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Transcriptional response study of auto inducer-2 regulatory system in *Escherichia coli* harboring bla_{NDM}

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Abstract

Background The emergence of carbapenem resistance in gram-negative bacteria such as *Escherichia coli* is one of the world's most urgent public health problems. *E. coli*, which encounter a diverse range of niches in host can rapidly adapt to the changes in surrounding environment by coordinating their behavior via production, release and detection of signal molecules called autoinducers through a cell density dependent communication system known as quorum sensing. Here, in this study we investigated whether imipenem, and acyl homoserine lactone quorum sensing signal molecules influence the transcriptional response within *lsr* and *lsrRK* operon which are associated with auto inducer-2 mediated quorum sensing in *E. coli*. Two *E. coli* isolates carrying bla_{NDM} were treated with 10% SDS for 20 consecutive days, resulting in the successful elimination of the bla_{NDM} encoding plasmid from one isolate. Plasmid was extracted from the isolate and was transformed into recipient *E. coli* DH5α by electroporation. The native type, plasmid-cured type, transformant, and *E. coli* DH5α were allowed to grow under eight different inducing conditions and the transcriptional responses of *lsr* and *lsrRK* operons were studied by quantitative real-time PCR method.

Results The findings of this study highlight the distinct effects of imipenem and AHL on the transcriptional responses of the *lsrB,lsrR*, and *lsrK* genes in native type, plasmid cured type, transformant, and *E. coli* DH5α.

Conclusion This study provides a basis for further research to elucidate different inducing conditions including antibiotics and autoinducers that could switch on the quorum sensing circuit in carbapenem non-susceptible *E. coli*, one of the world's most urgent public health threats.

Keywords Escherichia coli , AI2, SIC, lsr, Quorum sensing

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Introduction

The rise of carbapenem resistance in gram-negative bacteria like *Escherichia coli* is one of the most pressing public health issues globally [1, 2]. Research indicates that concentrations of carbapenem below the Minimum Inhibitory Concentrations (MIC) can be found in the human body, and these Sub-Inhibitory Concentrations (SIC) are also present in natural environments [3]. Such SICs of carbapenems may act as inducers allowing bacteria to adapt rapidly through gene expressions resulting in a variety of phenotypes [4, 5]. This is particularly vital in the case of gram-negative bacteria like *E. coli* which encounter a diverse range of niches [6, 7]. As such niches are diverse, these bacteria can rapidly adapt to the changes in surrounding environment by coordinating their behavior through a cell density dependent communication system known as quorum sensing. This communication is achieved via production, release, and detection of signal molecules called autoinducers [8]. Majority of autoinducers are Acyl Homoserine Lactone (AHL) molecules secreted by gram-negative bacteria like *Pseudomonas aeruginosa*, *Chromobacterium violaceum*, *and Acinetobacter baumanii* etc [9, 10]. Autoinducer-2 (AI-2) is a ubiquitous signal molecule which mediates communication at both intra and inter species level and is widely distributed among a large cohort of bacteria [11]. AI-2 has been detected in many bacteria, whereas the regulatory mechanism for AI-2 uptake has been detected in *Vibrio,Salmonella*, and *E. coli* [12]. In *E. coli*, the *lsr* operon consists of six genes (*lsrACDBFG*) that code an ATP binding cassette (ABC) transporter. The *lsrB* gene which codes for LsrB, one of the substrate binding proteins of the ABC transport system, is responsible for the import of extracellular AI-2 [13]. The regulatory network for AI-2 uptake is composed of two adjacently located genes, *lsrR* and *lsrK*, both of which are divergently transcribed from the *lsr* operon. LsrR is the repressor of the *lsr* operon and LsrK is a kinase which converts AI-2 to phospho-AI-2 that is required for relieving LsrR repression [13, 14].

E. coli occupies diverse habitats within hosts, including exposure to SICs of carbapenems during antibiotic therapy. Additionally, it interacts with various autoinducers, such as AI-2 and AHL, which are secreted by itself and other bacterial species. *E. coli* is also commonly found causing co-infection with *P. aeruginosa* in nosocomial and community acquired infections [15]. Here, in this study we have investigated whether imipenem and acyl homoserine lactone quorum sensing signal molecules influence the transcriptional response of genes in *lsr* and *lsrRK* operon that are associated with AI-2 mediated quorum sensing in *E. coli.* Our findings provide a basis for further studies into the regulatory role of *lsr* operon mediating AI-2 quorum sensing system in response to other classes of antibiotics and different autoinducers secreted by other bacteria.

Table 1 Description of bacterial strains used in the study

Bacterial Strains	Genotypic Description
E. coli (native type)	bla_{NDM}, *aac,ant*, and *aph* genes, IncFIC type plasmid
E. coli (plasmid-cured type)	Devoid of bla_{NDM}, *aac,ant*, and *aph* genes, IncFIC type plasmid
E. coli DH5α (transformant)	IncFIC plasmid harbouring bla_{NDM}
Pseudomonas aeruginosa	bla_{NDM}

Materials and methods

Bacterial strains, plasmids, primers, and growth conditions

The study was conducted using seven clinical isolates of *E. coli* that were non-susceptible to carbapenems. These isolates were obtained as secondary sources from Silchar Medical College and Hospital, a tertiary referral care facility in Silchar. The selection criteria for these isolates were based on their Minimum Inhibitory Concentrations (MICs) against imipenem and meropenem, as well as their resistance to cephalosporins (including ceftazidime, ceftriaxone, and cefepime) and aminoglycosides (such as gentamicin and tobramycin). Additionally, the presence of one or more carbapenemase genes was also considered (Table 1).

