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Characterization of AI-2/LuxS quorum sensing system in antibiotic resistance, pathogenicity of non carbapenemase-producing carbapenem-resistant *Escherichia coli*

Xiaohong Xu[1,2†], Meili Cai[1†], Huishan Lai[3], Siyan Lian[1], Liping Hu[4*] and Yingping Cao[1,2*]

Abstract

Background As a quorum sensing system, LuxS/AI-2 is closely associated with bacterial growth, biofilm formation, and virulence. As yet, it is not known how the *luxS* is associated with a diverse array of physiological activities in non- carbapenemase producing carbapenem resistant *Escherichia coli* (non-CP-CREC). The purpose of this study is to explore the characterization of AI-2/LuxS quorum sensing system in antibiotic resistance, pathogenicity of non-CP-CREC.

Methods A total of five non-CP-CREC isolates that did not have *ompC* and *ompF* deletions were collected from various clinical samples from January 2021 to December 2023. RT-qPCR was used to detect genes expression of *luxS*, *acrA*, *acrB*, *tolC*, *mdtB*, *mdtC*, *mdtE*, *mdtF*, *ompA*, *ompX*, IL-8, IL-6, and TNF-α. Homologous recombination was used to create the *luxS* knockout strain. Transcriptome sequencing was utilized to analyze gene expression changes before and after the *luxS* knockout. Biofilm formation was detected using crystal violet staining. Antimicrobial susceptibility test was used to determine drug resistance. Bacterial growth curves were used to detect the influence of the *luxS* on bacterial growth. A cell infection assay was used to detect the impact of the *luxS* on bacterial adhesion and the inflammatory response it induces.

Results Our results indicated that the expression of the *luxS* was significantly elevated in non-CP-CREC strains compared to the carbapenem antibiotics sensitive *E. coli* (CSEC), with CREC229 exhibiting the most pronounced difference. Consequently, CREC229 was chosen for the development of the *luxS* knockout strain (CREC229Δ*luxS*). The deletion of the *luxS* did not impact the growth of non-CP-CREC. RNA sequencing analysis revealed that 82 genes were differentially expressed, with notable alterations observed in genes associated with biofilm formation regulation and outer membrane proteins in the Δ*luxS* strain. Our transcriptomic results show that the expression of *bssS* associated with biofilm formation is significantly reduced in the Δ*luxS* strain, which in turn reduces i

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ts capacity for biofilm formation. In addition, the *luxS* deletion increased the expression of adhesion-related genes, such as *ompA* and *ompX*, enhanced HCT-8 adherence to CREC229, and promoted the secretion of the inflammatory cytokine IL-6. In terms of bacterial resistance, the deletion of *luxS* increased the sensitivity of non-CP-CRECs to aminoglycoside antibiotics.

Conclusions LuxS/AI-2 quorum sensing systems can alter pathogenicity and resistance in several ways.

Keywords LuxS/AI-2, Quorum sensing systems, Non-CP-CREC, Biofilm formation, IL-6

Introduction

Escherichia coli (*E. coli*) is an opportunistic pathogen that can cause a variety of serious infections, including meningitis, hemorrhagic colitis, pneumonia, urinary tract infections, and hemolytic uremic syndrome. *E. coli* is regarded as one of the most troublesome human pathogens in healthcare systems and animal industries worldwide [1]. In order to develop new antipathogenic strategies, it is essential to understand their virulence control mechanisms in depth. Quorum sensing (QS), a communication system dependent on cell density, regulates various bacterial functions such as virulence factors and drug resistance genes [2].

QS is a communication phenomenon among bacterial cells that is triggered by signals exchanged between them, influencing their behavior to adapt to the external environment. The QS system was first discovered and described in *Vibrio fischeri* (*V. fischeri*) and *Vibrio harveyi* (*V. harveyi*), which use autoinducer molecules to regulate genes expression in response to increased cell density [3]. There is evidence that QS plays a role in regulating bacterial cell density, growth characteristics, and various physiological and biochemical functions, such as bioluminescence, sporulation, motility, conjugation, antibiotic production, biofilm formation, and the secretion of virulence factors for infection or colonization [4]. There are three QS systems described for *E. coli*: a LuxR homologue (the SdiA regulator), a LuxS synthetase/autoinducer-2 (AI-2), and an autoinducer-3 (AI-3) [5].

In addition to mediating interspecies communication, AI-2 signaling molecules are thought to play an important role in the pathogenicity of various *E. coli* species. Increased adherence of pathogenic *E. coli* to epithelial cells has been reported to be induced by AI-2 signaling [6]. Additionally, AI-2 increases the expression of several genes associated with virulence and influences the survival of *E. coli* [7]. A study reported that AI-2 initially increased IL-8 expression and then significantly decreased it, suggesting an immune response modulation in nonpathogenic *E. coli* [8].

Since LuxS is involved in a very important regulatory system, some *luxS* gene mutant.

strains were generated to study the function of this gene in *Edwardsiella piscicida*, *C. jejuni*, and *Aggregatibacter actinomycetemcomitans* [9]. As far as we know, the AI-2/LuxS system in no-CP-CREC has not been available to explore its role in a wide range of physiological functions. Consequently, this research sought to clarify the impact of the QS-related *luxS* gene on no-CP-CREC's pathogenicity and its response to antimicrobials.

Methods

Bacterial strains and cultivation conditions

Our research group screened five strains of non-carbapenemase producing carbapenem resistant *E. coli* (non-CP-CREC) without porin missing at an early stage of the research from clinical isolates [10]. Bruker Biotyper™ (Bruker Daltonics Inc., Billerica, Massachusetts) was used to identify the bacterial species from the collected isolates by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS). All strains in this study were cultured on Columbia blood plates (Thermo Fisher, USA), or in MH agar plates and Luria–Bertani (LB) broth (Thermo Fisher, USA) at 37 °C.

Construction of isogenic △*luxS* mutants of non-CP-CREC

To construct a *luxS* knockout mutant of non-CP-CREC (△ *luxS*), a DNA fragment containing a upstream sequence of *luxS* was amplified using the primers *luxS*-up-F and *luxS*-up-R, a DNA fragment containing a downstream sequence of *luxS* was amplified using the primers *luxS*-down-F and *luxS*-down-R, and a DNA fragment containing CAT, which confers chloramphenicol resistance, was amplified using primers *luxS*-Cm-F and *luxS*-Cm-R. We ligated the *luxS* upstream and downstream sequences, as well as a chloramphenicol resistance DNA fragment, into pCVD442 (gift from Professor Lishan of Hubei University of Medicine) using the Clon Express MultiS One Step Cloning Kit (Vazyme, Nanjing, China), resulting in pCVD442-*luxS* KO, which was further transformed into *E. coli* DH5α. A colony PCR and Sanger sequence were then used to confirm the plasmid sequencing. After purification, pCVD442-*luxS* KO was electroporated into non-CP-CREC, and the bacteria were cultivated on chloramphenicol-containing

agar plates. By colony PCR and Sanger sequencing, *luxS* knockout mutants were further confirmed.

