clarke MB, Sperandio V. Transcriptional regulation of *flhDC* by QseBC and σ28 (FliA) in enterohaemorrhagic *Escherichia coli*. *Mol Microbiol* 2005;**57**:1734–49.
Connolly JPR, Finlay BB, Roe AJ. From ingestion to colonization: the influence of the host environment on regulation of the LEE encoded type III secretion system in enterohaemorrhagic *Escherichia coli*. *Front Microbiol* 2015;**6**:568.
Croxen MA, Finlay BB. Molecular mechanisms of *Escherichia coli* pathogenicity. *Nat Rev Microbiol* 2010;**8**:26–38.
Culler H, Couto S, Higa J *et al.* Role of SdiA on biofilm formation by atypical enteropathogenic *Esch e richia coli*. *Genes* 2018;**9**:253.
Curtis MM, Russell R, Moreira CG *et al.* QseC inhibitors as an antivirulence approach for gram-negative pathogens. *Mbio* 2014;**5**:e02165.
Defoirdt T. Quorum-sensing systems as targets for antivirulence therapy. *Trends Microbiol* 2018;**26**:313–28.
Delcaru C, Alexandru I, Podgoreanu P *et al.* Microbial biofilms in urinary tract infections and prostatitis: etiology, pathogenicity, and combating strategies. *Pathogens* 2016;**5**:65.
Denamur E, Clermont O, Bonacorsi S *et al.* The population genetics of pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2021;**19:** 37–54.
Di Martino P, Fursy R, Bret L *et al.* Indole can act as an extracellular signal to regulate biofilm formation of *Escherichia coli* and other indole-producing bacteria. *Can J Microbiol* 2003;**49**:443–9.
Diggle SP, Gardner A, West SA *et al.* Evolutionary theory of bacterial quorum sensing: when is a signal not a signal? *Phil Trans R Soc B* 2007;**362**:1241–9.
Ding Y, Zhang D, Zhao X *et al.* Autoinducer-2-mediated quorum-sensing system resists T4 phage infection in *Escherichia coli*. *J Basic Microbiol* 2021;**61**:1113–23.
Domka J, Lee J, Wood TK. YliH (BssR) and YceP (BssS) regulate *Escherichia coli* K-12 biofilm formation by influencing cell signaling. *Appl Environ Microbiol* 2006;**72**:2449–59.
Dong Y-H, Wang L-H, Xu J-L *et al.* Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. *Nature* 2001;**411**:813–7.
Dong Y-H, Wang L-H, Zhang L-H. Quorum-quenching microbial infections: mechanisms and implications. *Phil Trans R Soc B* 2007;**362:** 1201–11.
Dyszel JL, Soares JA, Swearingen MC *et al.* *E. coli* K-12 and EHEC genes regulated by SdiA. *PLoS One* 2010;**5**:e8946.
ECDC (European Centre for Disease Prevention and Control), 2022. *STEC Infection—Annual Epidemiological Report for 2021*. https://www.ecdc.europa.eu/en/publications-data/stec-infection-annual-epidemiological-report-2021 (29 May 2023, date last accessed).
Elliott SJ, Sperandio V, Giro´n JA *et al.* The locus of enterocyte effacement (LEE)-encoded regulator controls expression of both LEE- and non-LEE-encoded virulence factors in enteropathogenic and enterhemorrhagic *Escherichia coli*. *Infect Immun* 2000;**68**:6115–26.
Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med* 2002;**113**:5–13.
Freestone PPE, Haigh RD, Williams PH *et al.* Stimulation of bacterial growth by heat-stable, norepinephrine-induced autoinducers. *FEMS Microbiol Lett* 1999;**172**:53–60.
Fuqua C, Greenberg EP. Listening in on bacteria: acyl-homoserine lactone signalling. *Nat Rev Mol Cell Biol* 2002;**3**:685–95.
Gaimster H, Cama J, Hernández-Ainsa S *et al.* The Indole pulse: a new perspective on indole signalling in *Escherichia coli*. *PLoS One* 2014;**9**:e93168.
Gomes TA, Elias WP, Scaletsky IC *et al.* Diarrheagenic *Escherichia coli*. *Microbiol* 2016;**47**:3–30.