Strain construction

Plasmid curing

Two isolates harboring bla_{NDM} with high (64 µg/ml) and moderate (16 µg/ml) MIC against imipenem were treated with 10% Sodium Dodecyl Sulphate (SDS) for 20 consecutive days. The bacterial isolates were cured of plasmids that were harboring bla_{NDM} using 10% of SDS [16]. 10 µl of an overnight culture of bacterial cells were transferred to 5mL fresh Luria Bertani (LB) broths (HiMedia, India) containing 10% (w/v) SDS and was incubated at 37 °C in a shaker incubator at 220 rpm. Cells were plated on LB agar supplemented with 1 µg/ml imipenem after each SDS treatment. Plasmid elimination was estimated by calculating the number of cells growing on Luria Bertani (LB) agar (HiMedia, India) with and without imipenem. Furthermore, Polymerase Chain Reaction (PCR) assay was carried out after each treatment to check for the loss of bla_{NDM} gene. MIC of imipenem for the plasmid-cured type was checked by broth dilution method and the results were compared against their untreated native type.

Transformation

Plasmid was extracted from isolates harboring bla_{NDM} using QIAprep Spin Miniprep Kit (Qiagen, Germany) as per manufacturer's protocol. The extracted plasmid was transformed into recipient strain *E. coli* DH5α by

electroporation method with a pulse of 1.8 kV, 25 µF capacitance and 200 Ω resistance using a Gene Pulser Xcell electroporator (BioRad, USA). The transformants were selected on LB agar containing 0.5 µg/ml of imipenem (Merck, France). A Colony PCR was performed to confirm successful transformation of plasmid harboring bla_{NDM} into recipient strain *E. coli* DH5α.

PCR based replicon typing was done to characterize the plasmid encoding bla_{NDM} targeting 18 different replicon types viz., FIA, FIB, FIC, HI1, HI2, I1-Iγ, L/M, N, P, W, T, A/C, K, B/O, X, Y, F and FIIA through 5 multiplex PCR and 3 simple PCR. Resulting amplicons were visualized by gel electrophoresis.

RNA extraction and qRT-PCR

Transcriptional response analysis of *lsrB*, *lsrR*, and *LsrK* genes in native type, plasmid-cured type, transformant, and *E. coli* DH5α under different inducing conditions

The transcriptional responses of *lsrB*, *lsrR*, and *lsrK* genes in native type, plasmid-cured type, transformant, and *E. coli* DH5α were studied under different inducing conditions (Table 2) by quantitative real-time PCR method [17, 18]. The native type, plasmid-cured type, transformant, and *E. coli* DH5α were allowed to grow up to the log phase under the mentioned inducing conditions in fresh LB broths. The cells were grown at 37 °C with shaking at 200 rpm. OD_{600} readings of the bacterial cultures grown under different inducing conditions were taken at an interval of one hour for a period of 16 h. The natural logarithm of each OD reading and the time interval between two OD readings were calculated. All the different bacterial cultures grown under different inducing conditions had almost a similar growth rate with OD value ranging from 0.8 to 1. mRNA was extracted from cell pellets obtained after centrifugation using the RNeasy kit (Qiagen, Germany) following the manufacturer's protocol. RNase-free DNase I (Qiagen, Germany) was used to exclude any DNA contamination, and the DNase was efficiently removed in subsequent wash steps. Electrophoresis of extracted mRNA samples in 2% (w/v) agarose gel, stained with ethidium bromide with a DNA control further verified that the mRNA preparations did not contain DNA. The extracted mRNA was then immediately reverse transcribed to cDNA using the Quanti Tect Reverse Transcription kit (Qiagen, Germany) following the manufacturer's protocol. The synthesized cDNA was then assessed by running a semi-quantitative PCR using oligonucleotide primers of *lsrB*, *lsrR*, and *lsrK* genes (Table 3). The transcriptional responses of *lsrB*, *lsrR*, and *lsrK* genes were assessed in the native type, plasmid-cured type, transformant, and *E. coli* DH5α in Step One Plus real-time detection system (Applied Biosystem, USA) with the synthesized cDNA as templates. The target samples were the strains (native type, plasmid-cured type, transformant, and *E. coli* DH5α) cultured under different inducing conditions while the reference samples were the strains (native type, plasmid-cured type, transformant, and *E. coli* DH5α) cultured under untreated growth conditions. The experiment was performed in triplicate and the ct values were obtained. The relative quantity (RQ) of the expression of the genes was evaluated using the ΔΔct method. 30 S ribosomal protein subunit *rpsl* gene was used as a house-keeping gene whose expression is known to be stable under our experimental conditions and this gene was used to normalize the mRNA levels of genes of interest before the comparison between different samples by real-time PCR.