Cell lines, cultivation and co-culture of HCT-8 cells and bacteria

We cultured HCT-8 colorectal cancer cells (derived from human caecal cancer cells, purchased from Shanghai Fusheng Industrial Co., Ltd.) in DMEM/F12 (HyClone Laboratories Inc., Logan, UT, United States), with supplementation of 10% FBS (PANS, Aidenbach, Bayern, Germany) at 37℃ in a 5% CO_2 humidified atmosphere. As part of the non-CP-CREC infection assays, HCT-8 cells were grown in 6-well plates (NUNC, Thermo, DE, United States) containing 10% FBS in DMEM/F12 medium until confluence reached 75%. Supernatants were removed, and the cells were washed twice with phosphate-buffered saline (PBS) before being cultured in FBS-free DMEM/F12 for four hours. We first cultivated non-CP-CREC strains on agar plates, then resuspended the bacteria in broth at an initial OD_{600} of 0.1, followed by 24 h of culture. At a multiplicity of infection (MOI) of 100, bacterial cells were pelleted and washed twice with DMEM/F12 medium, resuspended in DMEM/F12 medium, and added to the HCT-8 cell culture.

Determination of bacterial growth rates

We examined the effects of LuxS on the growth of non-CP-CREC by growing it on Columbia agar plates for 16 h, collecting bacterial cells, and re-suspending them in LB broth with an initial $OD_{600}=0.01$. The bacteria were then cultured at 37 °C with agitation and their OD_{600} values were measured every 2 h. Each experiment was repeated at least three times.

RNA sequencing and data analysis

As part of the transcriptomic study, non-CP-CREC and Δ*luxS* cells were cultured in LB broth for 6 h until they reached the exponential phase in a shaker at 120 rpm and 37 °C. Trizol Reagent (Invitrogen Life Technologies) was used to isolate total RNA, and a NanoDrop spectrophotometer (Thermo Scientific) and Bioanalyzer 2100 (Agilent) were used for quality and integrity determinations. Total RNA was then processed using the Zymo-Seq RiboFree Total RNA Library Kit. To synthesize the first strand of cDNA, random oligonucleotides and Super Script III were used. DNA Polymerase I and RNase H were then used to synthesize the second strand cDNA. We converted the remaining overhangs into blunt ends by utilizing exonuclease and polymerase activities and removed the enzymes. In preparation for hybridization, Illumina PE adapter oligonucleotides were ligated to the 3′ ends of DNA fragments following adenylation. By using Beckman Coulter's AMPure XP system (Beverly Hills, California, USA), the library fragments were selected for 400–500 bp cDNA fragments. By using the Illumina PCR Primer Cocktail in a 15-cycle PCR reaction, DNA fragments with ligated adaptor molecules were selectively enriched. The purified products were analyzed using the Agilent Bioanalyzer 2100 system (Agilent) and quantified using the Agilent high-sensitivity DNA assay. The sequencing library was then sequenced by Shanghai Personal Biotechnology Co., Ltd. on its Nova Seq 6000 platform (Illumina). We designated genes differentially expressed which had an adjusted *P*-value of 0.05 as differentially expressed. The data were deposited in the NCBI database.

RNA isolation and quantitative RT-PCR

In order to prepare bacterial RNA samples, bacteria were grown in LB for 6 h, followed by extraction using an RNeasy Mini Kit (QIAGEN, Valencia, CA, United States). Following infection with CREC229or CREC229Δ*luxS*, HCT-8 cells or HCT-8 cells infected with CREC were collected using TRI Zol reagent (Life Technologies, Carlsbad, CA, United States) after co-culturing with bacteria in a 5% CO_2 humidified atmosphere at 37 °C, according to the manufacturer's instructions. The concentration and purity of RNA were then determined using spectrophotometry (NanoDrop One, Thermo Fisher Scientific, DE, United States). With the HiScript II Q RT SuperMix for qPCR (+gDNA wiper) kit (Vazyme, Nanjing, China), cDNA was prepared through reverse transcription from 1 μg of total RNA. In this study, the SYBR qPCR Master Mix kit was used (Vazyme, Nanjing, China). Table S1 shows specific primers designed with Primer 5.0 for each indicated gene. Experiments were repeated in triplicate for each condition, using *rrsG* as an endogenous control.

Adhesion and invasion tests

HCT-8 cells were seeded in 6-well plates at a density of $3.5\times10^{\wedge5}$cells/well with 2 ml of DMEM/F12 to form a confluent monolayer and then infected with non-CP-CREC at a MOI of 100. After 4 h of infection, the HCT-8 cells were washed three times with PBS to remove any unattached bacteria. To determine the number of adherent non-CP-CREC, the HCT-8 cells were lysed using 0.1% saponin for 20 min at room temperature. After a serial dilution, 50 μl of each diluted cell lysate containing bacteria was placed on a Columbia sheep blood agar plate. Subsequently, the bacteria were incubated under 5% CO_2 conditions for 18 h, and colonies were counted.

For the invasion experiment, complete medium with DMEM was added to 1 ml per well, then amikacin with a final concentration of 200 μg/ml was added and incubated at 37 °C for 1 h to kill extracellular bacteria, and

then gently washed with sterile PBS solution for 3 times. The remaining steps are the same as the adhesion test.

In vitro biofilm formation

Biofilm formation was quantified by the crystal violet assay. Cultures were grown to an optical density (OD_{600}) of approximately 0.1, diluted 1:1000, and added to the sterilized 96-well plate at a rate of 200 µl per well. After 24 h at 37 °C, each strain of bacteria was tested in triplicate. We discarded the culture medium and washed the wells three times with sterile PBS to eliminate loosely adhered bacteria. After the remaining bacteria that had attached to the wells were fixed with methanol for 30 min, each well was stained for 10 min at room temperature with a 1% crystal violet solution. The wells were rinsed multiple times with distilled water to remove excess crystal violet. Crystal violet associated with biofilm was solubilized in 95% (v/v) ethanol, and its optical density at 595 nm was measured.

Antibiotic susceptibility testing

According to the Clinical and Laboratory Standards Institute (CLSI) guidelines, we used the microbroth dilution method to determine the minimum inhibitory concentrations (MICs) of gentamicin, kanamycin, tobramycin, imipenem, meropenem, fosfomycin, levofloxacin, ciprofloxacin, tetracycline, and clarithromycin. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control standards.

Results

LuxS was highly expressed in non-CP-CREC strains

To investigate the effect of the QS system on the development of resistance and virulence in non-CP-CREC strains, our research group screened five out of 48 CREC strains that did not have *ompC* and *ompF* deletions at the early stages. Following that, RT-qPCR was used to detect the expression of the non-CP-CREC *luxS* gene in 5 strains. The results showed that the non-CP-CREC *luxS* gene mRNA was highly expressed compared to the control group, with CREC229 showing the most significant difference (Fig. 1A). Therefore, CREC229 was selected for the construction of the *luxS* knockout strain (CREC229Δ*luxS*).