González Barrios AF, Zuo R, Hashimoto Y *et al.*. Autoinducer 2 controls biofilm formation in *Escherichia coli* through a novel motility quorum-sensing regulator (MqsR, B3022). *J Bacteriol* 2006;**188**:305–16.
Gorelik O, Levy N, Shaulov L *et al.* *Vibrio cholerae* autoinducer-1 enhances the virulence of enteropathogenic *Escherichia coli*. *Sci Rep* 2019;**9**:4122.
Hadjifrangiskou M, Kostakioti M, Chen SL *et al.* A central metabolic circuit controlled by QseC inpathogenic *Escherichia coli*. *Mol Microbiol* 2011;**80**:1516–29.
Han X, Bai H, Liu L *et al.* The *luxS* gene functions in the pathogenesis of avian pathogenic *Escherichia coli*. *Microb Pathog* 2013;**55**:21–7.
Hegde M, Englert DL, Schrock S *et al.* Chemotaxis to the quorum-sensing signal AI-2 requires the Tsr chemoreceptor and the periplasmic LsrB AI-2-binding protein. *J Bacteriol* 2011;**193**:768–73.
Henke JM, Bassler BL. Bacterial social engagements. *Trends Cell Biol* 2004;**14**:648–56.
Hense BA, Schuster M. Core principles of bacteria autoinducer systems. *Microbiol Mol Biol Rev* 2015;**79**:153–69.
Hernandez DE, Sintim HO. Quorum sensing autoinducer-3 finally yields to structural elucidation. *ACS Cent Sci* 2020;**6**:93–96.
Herzberg M, Kaye IK, Peti W *et al.* YdgG (TqsA) controls biofilm formation in *Escherichia coli* K-12 through Autoinducer 2 transport. *J Bacteriol* 2006;**188**:587–98.
Houdt R, Aertsen A, Moons P *et al.* *N*-acyl-L-homoserine lactone signal interception by *Escherichia coli*. *FEMS Microbiol Lett* 2006;**256**:83–9.
Hsiao A, Ahmed AMS, Subramanian S *et al.* Members of the human gut microbiota involved in recovery from *Vibrio cholerae* infection. *Nature* 2014;**515**:423–6.
Hu J, Che C, Jiang W *et al.* Avian pathogenic *Escherichia coli* through Pfs affects the transcription of membrane proteins to resist β-lactam antibiotics. *Vet Sci* 2022;**9**:98.
Hughes DT, Terekhova DA, Liou L *et al.* Chemical sensing in mammalian host-bacterial commensal associations. *Proc Natl Acad Sci USA* 2010;**107**:9831–6.
Ismail AS, Valastyan JS, Bassler BL. A host-produced autoinducer-2 mimic activates bacterial quorum sensing. *Cell Host & Microbe* 2016;**19**:470–80.
Jani S, Seely AL, Peabody V GL *et al.* Chemotaxis to self-generated AI-2 promotes biofilm formation in *Escherichia coli*. *Microbiology (Reading)* 2017;**163**:1778–90.
Kanamaru K, Kanamaru K, Tatsuno I *et al.* SdiA, an *Escherichia coli* homologue of quorum-sensing regulators, controls the expression of virulence factors in enterohaemorrhagic *Escherichia coli* O157:H7. *Mol Microbiol* 2000;**38**:805–16.
Kaper JB, Nataro JP, Mobley HLT. Pathogenic *Escherichia coli*. *Nat Rev Microbiol* 2004;**2**:123–40.
Karavolos MH, Winzer K, Williams P *et al.* Pathogen espionage: multiple bacterial adrenergic sensors eavesdrop on host communication systems. *Mol Microbiol* 2013;**87**:455–65.
Kendall MM, Gruber CC, Parker CT *et al.* Ethanolamine controls expression of genes encoding components involved in interkingdom signaling and virulence in enterohemorrhagic *Escherichia coli* O157:H7. *Mbio* 2012;**3**:e00050–12.
Kendall MM, Rasko DA, Sperandio V. Global effects of the cell-to-cell signaling molecules autoinducer-2, autoinducer-3, and epinephrine in a *luxS* mutant of enterohemorrhagic *Escherichia coli*. *Infect Immun* 2007;**75**:4875–84.
Khera R, Mehdipour AR, Bolla JR *et al.* Cryo-EM structures of pentameric autoinducer-2 exporter from *Escherichia coli* reveal its transport mechanism. *EMBO J* 2022;**41**:e109990.