A carbapenem resistant clinical isolate of *P. aeruginosa* harboring bla_{NDM} gene and the native type, plasmid-cured type, and transformant were separately cultured overnight in fresh LB broths. The overnight cultures were diluted to a final optical density of 0.05 and inoculated in two fresh 5 mL LB broths at a 1:1 ratio, one of which was supplemented with 1 µg/ml imipenem. Co-cultures comprised 100 µL of *P. aeruginosa* and 100 µL of *E. coli*. To count the number of cells, the cultures were serially diluted 10-fold after 6, 12, and 24 h of incubation and plated onto Cetrimide agar (HiMedia, India) for *P. aeruginosa* and onto MacConkey agar (HiMedia, India) with 1 µg/mL imipenem for *E. coli*. Total viable cell counts were measured, and the results indicated that the CFU of *E. coli* and *P. aeruginosa* in co-culture remained

Table 2 Different inducing conditions under which native type, plasmid-cured type, transformant, and *E. coli* DH5α was grown

Bacterial Strains	Growth Conditions
E. coli (native type) *E. coli* (plasmid-cured type) *E. coli* (transformant) And *E. coli* DH5α	Untreated
	With 1 µg/ml of Oxododeconyl homoserine lactone (C12AHL)
	With 1 µg/ml of Butanoyl homoserine lactone (C4AHL)
	With both C4AHL and C12AHL at 1 µg/ml each, respectively
	With 1 µg/ml imipenem
	With both imipenem and C12AHL at 1 µg/ml each, respectively
	With both imipenem and C4AHL at 1 µg/ml each, respectively
	With imipenem, C12AHL, and C4AHL at 1 µg/ml each, respectively

Table 3 Oligonucleotide primers designed in the study

Primer pairs	Sequence (5′-3′)	Amplicon size (bp)	Source
lsrB (F)	GTCAGGTTGAAGTCTCGCCA	92	This study
lsrB (R)	GAATATCACGCGCTCCGGTA		
lsrR (F)	GCCTAAAATGTCGCCAACCG	94	This study
lsrR (R)	ACAGGACGATGCGACAATCA		
lsrK (F)	CGCACGTTCATTTCTGGGTC	74	This study
lsrK (R)	GTTCTTGGCGGCACATTCTG		

consistent with their respective single cultures. Given that *P. aeruginosa* naturally synthesizes acyl-homoserine lactones (AHLs), these compounds were not added to the growth media. The co-cultures were allowed to grow until they reached the log phase. mRNA was then extracted using RNeasy kit (Qiagen, Germany). Following extraction, the mRNA was immediately reverse transcribed into cDNA using the Quanti Tect Reverse Transcription Kit (Qiagen, Germany), in accordance with the manufacturer's protocol. The transcriptional responses of *lsrB*, *lsrR*, and *lsrK* genes were assessed in the Step One Plus real-time detection system (Applied Biosystem, USA) with synthesized cDNA as templates.

Statistical analysis

A two-way ANOVA was conducted using SPSS software to analyze the effects of different inducing conditions and strain types on the expression (RQ) of the *lsrB, lsrR*, and *lsrK* genes. Differences were deemed statistically significant when the P value was ≤ 0.05.

Results

The seven carbapenem non-susceptible clinical isolates of *E. coli* obtained for the present study had MIC values above breakpoint for imipenem, meropenem, and ertapenem. All the isolates were harboring bla_{NDM} gene. The study isolates were also resistant to all the tested aminoglycosides (gentamycin, tobramycin) and cephalosporins (ceftazidime, ceftriaxone, cefepime). No other classes of carbapenemase genes were found in any of the isolates.

Elimination of plasmid from the isolates harboring bla_{NDM}

The plasmid (carrying bla_{NDM}) was successfully eliminated from one isolate (with 64 µg/ml MIC) after 18th treatment. PCR analysis of the plasmid-cured type revealed the successful elimination of plasmid after 18th consecutive treatment. The MIC of imipenem and meropenem for the plasmid-cured type against its untreated native type also reduced from 64 µg/ml to 4 µg/ml indicating successful plasmid curing.

Transformation

Colony PCR confirmed successful transformation of plasmid into recipient strain *E. coli* DH5α. PCR Based Replicon Typing revealed that the plasmid belonged to IncFIC type and was approximately 62 Kb in size. The plasmid also harbored aminoglycoside resistance genes (*aph*, *ant*, and *aac*).

Up-regulation of *lsrB* gene in response to C12AHL, C4AHL, Imipenem and C12AHL, Imipenem and C4AHL in native type *E. coli*

The transcriptional response of *lsrB* gene in native type *E. coli* was studied under different inducing conditions. Up-regulation dominated over down-regulation in five different inducing conditions (C12AHL, C4AHL, Imipenem + C12AHL, Imipenem + C4AHL, and Imipenem + C12AHL + C4AHL) and the maximum up-regulation (5-fold increase) of the gene was observed when the native type *E.coli* was allowed to grow with imipenem in combination with C12AHL (Imipenem + C12AHL), and C4AHL (Imipenem + C4AHL), respectively (Fig. 1).