LuxS deletion did not affect the growth of non-CP-CREC

Before analyzing the effect of *luxS* on non-CP-CREC virulence and antimicrobial susceptibility, we first assessed whether it affected bacterial growth. The results showed that there was no difference between CREC229 and CREC229Δ*luxS* in the timing of entering the logarithmic phase and the stable phase, indicating that *luxS* did not affect the proliferation ability of CREC229 (Fig. 1B).

Transcriptomic profiling of gene expression in CREC229 and Δ*luxS* strains

Additionally, we performed RNAseq analysis to investigate which genes were differentially expressed between CREC229 and a Δ*luxS* strain. In the study, 82 genes were differentially expressed with a |fold change|> 1.5, including 59 genes that were upregulated and 23 genes that were downregulated in Δ*luxS* ($P < 0.05$). These genes are listed in Table 1 and Fig. 2. We found that genes involved in biofilm formation regulation and outer membrane proteins were significantly changed in Δ*luxS*. Since biofilm formation and porin play a significant role in antibacterial susceptibility and virulence, we will explore the effects of the *luxS* in more detail.

Gene Ontology (GO) describes the properties of genes by assigning them to biological processes, cellular

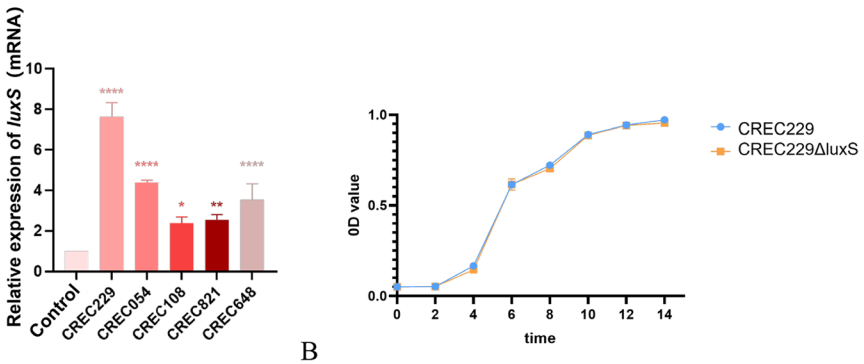

Fig. 1 mRNA level of *luxS* expressed in non-CP-CREC strains and the growth curve of CREC229. **A**Values represent the relative mRNA level of luxS normalized to control group: carbapenem antibiotics sensitive E. coli (CSEC). *** $P < 0.001$, ** $P < 0.01$, * $P < 0.05$. **B** The growth rates of CREC229 and luxS mutant strains were similar by measuring the optical density (OD 600) every 2 h over a period of 14 h. Data shown represent average means from three independent experiments, and standard deviations are also indicated

Table 1 Differentially expressed genes identified by RNA-seq

Gene expression	Gene name	Gene function annotations	fold change(CREC229△*luxS*/CREC229)
Up-regulated	*dnaK*	Molecular chaperone	1.778103764
	gcvR	Glycine cleavage system transcriptional repressor	1.939413775
	groL	Chaperonin	1.858460794
	htpG	Molecular chaperone	1.638489828
	gapA	Glyceraldehyde-3-phosphate dehydrogenase	1.839035122
	ybeL	Zinc ribbon-containing protein	1.643474072
	yfbU	YfbU family protein	1.716433179
	cfa	Cyclopropane fatty acyl phospholipid synthase	1.51792448
	cysK	Cysteine synthase A	2.499808058
	sbmC	DNA gyrase inhibitor	1.503377994
	dapA	4-hydroxy-tetrahydrodipicolinate synthase	1.516158267
	ydcL	DUF3313 domain-containing protein	1.577463296
	yggL	YggL family protein	1.560467747
	ompX	Outer membrane protein	1.576036977
	eno	Phosphopyruvate hydratase	1.626916188
	folE	GTP cyclohydrolase I	1.561396293
	rraB	Ribonuclease E inhibitor	1.521865481
	yhiM	DUF2776 domain-containing protein	1.616963222
	hslU	HslU–HslV peptidase ATPase subunit	1.790862953
	adk	Adenylate kinase	1.504177685
	cadC	Lysine decarboxylation/transport transcriptional activator	1.561328827
	pykF	Pyruvate kinase	1.658778447
	ghoS	Type V toxin-antitoxin system endoribonuclease antitoxin	1.598995385
	Predicted gene	Hypothetical protein	1.668042979
	msyB	Acidic protein	1.64274808
	sixA	Phosphohistidine phosphatase	1.583498234
	yajO	1-deoxyxylulose-5-phosphate synthase	1.727643135
	yceD	23S rRNA accumulation protein	2.187966073
	artJ	Arginine ABC transporter substrate-binding protein	1.580250377
	ydgA	YdgA family protein	1.690939272
	ahpC	Alkyl hydroperoxide reductase subunit C	2.097823882
	cbpA	Curved DNA-binding protein	1.514251801
	nusB	Transcription antitermination factor	1.51748717
	rpsA	30S ribosomal protein S1	1.518159664
	Predicted gene	Hypothetical protein	1.549160528
	yjdN	VOC family metalloprotein	1.616287367
	groS	Co-chaperone	1.5956544
	tolC	Outer membrane channel protein	1.699549624

Table 1 (continued)

Gene expression	Gene name	Gene function annotations	fold change(CREC229△*luxS*/CREC229)
	hdhA	7-alpha-hydroxysteroid dehydrogenase	1.537081732
	gpmA	2,3-diphosphoglycerate-dependent phosphoglycerate mutase	1.693070404
	gndA	NADP-dependent phosphogluconate dehydrogenase	1.778713394
	yiaF	DUF3053 domain-containing protein	1.540483049
	proQ	RNA chaperone	1.534832015
	tuf	Elongation factor Tu	1.921636321
	msrA	Peptide-methionine (S)-S-oxide reductase	1.822715993
	tpiA	Triose-phosphate isomerase	1.826441075
	tuf	Elongation factor Tu	2.251483796
	yrdA	Gamma carbonic anhydrase family protein	1.889635253
	ppiB	Peptidylprolyl isomerase B	2.145395399
	Predicted gene	SDR family oxidoreductase	1.735258491
	rbsD	D-ribose pyranase	1.528434932
	fabI	Enoyl-ACP reductase FabI	2.086920616
	otsB	Trehalose-phosphatase	1.690611824
	yebY	YebY family protein	1.513017051
	nadE	Ammonia-dependent NAD(+) synthetase	1.596716128
	btuE	Bifunctional thioredoxin/glutathione peroxidase	1.718766122
	skp	Molecular chaperone	1.907532761
	yhbO	Protein/nucleic acid deglycase	2.217366509
	ppa	Inorganic diphosphatase	1.613147841
Down regulated	*luxS*	S-ribosylhomocysteine lyase	0
	yebO	YebO family protein	0.452054644
	bssS	Biofilm formation regulator	0.404172193
	ypfN	YpfN family protein	0.165855441
	alaE	L-alanine exporter	0.44819015
	yraN	YraN family protein	0.533376313
	hcaC	Bifunctional 3-phenylpropionate/cinnamic acid dioxygenase ferredoxin subunit	0.319994141
	Predicted gene	Hypothetical protein	0.33296316
	nrfF	Heme lyase NrfEFG subunit	0.522565817
	ybdF	MmcQ/YjbR family DNA-binding protein	0.494769692
	Predicted gene	Phage baseplate assembly protein V	0.609886333
	frdD	Fumarate reductase subunit	0.530827339
	fimI	Type 1 fimbrial protein	0.628853892
	yjfM	DUF1190 domain-containing protein	0.592054039
	tomB	Hha toxicity modulator TomB	0.630415317
	yobH	YobH family protein	0.045701361
	yjfN	DUF1471 family protease activator	0.261184319
	pspB	DUF1471 family protease activator	0.266646538