Kim CS, Gatsios A, Cuesta S *et al.* Characterization of autoinducer-3 structure and biosynthesis in *E. coli*. *ACS Cent Sci* 2020;**6**:197–206.
Kim J, Park W. Indole: a signaling molecule or a mere metabolic byproduct that alters bacterial physiology at a high concentration? *J Microbiol* 2015;**53**:421–8.
Kim T, Duong T, Wu C-A *et al.* Structural insights into the molecular mechanism of *Escherichia coli* SdiA, a quorum-sensing receptor. *Acta Crystallogr D Biol Crystallogr* 2014;**70**:694–707.
Kumar A, Russell RM, Hoskan MA *et al.* Indole sensing regulator (IsrR) promotes virulence gene expression in enteric pathogens. *Mbio* 2022;**13**:e0193922.
Kumar A, Sperandio V. Indole signaling at the host-microbiota-pathogen interface. *Mbio* 2019;**10**:e01031–19.
Laganenka L, Colin R, Sourjik V. Chemotaxis towards autoinducer 2 mediates autoaggregation in *Escherichia coli*. *Nat Commun* 2016;**7**:12984.
Laganenka L, Lee JW, Malfertheiner L *et al.* Chemotaxis and autoinducer-2 signalling mediate colonization and contribute to co-existence of *Escherichia coli* strains in the murine gut. *Microbiol* 2023;**8**:204–17.
Laganenka L, Sourjik V. Autoinducer 2-dependent *Escherichia coli* biofilm formation is enhanced in a dual-species coculture. *Appl Environ Microbiol* 2018;**84**:e02638–17.
Lane MC, Lockatell V, Monterosso G *et al.* Role of motility in the colonization of uropathogenic *Escherichia coli* in the urinay tract. *Infect Immun* 2005;**73**:7644–56.
Lasarre B, Federle MJ. Exploiting quorum sensing to confuse bacterial pathogens. *Microbiol Mol Biol Rev* 2013;**77**:73–111.
Laverty G, Gorman S, Gilmore B. Biomolecular mechanisms of *Pseudomonas aeruginosa* and *Escherichia coli* biofilm formation. *Pathogens* 2014;**3**:596–632.
Lee J, Jayaraman A, Wood TK. Indole is an inter-species biofilm signal mediated by SdiA. *BMC Microbiol* 2007;**7**:42.
Lee J, Maeda T, Hong SH *et al.* Reconfiguring the quorum-sensing regulator SdiA of *Escherichia coli* to control biofilm formation via indole and *N*-acylhomoserine lactones. *Appl Environ Microbiol* 2009;**75**:1703–16.
Li J, Attila C, Wang L *et al.* Quorum sensing in *Escherichia coli* is signaled by AI-2/LsrR: effects on small RNA and biofilm architecture. *J Bacteriol* 2007;**189**:6011–20.
Liu H, Fan K, Li H *et al.* Synthetic gene circuits enable *Escherichia coli* to use endogenous H2S as a signaling molecule for quorum sensing. *ACS Synth Biol* 2019;**8**:2113–20.
Liu Y, Wu L, Yan Y *et al.* Lactic acid and peroxyacetic acid inhibit biofilm of *Escherichia coli* O157:H7 formed in beef extract. *Foodborne Pathog Dis* 2021;**18**:744–51.
Lustri BC, Sperandio V, Moreira CG. Bacterial chat: intestinal metabolites and signals in host-microbiota-pathogen interactions. *Infect Immun* 2017;**85**:e00476–17. doi: 10.1128/IAI.00476-17.
Ma X, Zhang S, Xu Z *et al.* SdiA improves the acid tolerance of *E. coli* by regulating GadW and GadY expression. *Front Microbiol* 2020;**11**:1078. doi: 10.3389/fmicb.2020.01078
Machado Ribeiro TR, Cardinali Lustri B, Elias WP *et al.* QseC signaling in the outbreak O104:H4 *Escherichia coli* strain combines multiple factors during infection. *J Bacteriol* 2019;**201**:e00203–19.
Mayer C, Romero M, López-Martin M *et al.* Quorum sensing in *Acinetobacter* virulence. In: Dhiman SS (ed.), *Quorum Sensing: Microbial Rules of Life*. Washington: ACS Symposium Series, 2020;**7:** 115–37.
Mayer C, Romero M, Muras A *et al.* Aii20J, a wide-spectrum thermostable *N*-acylhomoserine lactonase from the marine bacterium *tenacibaculum* sp. 20 J, can quench AHL-mediated acid resistance in *Escherichia coli*. *Appl Microbiol Biotechnol* 2015;**99**:9523–39.