The *lsrB* gene response is down-regulated by Imipenem and the combinations of C12AHL and C4AHL

Compared to the transcriptional response of *lsrB* gene in native type *E. coli* grown without any treatment, a five-fold decrease in expression was observed when the isolate was subjected to grow with 1 µg/ml imipenem exposure. Exposure to C12AHL and C4AHL in combination (C12AHL + C4AHL) significantly decreased the transcriptional response of the gene by multiple folds. However, combining AHLs with imipenem (C4AHL + C12AHL + imipenem) did not lead to a significant reduction in the transcriptional response. (Fig. 1).

Transcriptional response of *lsrR* and *lsrK* genes of *LsrRK* Operon in native type *E. coli*

The *lsrR* gene which codes for the repressor of *lsr* operon was found to be down-regulated in native type *E. coli* under all inducing conditions. The transcriptional response decreased multiple folds in comparison to its response under untreated growth condition (Fig. 2). Down-regulation was observed in the transcriptional response of *lsrK* gene which codes for a kinase when the isolate was grown with imipenem, C12AHL, C4AHL, C12AHL + C4AHL, and C12AHL + Imipenem. The gene's response was found to be up-regulated when treated with C4AHL in combination with imipenem (C4AHL + imipenem), as well as with the combination of C4AHL, C12AHL, and imipenem (C12AHL + C4AHL + Imipenem) (Fig. 3).

Up-regulation of *lsrB* in plasmid-cured type

When the plasmid-cured type was allowed to grow under various inducing conditions, the transcriptional response of the *lsrB* gene was found to increase by twenty-seven-fold in response to C12AHL and thirteen-fold in response to C4AHL. Up-regulation was also observed in response to the combination of C4AHL and imipenem (C4AHL + Imipenem) as well as C12AHL and imipenem (C12AHL + Imipenem) (Fig. 1).

Down-regulation of *lsrB* in response to Imipenem, AHLs in combination, and Imipenem and AHLs, all in combination in plasmid-cured type

In comparison to the transcriptional response observed in the plasmid-cured type under untreated growth

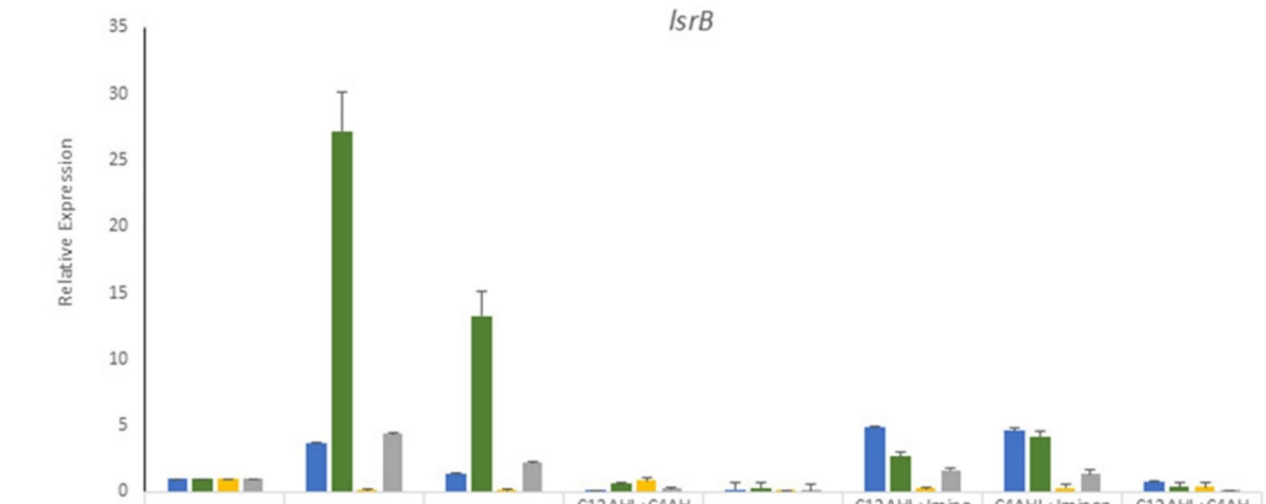


Fig 1: *lsrB* gene expression in response to inducing growth conditions in native type, plasmid-cured type, transformant, and *E. coli* DH5α. The means of the relative expression of genes are plotted in Y-axis against different inducing conditions plotted on X-axis. The error bars represent standard deviation (P value, <0.001)

Fig. 1 Fig 1: *lsrB* gene expression in response to inducing growth conditions in native type, plasmid-cured type, transformant, and *E. coli* DH5α. The means of the relative expression of genes are plotted in Y-axis against different inducing conditions plotted on X-axis. The error bars represent standard deviation, (P value, <0.001)

conditions, the transcriptional response of the *lsrB* gene decreased three fold when the plasmid-cured type was exposed to 1 µg/ml of imipenem. Additionally, the expression of the *lsrB* gene also declined in response to a combination of AHLs (C4AHL + C12AHL), as well as in response to both imipenem and AHLs combined (Imipenem + C4AHL + C12AHL) (Fig. 1).