Table 1 (continued)

Gene expression	Gene name	Gene function annotations	fold change(CREC229△*luxS*/CREC229)
	atpI	F0F1 ATP synthase subunit I	0.54899665
	rpmB	50S ribosomal protein L28	0.256976622
	Predicted gene	DNA methylase	0.583309846
	phnN	Ribose 1,5-bisphosphokinase	0.586564218
	sdiA	Transcriptional regulator SdiA	0.601258628

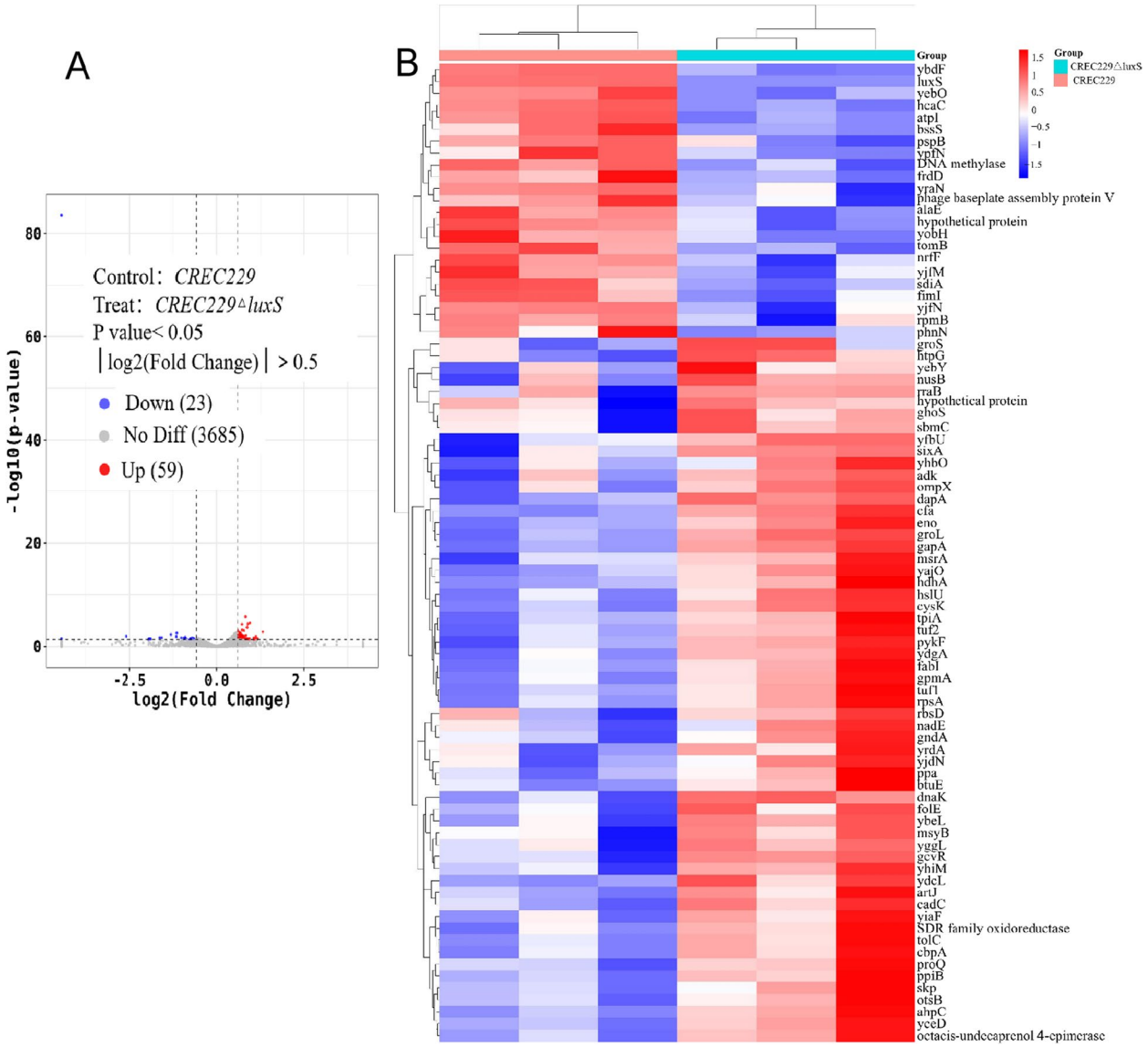

Fig. 2 Differentially expressed genes between CREC229 and Δ*luxS* by RNA sequencing. **A** Volcano plot of gene expression in CREC229 and Δ*luxS*. The Y-axis represents-log10 (*P*-value), and X-axis represents log2 (fold change). Positive values represent genes upregulated in Δ*luxS*, while negative values represent genes downregulated in Δ*luxS*. The horizontal dashed line represents $P = 0.05$. Red dots represent those genes with expression in Δ*luxS* higher than CREC229, with Log2 (fold change) > 0.5 and $P < 0.05$. Blue dots represent genes with lower expression in Δ*luxS* compared with CREC229, with Log2 (fold change) < –0.5 and $P < 0.05$. **B** Hierarchical cluster analysis of genes expression in CREC229 and Δ*luxS* strains

components, and molecular functions. By using the blast2GO software, the differentially expressed genes (DEGs) were grouped into molecular functions, biological processes, and cellular components. The vast majority of differential genes are associated with metabolic, catalytic activity and cellular processes (Fig. S1).