Michael B, Smith JN, Swift S *et al.* SdiA of *Salmonella enterica* is a LuxR homolog that detects mixed microbial communities. *J Bacteriol* 2001;**183**:5733–42.
Miller MB, Bassler BL. Quorum sensing in bacteria. *Annu Rev Microbiol* 2001;**55**:165–99.
Minagawa S, Inami H, Kato T *et al.* RND type efflux pump system MexABOprM of *Pseudomonas aeruginosa* selects bacterial languages, 3-oxo-acyl-homoserine lactones, for cell-to-cell communication. *BMC Microbiol* 2012;**12**:70.
Mobley HLT, Donnenberg MS, Hagan EC. . *EcoSal Plus* 2009;**3**. https://doi.org/10.1128/ecosalplus.8.6.1.3.
Moons P, Van Houdt R, Aertsen A *et al.* Role of quorum sensing and antimicrobial component production by *Serratia plymuthica* in formation of biofilms, including mixed biofilms with *Escherichia coli*. *Appl Environ Microbiol* 2006;**72**:7294–300.
Mukherjee S, Bassler BL. Bacterial quorum sensing in complex and dynamically changing environments. *Nat Rev Microbiol* 2017;**17**:371–82.
Ng W-L, Bassler BL. Bacterial quorum-sensing network architectures. *Annu Rev Genet* 2009;**43**:197–222.
Nguyen Y, Nguyen NX, Rogers JL *et al.* Structural and mechanistic roles of novel chemical ligands on the SdiA quorum-sensing transcription regulator. *Mbio* 2015;**6**: e02429–14.
Nguyen Y, Sperandio V. Enterohemorragic *E. coli* (EHEC) pathogenesis. *Front Cell Infect Microbiol* 2012;**2**:90.
Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. *Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 2014;**27**:543–74.
Nicolle LE. Catheter-related urinary tract infection. *Antimicrob Resist Infect Control* 2005;**22**:627–39.
Nicolle LE. Urinary catheter-associated infections. *Infect Dis Clin North Am* 2012;**26**:13–27.
Niu C, Robbins CM, Pittman KJ *et al.* LuxS influences *Escherichia coli* biofilm formation through autoinducer-2-dependent and autoinducer-2-independent modalities. *FEMS Microbiol Ecol* 2013;**83**:778–91.
Papenfort K, Bassler BL. Quorum sensing signal-response systems in gram-negative bacteria. *Nat Rev Microbiol* 2016;**14**:576–88.
Park H. Autoinducer-2 quorum sensing influences viability of *Escherichia coli* O157:H7 under osmotic and *In vitro* gastrointestinal stress conditions. *Front Microbiol* 2017;**8**:1077.
Park S, Wolanin PM, Yuzbashyan EA *et al.* Motion to form a quorum. *Science* 2003;**301**:188.
Platenkamp A, Mellies JL. Environment controls LEE regulation in enteropathogenic *Escheric h ia coli*. *Front Microbiol* 2018;**9**:1694.
Prescott RD, Decho AW. Flexibility and adaptability of quorum sensing in nature. *Trends Microbiol* 2020;**28**:436–44.
Rahmati S, Yang S, Davidson AL *et al.* Control of the AcrAB multidrug efflux pump by quorum-sensing regulator SdiA. *Mol Microbiol* 2002;**43**:677–85.
Rasko DA, Moreira CG, Li DR *et al.* Targeting QseC signaling and virulence for antibiotic development. *Science* 2008;**321**:1078–80.
Reading NC, Rasko DA, Torres AG *et al.* The two-component system QseEF and the membrane protein QseG link adrenergic and stress sensing to bacterial pathogenesis. *Proc Natl Acad Sci USA* 2009;**106**:5889–94.
Reisner A, Maierl M, Jorger M *et al.* Type 1 fimbriae contribute to catheter-associated urinary tract infections caused by *Escherichia coli*. *J Bacteriol* 2014;**196**:931–9.
Riley LW. Distinguishing pathovars from nonpathovars: *E scherichia coli*. *Microbiol Spectr* 2020;**8**. https://doi.org/10.1128/microbiolspec.AME-0014-2020.