Transcriptional response of *lsrR* and *lsrK* gene of *LsrRK* Operon in plasmid-cured type

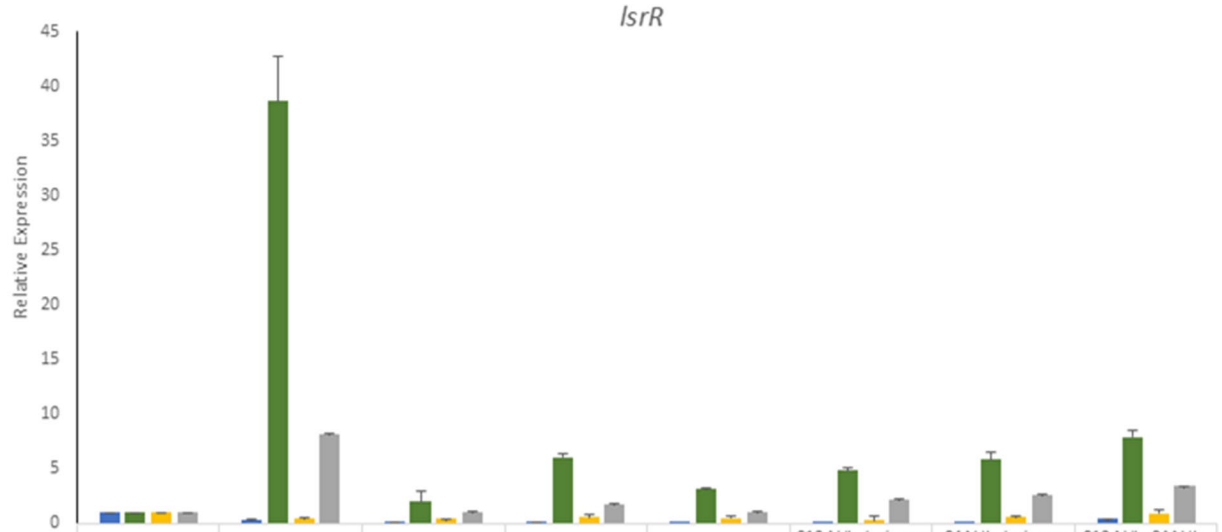
The transcriptional response of the *lsrR* gene increased significantly compared to its expression under untreated growth conditions. The expression of the *lsrR* gene was found to be maximally enhanced (38-fold) in response to C12AHL (Fig. 2). In contrast, the expression of the *lsrK* gene remained unchanged when treated with C12AHL combined with C4AHL (C12AHL + C4AHL). However, the expression of the *lsrK* gene was upregulated in all other treated conditions, although only a marginal increase was observed in response to imipenem and C4AHL, respectively (Fig. 3).

Down-regulation of *lsrB* gene, down-regulation of *lsrR* gene, and up-regulation of *LsrK* in the transformant

In the transformant, the transcriptional response of the *lsrB* gene was down-regulated under all conditions when compared to its response in untreated growth conditions. The *lsrR* gene of the *lsrRK* operon was also found to be down-regulated. However, the *lsrK* gene was up-regulated in response to all inducing conditions, showing a marginal increase in response to C4AHL. In contrast, there was no change in expression of *lsrK* gene in response to C12AHL (Fig. 3).

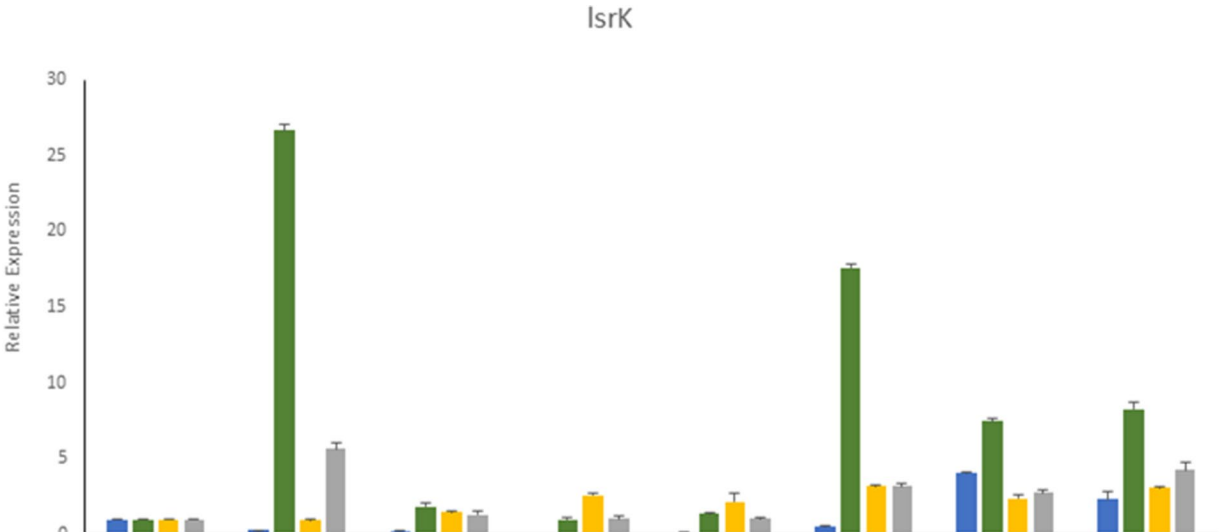
Down-regulation of *lsrB* in response to Imipenem, Imipenem and AHLs, all in combination, and up-regulation of *lsrR* and *lsrK* genes in *E. coli* DH5α