LuxS deletion increased the sensitivity of CREC to aminoglycoside antibiotics

Next, we further investigated whether the *luxS* would influence the sensitivity of CREC229 to commonly used antimicrobials. The microbroth dilution method was used to determine the susceptibility of CREC229 and CREC229Δ*luxS* to 13 antibiotics: imipenem, meropenem, gentamicin, kanamycin, fosfomycin, tobramycin, streptomycin, levofloxacin, ciprofloxacin, rifampicin, tetracycline, clarithromycin, and apramycin. *LuxS* mutations leading to increased sensitivity to gentamicin, kanamycin, and tobramycin are shown. No difference in susceptibility to imipenem, meropenem, fosfomycin, apramycin, levofloxacin, ciprofloxacin, rifampicin, tetracycline, and clarithromycin between CREC229 and CREC229Δ*luxS* was observed (Table 2). A RT-qPCR assay was used to detect expression differences among common efflux pump genes, but all other genes except TolC had no significant differences in expression (Fig. 3).

Table 2 Difference in drug sensitivity between CREC229 and CREC229Δ*luxS*

Antimicrobial drug	CREC229 (MIC:μg /ml)	CREC229Δ*luxS* (MIC:μg /ml)
MEM	8	8
IPM	4	4
GEN	> 256	16
Kana	256	16
FOS	32	16
TOB	128	16
APR	8	8
SM	128	128
LVX	128	128
CIP	128	128
RFP	16	16
TET	256	256
CLR	128	128

MEM Meropenem, *IPM* Imipenem, *GEN* Gentamicin, *Kana* kanamycin, *FOS* Fosfomycin, *TOB* Tobramycin, *APR* Amprimycin, *SM* Streptomycin, *CIP* Ciprofloxacin, *LEV* Levofloxacin, *RFP* Rifampicin, *TET* Tetracycline, *CLR* Clarithromycin

LuxS deletion enhanced the biofilm formation ability of CREC229

Our transcriptomic study revealed that *BssS* related to biofilm formation were differentially expressed in the Δ*luxS* strain. This suggests that the *luxS* might play an important role in biofilm formation. We quantified biofilm formation using a microtiter plate assay to compare CREC229 and the Δ*luxS* mutant strains for differences in biofilm formation. Compared to CREC229, the biofilm formation ability of CREC229Δ*luxS* was reduced to 1.82 ± 0.017 times ($P < 0.05$). These data indicate that the *luxS* is involved in the formation of CREC229 biofilms (Fig. 4).

LuxS deletion enhance the adhesion of CREC229 to colorectal cancer cell HCT-8

Our transcriptomic study revealed that several outer membrane proteins related to adhesion were differentially expressed in the Δ*luxS* strain. Based on these findings, LuxS may play an important role in bacterial adhesion. First, we confirmed the presence of the OMPs required for bacterial adhesion in order to test

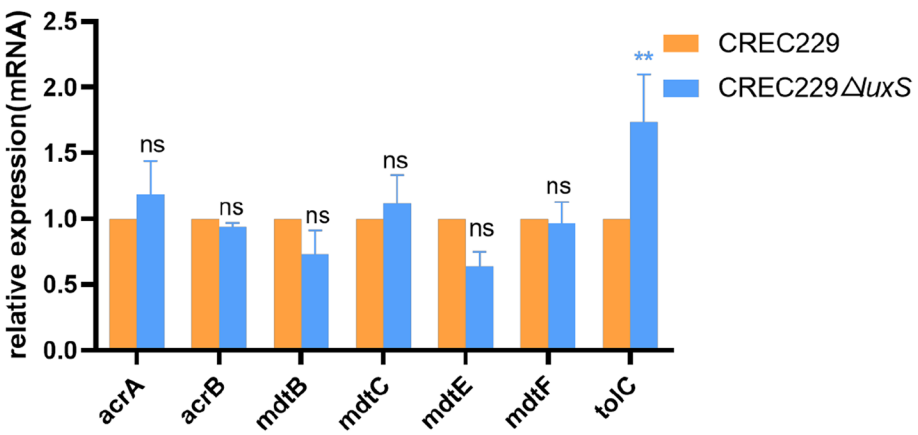

Fig. 3 Effects of the *luxS* on the expression of efflux pump. Values are shown as averages ± SD ($n = 3$). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

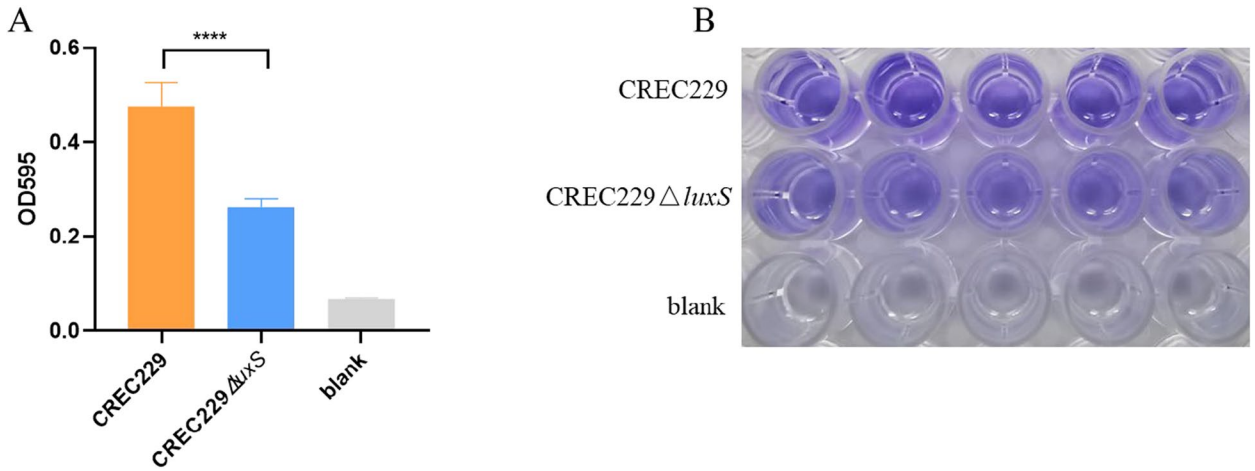

Fig. 4 Effects of the *luxS* on the Biofilm formation capacity. Data represent are shown as the mean ± SD ($n = 3$). ***$P < 0.001$, **$P < 0.01$

this hypothesis. In our study, we found that in Δ*luxS*, the adhesion genes (*ompA* and *ompX*) were expressed at higher levels than in CREC229 (Fig. 5A). This suggested that LuxS caused a lower expression of OMPs. Next, to verify whether the *luxS* altered the adhesion of CREC229 to HCT-8 cells, HCT-8 cells were infected both with both CREC229 and Δ*luxS* cells. Subsequently, we first investigated the number of bacteria bound to the HCT-8 cells. Our results showed that Δ*luxS* cells had a higher binding capacity compared to CREC229 cells (Fig. 5B). Meanwhile, to determine whether the *luxS* participates in mediating bacterial invasion of host cells, we further conducted invasion assays in HCT-8. We infected HCT-8 cells with CREC229 and its mutants Δ*luxS* for 4 h, and found that invasion frequencies of the CREC229 were not significantly different from the mutant strains Δ*luxS* ($p > 0.05$) (Fig. 5C). This suggests that in CREC229, LuxS reduces the adhesion of CREC229 to HCT-8 cells but did not affect its invasion.