Rutherford ST, Bassler BL. Bacterial quorum sensing: its role in virulence and possibilities for its control. *Cold Spring Harb Perspect Med* 2012;**2**:a012427.

Sabag-Daigle A, Dyszel JL, Gonzalez JF *et al.* Identification of *sdiA*-regulated genes in a mouse commensal strain of *E n terobacter cloacae*. *Front Cell Infect Microbiol* 2015;**5**:47.

Sabag-Daigle A, Soares JA, Smith JN *et al.* The acyl homoserine lactone receptor, SdiA, of *Escherichia coli* and *Salmonella enterica* serovar Typhimurium does not respond to indole. *Appl Environ Microbiol* 2012;**78**:5424–31.

Sarkar S, Vagenas D, Schembri MA *et al.* Biofilm formation by multidrug resistant *Escherichia coli* ST131 is dependent on type 1 fimbriae and assay conditions. *Pathogens and Disease* 2016;**74**:ftw013.

Sharma G, Sharma S, Sharma P *et al. Escherichia coli* biofilm: development and therapeutic strategies. *J Appl Microbiol* 2016;**121**:309–19.

Sharma VK, Bearson SMD, Bearson BL. Evaluation of the effects of *sdiA*, a *luxR* homologue, on adherence and motility of *Escherichia coli* O157:H7. *Microbiology (Reading)* 2010;**156**:1303–12.

Sharma VK, Bearson SMD. Evaluation of the impact of quorum sensing transcriptional regulator SdiA on long-term persistence and fecal shedding of *Escherichia coli* O157:H7 in weaned calves. *Microb Pathog* 2013;**57**:21–6.

Sheng H, Nguyen YN, Hovde CJ *et al.* SdiA aids enterohemorrhagic *Escherichia coli* carriage by cattle fed a forage or grain diet. *Infect Immun* 2013;**81**:3472–8.

Sircili MP, Walters M, Trabulsi LR *et al.* Modulation of enteropathogenic *Escherichia coli* virulence by quorum sensing. *Infect Immun* 2004;**72**:2329–37.

Sitkinov DM, Schineller JB, Baldwin TO. Control of cell division in *Escherichia coli*: regulation of transcription of *ftsQA* involves both *rpoS* and SdiA-mediated autoinduction. *Proc Natl Acad Sci USA* 1996;**93**:336–41.

Smith JL, Fratamico PM, Yan X. Eavesdropping by bacteria: the role of SdiA in *Escherichia coli* and *Salmonella enterica* serovar typhimurium quorum sensing. *Foodborne Pathog Dis* 2011;**8**:169–78.

Soares JA, Ahmer BM. Detection of acyl-homoserine lactones by *Escherichia* and *Salmonella*. *Curr Opin Microbiol* 2011;**14**:188–93.

Soni KA, Lu L, Jesudhasan PR *et al.* Influence of autoinducer-2 (AI-2) and beef sample extracts on *E. coli* O157:H7 survival and gene expression of virulence genes *yadK* and *hhA*. *J Food Science* 2008;**73**:M135–9.

Sperandio V, Li CC, Kaper JB. Quorum-sensing *Escherichia coli* regulator A: a regulator of the LysR family involved in the regulation of the locus of enterocyte effacement pathogenicity island in enterohemorrhagic *E. coli*. *Infect Immun* 2002a;**70**:3085–93.

Sperandio V, Mellies JL, Nguyen W *et al.* Quorum sensing controls expression of the type III secretion gene transcription and protein secretion in enterohemorrhagic and enteropathogenic *Escherichia coli*. *Proc Natl Acad Sci USA* 1999; **96**:15196–201.

Sperandio V, Torres AG, Giro´n JA *et al.* Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. *J Bacteriol* 2001;**183**:5187–97.

Sperandio V, Torres AG, Jarvis B *et al.* Bacteria-host communication: the language of hormones. *Proc Natl Acad Sci USA* 2003;**100**:8951–6.

Sperandio V, Torres AG, Kaper JB. Quorum sensing *Escherichia coli* regulators B and C (QseBC): a novel two-component regulatory system involved in the regulation of flagella and motility by quorum sensing in *E. coli*. *Mol Microbiol* 2002b;**43**:809–21.

Sperandio V. SdiA sensing of acyl-homoserine lactones by enterohemorrhagic *E. coli* (EHEC) serotype O157:H7 in the bovine rumen. *Gut Microbes* 2010;**1**:432–5.