In *E. coli* DH5α, a down-regulation of the *lsrB* gene was observed in response to imipenem, various AHLs in combination, and the combination of imipenem with AHLs. Conversely, an up-regulation of the *lsrB* gene occurred in response to C12AHL and C4AHL, respectively. Additionally, a marginal up-regulation was noted when combining C4AHL with imipenem (C4AHL + imipenem) and when combining C12AHL with imipenem



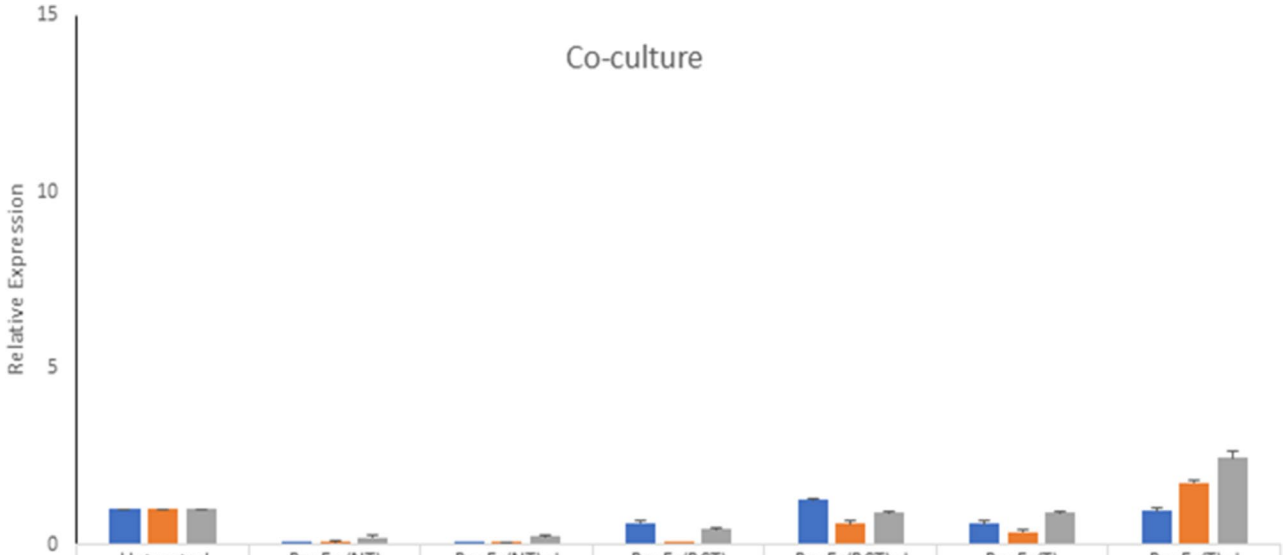
	Untreated	C12AHL	C4AHL	C12AHL+C4AHL	Imipenem	C12AHL+Imipenem	C4AHL+Imipenem	C12AHL+C4AHL+Imipenem
Native	1	0.307	0.001	0.001	0.011	0.005	0.006	0.42
Plasmid cured	1	38.604	2.034	6.101	3.236	4.89	5.866	7.937
Transformant	1	0.431	0.426	0.622	0.544	0.33	0.619	0.936
E coli DH5α	1	8.23	1.108	1.77	1.08	2.21	2.66	3.42

Fig. 2 lsrR gene expression in response to inducing growth conditions in native type, plasmid-cured type, transformant, and E. coli DHα5. The means of the relative expression of genes are plotted in the Y-axis against different inducing conditions plotted on X-axis. The error bars represent standard deviation (P value, <0.001)



	Untreated	C12AHL	C4AHL	C12AHL+C4AHL	Imipenem	C12AHL+Imipenem	C4AHL+Imipenem	C12AHL+C4AHL+Imipenem
Native	1	0.252	0.25	0.045	0.076	0.499	4.021	2.337
Plasmid-cured	1	26.652	1.812	0.991	1.41	17.61	7.467	8.246
Transformant	1	0.943	1.505	2.59	2.18	3.15	2.364	3.047
E coli DH5α	1	5.62	1.28	1.04	1.065	3.16	2.76	4.26

Fig. 3 lsrK gene expression in response to inducing growth conditions in native type, plasmid-cured type, transformant, and E. coli DH5α. The means of the relative expression of genes are plotted in the Y-axis against different inducing conditions plotted on X-axis. The error bars represent standard deviation (P value, <0.001)



	Untreated	Pa+Ec (NT)	Pa+Ec(NT)+I	Pa+Ec(PCT)	Pa+Ec(PCT)+I	Pa+Ec(T)	Pa+Ec(T)+I
lsrR	1	0.003	0.002	0.609	1.248	0.58	0.95
lsrK	1	0.039	0.011	0.006	0.592	0.338	1.744
lsrB	1	0.19	0.21	0.44	0.88	0.89	2.44

Fig. 4 lsrB, lsrR, and lsrK gene expression in co-cultures of P. aeruginosa andE. coli (native type, plasmid-cured type, transformant). The means of the relative expression of genes are plotted in Y-axis against untreated and treated (1 g/m1) conditions plotted on X-axis.. The means of the relative expression of genes are plotted in the Y-axis against different inducing conditions plotted on X-axis. The error bars represent standard deviation (P value, <0.001). NT= native type, PCT=plasmid-cured type, T=transformant

(C12AHL + imipenem) (Fig. 1). The expression of the *lsrR* gene exhibited an overall increase across all treated conditions. A marginal increase was noted in response to C12AHL and C4AHL in combination. However, no significant increase was observed with C4AHL and imipenem, respectively (Fig. 2). The *lsrK* gene was found to be up-regulated compared to its expression under untreated conditions. A marginal increase was observed in response to C4AHL, but no significant up-regulation was detected with the combination of C12AHL and C4AHL, nor with imipenem (Fig. 3).