LuxS deletion promote secretion of inflammatory factors IL-6

Inflammatory cytokines are secreted when bacteria successfully adhere to cells. Following this, we examined the expression of IL-8, IL-6, and TNF-α in HCT-8 cells infected with CREC229 using RT-qPCR. According to the results, IL-6 expression was significantly higher in HCT-8 cells induced by theΔ*luxS* strain than in those infected with CREC229. However, IL-8 and TNF-α expressions were not significantly different (Fig. 6).

Discussion

E. coli is a gram-negative opportunistic pathogen. Various medical device-associated infections, such as urethral, intravascular catheter infections, prosthetic joints, shunts, and prosthetic graft infections, are frequently caused by certain strains of *E. coli* [11]. In many bacterial species, cell density-dependent gene regulation systems called QS coordinate important biological functions, such as the regulation of virulence factors, antibiotic

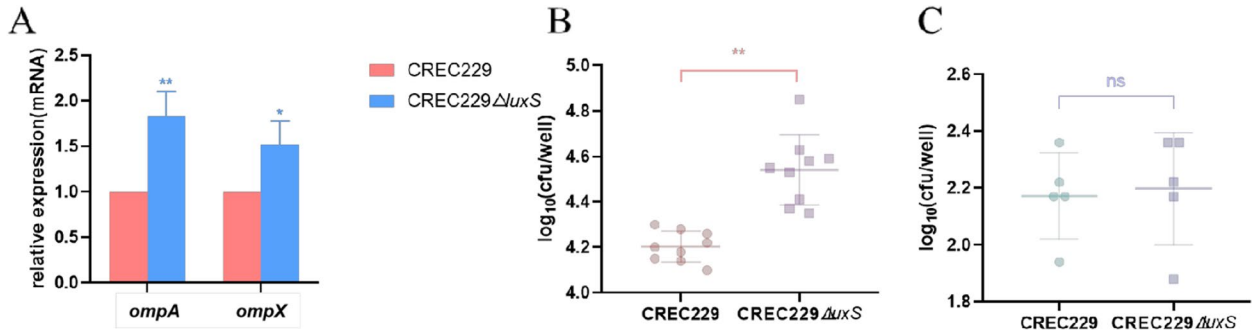

Fig. 5 Effect of *luxS* on adhesion and invasion ability. **A** mRNA level of *ompA* and *ompX* expressed in CREC229 and *luxS* mutation; **B** Effects of *luxS* on the adherence of CREC229 to HCT-8 colorectal cancer cells. Effects of *luxS* on the invasion of CREC229 to HCT-8 colorectal cancer cells. Data shown are the average values from three independent experiments, and bars represent standard deviations. **$P < 0.01$, *$P < 0.05$

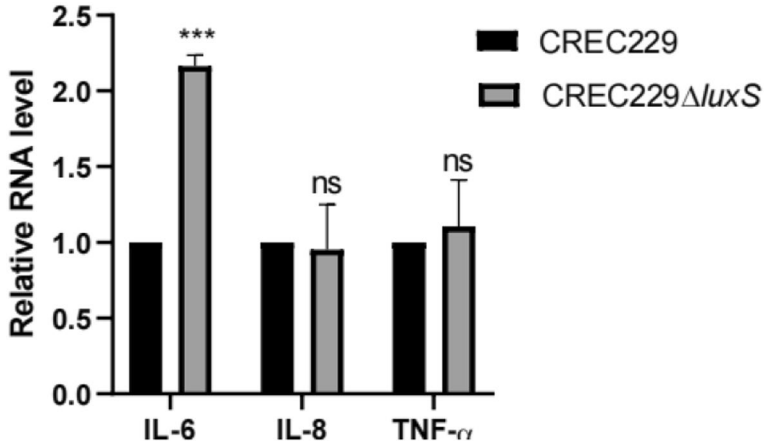

Fig. 6 Inflammatory factor production induced by CREC229 and its isogenic *luxS* mutant strains. HCT-8 cells were infected with CREC229 and its isogenic *luxS* mutant strains for 4 h with an MOI of 100. Data represent are shown as the mean ± SD ($n = 3$). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

biosynthesis, plasmid transfer, luminescence, and biofilm formation [12]. LuxS is an enzyme involved in quorum sensing and is present in various gram-positive and gram-negative bacterial species [13]. It has been confirmed by a large number of studies that the AI-2/LuxS system plays a critical role in detecting environmental changes in Gram-positive and Gram-negative bacteria [2, 14]. To our knowledge, this study is the first to construct a *luxS* gene deletion mutant of non-CP-CREC. This investigation focused on how the AI-2/LuxS system affects growth curves, *acrAB-tolC* efflux pump expression, bacterial resistance, biofilm formation, and virulence in non-CP-CREC. According to all findings, LuxS plays a critical role in many physiological activities in non-CP-CRECs.

Next, A mutant strain of *luxS* was constructed, and its knockout effect was verified using a variety of methods. This study showed that the mutant *luxS* strain did not produce LuxS, demonstrating that LuxS production is significantly affected by the deletion of the *luxS* gene, which is necessary for LuxS function. To ascertain whether the knockout of the *luxS* gene in *E. coli* influences bacterial growth, this study compared the growth of wild-type *E. coli* and its *luxS* gene knockout strains in LB medium. The growth curve analysis indicated no significant difference between the two strains, which is in agreement with prior studies on the growth patterns of *Lactobacillus paraplantarum* L-ZS9 [15], which is either *luxS*-negative or *luxS*-positive. Consequently, it was concluded that the subsequent experimental outcomes could not be attributed to the impact of *luxS* gene knockout on the normal growth of the bacteria. Nevertheless, studies indicate AI-2 is involved in metabolic processes that slow growth in the stationary phase and might interact with other components to "sense" population growth conditions [16]. Based on these results, AI-2/LuxS may have different effects on different bacterial species.

In addition,this study utilized transcriptional mapping to further investigate the relationship between LuxS and *E. coli* gene expression. The results of RNA-seq showed that genes involved in metabolic processes, cellular processes, and catalytic activities exhibited significant differential expression. Transcriptome sequencing results showed significant differences in biofilm-related genes (*bssS*), efflux pump-related genes (*tolC*), and virulence-related genes (*ompA* and *ompX*), suggesting the *luxS* may be associated with *E. coli* pathogenicity and drug resistance.