Spurbeck RR, Alteri CJ, Himpsl SD *et al.* The multifunctional protein YdiV represses P fimbria-mediated adherence in uropathogenic *Escherichia coli*. *J Bacteriol* 2013;**195**:3156–64.

Stephens K, Zargar A, Emamian M *et al.* Engineering *Escherichia coli* for enhanced sensitivity to the autoinducer-2 quorum sensing signal. *Biotechnol Prog* 2019;**35**:e2881.

Striednig B, Hilbi H. Bacterial quorum sensing and phenotypic heterogeneity: how the collective shapes the individual. *Trends Microbiol* 2022;**30**:379–89.

Styles MJ, Early SA, Tucholski T *et al.* Chemical control of quorum sensing in *E. coli*: identification of small molecule modulators of SdiA and mechanistic characterization of a covalent inhibitor. *ACS Infect Dis* 2020;**6**:3092–103.

Suzuki K, Wang X, Weilbacher T *et al. J Bacteriol* 2002;**184**:5130–40.

Swearingen MC, Sabag-Daigle A, Ahmer BMM. Are there acyl-homoserine lactones within mammalian intestines? *J Bacteriol* 2013;**195**:173–9.

Tavio MM, Aquili VD, Poveda JB *et al.* Quorum-sensing regulator *sdiA* and *marA* overexpression is involved in in vitro-selected multidrug resistance of *Escherichia coli*. *J Antimicrob Chemother* 2010;**65**:1178–86.

Thompson JA, Oliveira RA, Djukovic A *et al.* Manipulation of the quorum sensing signal AI-2 affects the antibiotic-treated gut microbiota. *Cell Rep* 2015;**10**:1861–71.

Vinothkannan R, Muthu Tamizh M, David Raj C *et al.* Fructose furoic acid ester: an effective quorum sensing inhibitor against uropathogenic *Escherichia coli*. *Bioorg Chem* 2018;**79**:310–8.

Walters M, Sircili MP, Sperandio V. AI-3 synthesis is not dependent on *luxS* in *Escherichia coli*. *J Bacteriol* 2006;**188**:5668–81.

Walters M, Sperandio V. Autoinducer 3 and epinephrine signaling in the kinetics of locus of enterocyte effacement gene expression in enterohemorrhagic *Escherichia coli*. *Infect Immun* 2006a;**74**:5445–55.

Walters M, Sperandio V. Quorum sensing in *Escherichia coli* and *Salmonella*. *Int J Med Microbiol* 2006b;**296**:125–31.

Wang D, Ding X, Rather PN. Indole can act as an extracellular signal in *Escherichia coli*. *J Bacteriol* 2001;**183**:4210–6.

Wang H, Shang F, Shen J *et al.* LsrR, the effector of AI-2 quorum sensing, is vital for the H_2O_2 response in mammary pathogenic *Escherichia coli*. *Vet Res* 2021;**52**:127.

Wang L, Li J, March JC *et al. luxS*-dependent gene regulation in *Escherichia coli* K-12 revealed by genomic expression profiling. *J Bacteriol* 2005;**187**:8350–60.

Wang XD, De Boer PA, Rothfield LI. A factor that positively regulates cell division by activating transcription of the major cluster of essential cell division genes of *Escherichia coli*. *EMBO J* 1991;**10**:3363–72.

Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol* 2005;**21**:319–46.

Watve S, Barrasso K, Jung SA *et al.* Parallel quorum-sensing system in *Vibrio cholerae* prevents signal interference inside the host. *PLoS Pathog* 2020;**16**:e1008313.

Weatherspoon-Griffin N, Yang D, Kong W *et al.* The CpxR/CpxA two-component regulatory system up-regulates the multidrug resistance cascade to facilitate *Escherichia coli* resistance to a model antimicrobial peptide. *J Biol Chem* 2014;**289**:32571–82.

Wei Y, Lee J-M, Smulski DR *et al.* Global impact of *sdiA* amplification revealed by comprehensive gene expression profiling of *Esch e richia coli*. *J Bacteriol* 2001;**183**:2265–72.

Whitehead NA, Barnard AML, Slater H *et al.* Quorum-sensing in gram-negative bacteria. *FEMS Microbiol Rev* 2001;**25**:365–404.

Whiteley M, Diggle SP, Greenberg EP. Bacterial quorum sensing: the progress and promise of an emerging research area. *Nature* 2017;**551**:313–20.