Down-regulation of *lsrB, lsrR*, and *lsrK* genes in *E. coli* (native type, plasmid-cured type, and transformant) when co-cultured with *Pseudomonas aeruginosa*

Native type, plasmid-cured type, and transformant were respectively co-cultured with a carbapenem resistant *P. aeruginosa* isolate in a 1:1 ratio in three separate 5 ml LB broths. Since *P. aeruginosa* synthesizes AHL, no exogenous AHL was added to the growth media. The transcriptional responses of the *lsrB*,*lsrR*, and *lsrK* genes were down-regulated compared to the untreated growth conditions. To determine whether imipenem at a concentration of 1 µg/ml alters the transcriptional response of the genes, we co-cultured the native type, plasmid-cured type, and transformant with *P. aeruginosa* in LB broth supplemented with 1 µg/ml imipenem. Our findings revealed that the transcriptional responses of the *lsrB* and *lsrK* genes decreased in both the native and plasmid-cured type, while their expression increased in the transformant. Additionally, the expression of the *lsrR* gene decreased in the native type, slightly increased in the plasmid-cured type, and remained unchanged in the transformant (Fig. 4).

Statistical analysis

Two-way ANOVA analysis revealed that there was a statistically significant interaction between the effects of different inducing conditions and strain type ($p = < 0.001$) on RQ values of *lsrB*,*lsrR*, and *lsrK* genes.

Discussion and conclusion

Quorum sensing is an important communication process that coordinates the cooperative behavior of bacteria in response to cell density [19]. In bacteria like *E. coli* there are intricate regulatory pathways that allow a signal from one autoinducer, such as AHL, to influence the expression of genes within an AI-2 regulatory operon, potentially via intermediary regulatory proteins [20]. In our study, we aimed to deepen our understanding of how imipenem and AHLs (C4AHL and C12AHL) affect the transcriptional response of the *lsr* and *lsrRK* operons which are crucial for regulating AI-2-mediated quorum sensing in *E. coli*. We performed experiments with a range of strains, including the native type, plasmid-cured type, transformant, and *E. coli* DH5α, under both

untreated and various inducing conditions. Our results indicate distinct differences in expression levels among the various strains in response to imipenem and AHLs. We also observed variations when these strains were co-cultured with *P. aeruginosa* under imipenem stress.

In the native type *E. coli*,*lsrB* gene was up-regulated in response to C12AHL + Imipenem, C4AHL + Imipenem, C12AHL and C4AHL; whereas the response was down-regulated in response to 1 µg/ml imipenem and C4AHL + C12AHL. The up-regulation in the response of *lsrB* gene indicates the import of extracellular AI-2 molecules into the cell by LsrB transporter protein. As reported in previous studies, once inside the cell, AI-2 is phosphorylated and converted to phospho-AI-2 by *lsrK* which codes for a kinase [19, 21]. This phospho-AI-2 is required for relieving LsrR repression of the *lsr* operon [13, 14]. In the present study, the expression of *lsrR* gene in the native type *E. coli* was found to be down-regulated multiple folds in response to all the inducing growth conditions, and the expression of *lsrK* gene was down-regulated in 5 of 7 inducing growth conditions. These data indicate that the increased expression of LsrB transporter in the already mentioned inducing conditions would facilitate the import of extracellular AI-2 into the cell, however the phosphorylation of AI-2 would be decreased due to decrease in *lsrK* expression in these conditions. However, increase in expression of both *lsrB* and *lsrK* genes in response to C4AHL in combination with imipenem (C4AHL + imipenem) indicate uptake and phosphorylation of AI-2 in this condition. This results in a positive feedback loop where uptake and phosphorylation of AI-2 enhance expression of transporter which in turn enhances more signal uptake and further induction of *lsr* [22].