It was previously identified that the *luxS* gene contributes to pathogenicity in bacteria such as *E. coli* and *A. pleuropneumoniae* [17]. Most bacteria inhabiting natural and clinical environments form biofilms rather than free-living or "planktonic" cells, as reported in previous studies [17]. This study showed that AI-2 molecules synthesized by the LuxS enhance biofilm formation, which is consistent with previous studies on *E. coli* [18] and *Streptococcus pneumoniae* [19]. Despite this, previous studies reported that AI-2 moleculars could inhibit the ability of biofilm formation in some bacteria, such as *Haemophilus parasuis* [20] and *Lactobacillus reuteri* 100-23C [21]. This may be explained by the multifactors involved in adherence, metabolism, quorum sensing, stress reactions, and other processes during biofilm formation. This study proposes two potential mechanisms through which the reduction in biofilm formation ability may occur due to *luxS* knockout. Firstly, AI-2, an important signaling molecule that directly stimulates biofilm formation, is synthesized by LuxS. Therefore, the knockout of the *luxS* results in decreased AI-2 synthesis, thereby weakening biofilm formation. Researchers have found that

bacteria lacking the *luxS* are unable to produce QS signal AI-2 [17]. Secondly, the knockout of the *luxS* leads to the downregulation of *bssS*, a regulatory gene that positively influences biofilm formation [22]. Therefore, it may not be solely *luxS* that is responsible for regulating biofilm formation; other genes may also be involved. The regulation of the LuxS/AI-2 QS system on biofilm formation in various bacteria remains an open question.

It has been shown that quorum sensing signaling molecules play a role in maintaining barrier function, regulating inflammatory processes, and regulating resistance to pathogens through their interaction with receptors [23]. Therefore, a detailed understanding of AI-2/LuxS QS will help us better understand *E. coli* pathogenicity. Bacteria adhering to host cell surfaces are essential for colonization and cellular invasion, leading to persistent infection in the host and eventually systemic diseases [24]. The OMPs play an important role in colonization [25]. In this study, the *luxS* knockout strain expressed significantly more *ompA* and *ompX*, along with a significantly higher adhesion ability, compared to CREC229, but its invasion abilities were not significantly altered. According to other studies, the adhesion rate of bacteria decreased after the mutation of *luxS* [26, 27].This could be attributed to strain differences. In this study, multidrug-resistant bacteria from patients were analyzed, unlike other studies that looked at *Campylobacter jejuni* or standard strains. Under certain conditions, bacteria can induce inflammation and cause infectious diseases after successfully colonizing the host body. The AI-2/LuxS quorum sensing system is also capable of impacting gut gene expression, thus regulating the immune response in mammalian cells [8]. In this study, as a result of the *luxS* deletion, IL-6 expression increased, which may be closely related to the increased expression of OmpA and OmpX and enhanced adhesion. According to Zargar et al., AI-2/LuxS upregulated the production of IL-8, which subsequently decreased significantly, indicating that AI-2/LuxS modulated immune response [8]. Together, these results suggest that QS regulated effectors may affect the non-CP-CREC-induced inflammation in different ways.

Additionally, the investigation explored the role of the *luxS* in affecting antimicrobial susceptibility. Our results found that the *luxS* mutation resulted in increased susceptibility to tobramycin, gentamicin, and kanamycin. NIBRAS [28] found that *Streptococcus anginosus* was more susceptible to erythromycin and ampicillin when the *luxS* was mutated. Other studies suggest that *E. coli* with the *luxS* mutations might show reduced susceptibility to kanamycin, tobramycin, gentamicin, colistin, tetracycline, fluoroquinolones, chloramphenicol, and macrolides due to increased expression of its efflux pump [18]. Afterthe *luxS* was knocked out, *S. aureus* exhibited decreased susceptibility to antibiotics and elevated *vraSR* levels [29]. All of these factors might be closely linked to the increased expression of its efflux pump due to *luxS*. However, the regulatory protein controls the expression of thee fflux pump genes. SdiA has been shown to enhance the expression of the AcrAB, AcrAD, and AcrEF efflux pumps, which contribute to antibiotic resistance against β-lactams, quinolones, tetracyclines, chloramphenicol and so on [30]. In ESBL-positive *E. coli*, the TEM antibiotic resistance gene might be regulated by AI-2 via a mechanism dependent on LsrR [31]. Additionally, quorum-sensing regulation of production phenazine enhances *Pseudomonas aeruginosas's* resistance to ciprofloxacin [32]. This study found that despite the downregulation of the *sidA*, the other efflux pump expression remained unchanged, except for *tolC*. TolC is an outer membrane channel protein that, along with the inner membrane AcrB and periplasmic AcrA, forms the AcrAB-TolC ternary efflux pump. Suggesting that variations in antimicrobial susceptibility may not be linked to efflux pump activity. Research indicates that *Pseudomonas aeruginosas* exists as a biofilm in infectious contexts, and pyruvate, which plays a role in carbon metabolism, can attach to these biofilms, influencing the development of resistance to antimicrobials [32]. This study found that the *luxS* can increase resistance to aminoglycoside antibiotics, which may be closely related to biofilm formation. The relationship may involve quorum sensing impacting the formation of small molecules, which mediates alterations in biofilms and thus influences their resistance to drugs. But additional research is needed to determine the specific gene regulatory mechanism involved.

In summary, the results of our study showed that the deletion of the *luxS* in *E. coli* did not affect its growth, but could reduce biofilm formation, promote bacterial adhesion to the cell surface, enhance IL-6 secretion, and increase its sensitivity to amino- glycoside antibiotics. In all cases, the LuxS/AI-2 quorum sensing system in *E. coli* contributes to the pathogenicity and resistance of the organism by modulating a variety of physiological functions. There is evidence that the AI-2 QS system can be very important in adapting to a changing environment in *E. coli*.

Abbreviations

E coli	*Escherichia coli*
CREC	Carbapenem-resistant *E.coli*
non-CP-CREC	Non carbapenemase-producing CREC
CSEC	Carbapenem sensitive *E.coli*
MIC	Minimum inhibitory concentration
QS	Quorum sensing
V. fischeri	*Vibrio fischeri*
V. harveyi	*Vibrio harveyi*
AI-2	Autoinducer-2
AI-3	Autoinducer-3

MALDI-TOF/MS	Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry
LB	Luria-Bertani
MOI	Multiplicity of infection
CLSI	Clinical and Laboratory Standards Institute

Supplementary Information

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Supplementary Material 1

Authors' contributions

Study design: Yingping Cao and Xiaohong Xu. Study conduct: Meili Cai, Siyan Lian, and Huishan Lai. Data collection: Siyan Lian, Liping Hu and Meili Cai. Data analysis: Meili Cai and Xiaohong Xu. Data interpretation: Huishan Lai and Meili Cai. Drafting manuscript: Xiaohong Xu. Revising manuscript content: Yingping Cao and Liping Hu. Approving the final version of the manuscript: Yingping Cao and Liping Hu. All authors read and approved the final manuscript.