WHO (World Health Organization), WHO estimates of the global burden of foodborne diseases. 2015. https://apps.who.int/iris/bitstream/handle/10665/199350/9789241565165_eng.pdf?sequence=1 (29 May 2023, date last accessed).

WHO (World Health Organization). 2017. WHO publishes list of bacteria for which new antibiotics are urgently needed. https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed (3 May 2023, date last accessed).

Williams P, Winzer K, Chan WC *et al.* Look who's talking: communication and quorum sensing in the bacterial world. *Phil Trans R Soc B* 2007;**362**:1119–34.

Winzer K. Bacteria cell-to-cell communication: sorry, can't talk now—gone to lunch!. *Curr Opin Microbiol* 2002;**5**:216–22.

Witsø IL, Valen Rukke H, Benneche T *et al.* Thiophenone attenuates enteropathogenic *Escherichia coli* O103:H2 virulence by interfering with AI-2 signaling. *PLoS One* 2016;**11**:e0157334.

Xavier KB, Bassler BL. Regulation of uptake and processing of the quorum-sensing autoinducer AI-2 in *Escherichia coli*. *J Bacteriol* 2005;**187**:238–48.

Xue T, Yu L, Shang F *et al.* Short communication: the role of autoinducer 2 (AI-2) on antibiotic resistance regulation in an *Escherichia coli* strain isolated from a dairy cow with mastitis. *J Dairy Sci* 2016;**99**:4693–8.

Yamamoto K, Yata K, Fujita N *et al.* Novel mode of transcription regulation by SdiA, an *Escherichia coli* homologue of the quorum-sensing regulator. *Mol Microbiol* 2001;**41**:1187–98.

Yang K, Meng J, Huang Y-C *et al.* The role of the QseC quorum-sensing sensor kinase in epinephrine-enhanced motility and biofilm formation by *Escherichia coli*. *Cell Biochem Biophys* 2014;**70**:391–8.

Yang S-C, Lin C-H, Aljuffali IA *et al.* Current pathogenic *Escherichia coli* foodborne outbreak cases and therapy development. *Arch Microbiol* 2017;**199**:811–25.

Yang X, Chen H, Zheng Y *et al.* Disease burden and long-term trends of urinary tract infections: a worldwide report. *Front Public Health* 2022;**10**:888205.

Yang Y, Zhang X, Zhang B *et al.* Quorum sensing-1 signaling of N-hexanoyl-L-homoserine lactone contributes to virulence in avian pathogenic *Escherichia coli*. *Arch Microbiol* 2021;**203**:6079–89.

Yang Y, Zhou M, Hardwidge PR *et al.* Isolation and characterization of *N*-acyl homoserine lactone-producing bacteria from cattle rumen and swine intestines. *Front Cell Infect Microbiol* 2018;**8**:155.

Yates EA, Philipp B, Buckley C *et al.* *N*-acylhomoserine lactones undergo lactonolysis in a pH-, temperature-, and acyl chain length-dependent manner during growth of *Yersinia pseudotuberculosis* and *Pseudomonas aeruginosa*. *Infect Immun* 2002;**70**:5635–46.

Yu L, Li W, Zhang M *et al.* Imidazole decreases the ampicillin resistance of an *Escherichia coli* strain isolated from a cow with mastitis by inhibiting the function of autoinducer 2. *J Dairy Sci* 2018;**101**:3356–62.

Zargar A, Quan DN, Carter KK *et al.* Bacterial secretions of nonpathogenic *Escherichia coli* elicit inflammatory pathways: a closer investigation of interkingdom signaling. *Mbio* 2015;**6**:e00025.

Zarkan A, Liu J, Matuszewska M *et al.* Local and universal action: the paradoxes of indole signalling in bacteria. *Trends Microbiol* 2020;**28**:566–77.

Zhang L, Li S, Liu X *et al.* Sensing of autoinducer-2 by functionally distinct receptors in prokaryotes. *Nat Commun* 2020;**11**:5371.

Zhou X, Meng X, Sun B. An EAL domain protein and cyclic AMP contribute to the interaction between the two quorum sensing systems in *Escherichia coli*. *Cell Res* 2008;**18**:937–48.

Zuo J, Yin H, Hu J *et al.* Lsr operon is associated with AI-2 transfer and pathogenicity in avian pathogenic *Escherichia coli*. *Vet Res* 2019;**50**:109.

Received 2 September 2022; **revised** 2 June 2023; **accepted** 12 June 2023