P. aeruginosa serves as an important model organism for studying quorum sensing systems. Research has shown that the SdiA protein in *E. coli* can receive acyl-homoserine lactones (AHLs) from neighboring bacteria, in addition to its own signaling molecule, AI-2 [23]. The co-occurrence of *E. coli* and *P. aeruginosa* in hospital environments frequently contributes to co-infections, both in nosocomial and community-acquired contexts [24]. Moreover, *P. aeruginosa* synthesizes and releases AHLs, referred to as C12AHL and C4AHL [25]. Given these dynamics, this study aims to enhance our understanding of the interactions between *P. aeruginosa* and *E. coli*, which could provide valuable insights for addressing infections in clinical settings. The analysis of the transcriptional response provides valuable insights into the behavior of the *lsrRK* and *lsr* operons under various conditions. Under untreated growth conditions, both operons were repressed in the native type, plasmid-cured type, and transformant, which sets a foundational understanding of their regulation. When imipenem was introduced, the native type exhibited down-regulation of both the *lsrRK* and *lsr* operons, indicating a potential adaptive response to antimicrobial stress. In the plasmid-cured type, the expression of the *lsrR* gene remained unchanged in the presence of imipenem, while the *lsrB* and *lsrK* genes were down-regulated. This suggests a nuanced regulatory mechanism in this variant that warrants further exploration. Conversely, in the transformant, even though *lsrR* expression did not significantly change, the up-regulation of both *lsrB* and *lsrK* genes in response to imipenem are noteworthy. This variation indicates distinct regulatory pathways at play and highlights the transformant's potential adaptability to antibiotic pressure.

In the plasmid-cured type, the *lsrB* gene was over-expressed in response to C4AHL, C12AHL, C4AHL + Imipenem, C12AHL + Imipenem and under-expressed in response to imipenem, C4AHL + C12AHL and C4AHL + C12AHL + Imipenem. A similar pattern of expression of *lsrB* gene was also observed in *E. coli* DH5α. The *lsrR* gene associated with repression of AI-2 mediated quorum sensing was found to be over-expressed in response to all conditions, unlike in the native type *E. coli*, where the expressions of *lsrR* gene were under-expressed. This may be due to the divergence between these two strain types that has occurred due to the elimination of plasmid. As suggested by a previous study [26], other genes within the plasmid might also have a role. The *lsrK* gene was up-regulated in four of the seven conditions. Its expression remained unchanged in the presence of C12AHL + C4AHL, and was only marginally elevated with C4AHL and imipenem, respectively. These data indicate that the *lsr* operon associated with coding LsrB transporter protein expresses itself in response to C12AHL, C4AHL, C12AHL + imipenem, C4AHL + imipenem. Although imipenem, C12AHL + C4AHL, and C12AHL + C4AHL + imipenem had an inhibitory effect on the response of *lsrB* gene, which codes for transport apparatus for internalization of AI-2, the response of *lsrR* gene increased in response to these conditions. Increase in expression of *lsrR* gene pertains to repression in the transcription of *lsr* operon which might be the reason behind decrease in expression of *lsrB* gene in these conditions.

In *E. coli* DH5α, we observed a noteworthy increase in the expression of the *lsrR* and *lsrK* genes under various conditions, including C12AHL, C4AHL combined with imipenem, C12AHL combined with imipenem, and C4AHL combined with C12AHL and imipenem. There was a slight increase in the expression of *lsrR* gene in response to the combination of C12AHL and C4AHL. However, no significant increase could be detected with C4AHL and imipenem. A slight increase was detected in response of *lsrK* gene to C4AHL; however, no significant

up-regulation was observed with the combination of C12AHL and C4AHL, nor with imipenem.

In the transformant, however, the transcriptional response of the *lsrB* gene was consistently downregulated across all conditions. Interestingly, while the *lsrR* gene of the *lsrRK* operon also showed down-regulation, the *lsrK* gene exhibited over expression in response to most inducing conditions, except for C12AHL, where its expression remained unchanged. The enhanced expression of the *lsrK* gene could potentially be influenced by other regulatory elements, such as the cAMP-CRP complex [27], suggesting areas for further exploration. This study highlights the complexity of gene expression patterns, as we found no consistent correlation in the expression levels of genes within the *lsr* and *lsrRK* operons across the different strain types. The expressions of the *lsrB*, *lsrR*, and *lsrK* genes in the clinical strain did not predictably align with the transformant's expression patterns. Similarly, the plasmid-cured strain exhibited different expression levels compared to the laboratory strain, *E. coli* DH5α. These observations suggest that variations in genetic makeup may lead to independent regulatory mechanisms governing gene expression, even when subjected to similar inducing conditions [28]. Further investigation into these differences could provide valuable insights into genetic regulation and its implications in different *E. coli* strains.

In conclusion, the findings of this study demonstrated that imipenem and AHL have distinct effects on the transcriptional responses of the *lsrB*,*lsrR*, and *lsrK* genes in different strains, including native type, plasmid-cured type, transformant, and *E. coli* DH5α. This research lays the groundwork for further investigation into the various conditions, such as the presence of antibiotics and autoinducers, that can activate the quorum sensing circuit in *E. coli*. Continued exploration in this area will enhance our understanding of the *lsr* operon-regulated AI-2 quorum sensing system in gram-negative bacteria like *E. coli* and could lead to the development of new strategies for targeting this complex quorum sensing network.

Abbreviations

MIC	Minimum Inhibitory Concentrations
SIC	Sub-inhibitory concentrations
AHL	Acyl homoserine lactone
AI2	Autoinducer-2
SDS	Sodium Dodecyl Sulphate
PCR	Polymerase Chain Reaction
RQ	Relative Quantity

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Data availability

The data underlying this article are available in the article.

Declarations

Ethics approval and consent to participate

The study was approved as review exempted category as the study isolates were collected from a secondary source.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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