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Data availability

The original contributions presented in the study are included in the article /Supplementary Material. Further inquiries can be directed to the corresponding authors. The original contributions generated for this study are publicly available. This data can be found here: NCBI Gene Expression Omnibus database (https://www.ncbi.nlm.nih.gov/sra/PRJNA1185920) under accession number PRJNA1185920.

Declarations

Ethics approval and consent to participate

The Medical Ethics Committee of Fujian Medical University Union Hospital (2024KY077) thoroughly reviewed and granted approval for all procedures pertaining to human subjects in this study. We affirm that the execution of this study adhered to the principles outlined in the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Erick D, Olivier C, Stéphane B, et al. The population genetics of pathogenic *Escherichia coli* Nat Rev Microbiol, 2020, 19(1): 37–54.
2. Papenfort K, Bassler B. Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol. 2016;14(9):576–88.
3. K H N, J W H J M R. Bacterial bioluminescence: its control and ecological significance. Microbiol Rev, 1979, 43(4):496–518.
4. Waters C, Bassler B. Quorum sensing: cell-to-cell communication in bacteria. Annu Rev Cell Dev Biol. 2005;21:319–46.
5. Chung SUB K, Alexandra G, Santiago C, et al. Characterization of Autoinducer-3 Structure and Biosynthesis in *E. coli*. ACS Cent Sci, 2020, 6(2): 197–206.
6. Sircili M, Walters M, Trabulsi L, et al. Modulation of enteropathogenic *Escherichia coli* virulence by quorum sensing. Infect Immun. 2004;72(4):2329–37.
7. Soni K, Lu L, Jesudhasan P, 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 Sci, 2008, 73(3): M135–9.
8. Zargar A, Quan D, Carter K, et al. Bacterial secretions of nonpathogenic *Escherichia coli* elicit inflammatory pathways: a closer investigation of interkingdom signaling. mBio, 2015, 6(2): e00025.
9. Yongcan S, Yu L, Qian L, et al. LuxS/AI-2 Quorum Sensing System in Edwardsiella piscicida Promotes Biofilm Formation and Pathogenicity. 2020, 88(5): e00907–19.
10. Lian S, Liu C, Cai M, et al. Risk factors and molecular characterization of carbapenem resistant *Escherichia coli* recovered from a tertiary hospital in Fujian, China from 2021 to 2023. BMC Microbiol. 2024;24(1):374.
11. Andreas R, Mario M, Michael J, et al. Type 1 fimbriae contribute to catheter-associated urinary tract infections caused by *Escherichia coli*. J Bacteriol. 2013;196(5):931–9.
12. Kai P, Bonnie LB. Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol. 2016;14(9):576–88.
13. Xiangyong Z, Yunman Z, Jia Z, et al. Quorum sensing-mediated microbial interactions: Mechanisms, applications, challenges and perspectives. Microbiol Res. 2023;273: 127414.
14. Monnet V, Gardan R. Quorum-sensing regulators in Gram-positive bacteria: "cherchez le peptide". Mol Microbiol. 2015;97(2):181–4.
15. Xin L, Tengfei L, Yuebin Z, et al. Structural Insights into the Thermophilic Adaption Mechanism of Endo-1,4-β-Xylanase from *Caldicellulosiruptor owensensis*. J Agric Food Chem. 2017;66(1):1–13.
16. Sperandio V, Torres A, Girón J, et al. Quorum sensing is a global regulatory mechanism in enterohemorrhagic *Escherichia coli* O157:H7. J Bacteriol, 2001, 183(17): 5187–97.
17. Xiangan H, Hao B, Lei L, et al. The luxS gene functions in the pathogenesis of avian pathogenic *Escherichia coli*. Microb Pathog. 2012;55:21–7.
18. Amandeep K, Neena C, Prince S. Expression of Meiothermus ruber *luxS* in *E. coli* alters the antibiotic susceptibility and biofilm formation. Appl Microbiol Biotechnol, 2020, 104(10): 4457–4469.
19. Krystle B-C, Cecilia A H, Ramya A S B, et al. *Streptococcus pneumoniae* biofilm formation is strain dependent, multifactorial, and associated with reduced invasiveness and immunoreactivity during colonization. mBio, 2013, 4(5):e00745–13.
20. Zhang B, Ku X, Zhang X, et al. luxSThe AI-2/ Quorum Sensing System Affects the Growth Characteristics, Biofilm Formation, and Virulence of *Haemophilus parasuis*. Front Cell Infect Microbiol. 2019;9:62.
21. Tannock G, Ghazally S, Walter J, et al. Ecological behavior of *Lactobacillus reuteri* 100–23 is affected by mutation of the *luxS* gene. Appl Environ Microbiol. 2005;71(12):8419–25.
22. Domka J, Lee J, Wood T. YliH (BssR) and YceP (BssS) regulate *Escherichia coli* K-12 biofilm formation by influencing cell signaling. Appl Environ Microbiol. 2006;72(4):2449–59.
23. Krasulova K, Illes P. Intestinal interplay of quorum sensing molecules and human receptors. Biochimie. 2021;189:108–19.
24. Fan H, Chen Z, Lin R, et al. In Vitro *Bacteroides fragilis* Strain ZY-312 Defense against *Cronobacter sakazakii*-Induced Necrotizing Enterocolitis and in a Neonatal Rat Model. mSystems, 2019, 4:1–4.
25. Horne J, Brockwell D, Radford S. Role of the lipid bilayer in outer membrane protein folding in Gram-negative bacteria. J Biol Chem. 2020;295(30):10340–67.
26. Martin T, Ekaterina S, Lucie V, et al. Mutagenic strategies against *luxS* gene affect the early stage of biofilm formation of *Campylobacter jejuni*. J Appl Genet. 2021;63(1):145–57.
27. Zhaoxi D, Kangwei H, Teresa GV, et al. AI-2/LuxS Quorum Sensing System Promotes Biofilm Formation of *Lactobacillus rhamnosus* GG and Enhances the Resistance to Enterotoxigenic *Escherichia coli* in Germ-Free Zebrafish. Microbiol Spectr. 2022;10(4): e0061022.

28. Nibras A A M A, Fernanda C P, Anne Aamdal S. AI-2 quorum sensing affects antibiotic susceptibility in *Streptococcus anginosus*. J Antimicrob Chemother, 2007, 60(1):49–53.
29. S G, S W W, S G, et al. Role of VraSR in antibiotic resistance and antibiotic-induced stress response in *Staphylococcus aureus*. Antimicrob Agents Chemother, 2006, 50(10):3424–34.
30. JOãO A, Matthew P M, Séamus F, et al. The ins and outs of RND efflux pumps in *Escherichia coli*. Front Microbiol, 2015, 6(0):587.
31. Ting X, Lumin Y, Fei S, 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(6):4693–8.
32. Xia L, Li Y, Wang Y, et al. Quorum-sensing regulation of phenazine production heightens resistance to ciprofloxacin in *Pseudomonas aeruginosa*. Antimicrob Agents Chemother. 2024;68(5): e0011824.

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