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Modulation of BarA/UvrY Two-Component System Expression in *Escherichia coli* Induced by Ceftazidime Treatment

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Abstract

Uropathogenic Escherichia coli (UPEC) remains the most common etiologic agent of community-acquired urinary tract infections (UTIs), largely due to its extensive virulence arsenal and adaptive regulatory mechanisms. In this study, we examined the phenotypic regulation of major virulence factors—hemolysin production, biofilm, swarming motility, and adhesion—under exposure to sub-inhibitory concentrations of Ceftazidime, an extended-spectrum β-lactam antibiotic. The transcriptional response of the BarA/UvrY two-component regulatory system was also examined to unravel its role in response to antibiotic stress. Using an extensive range of microbiological, molecular, and phenotypic tests, we unveiled differential profiles of virulence traits. Exposure to sub-MICs Ceftazidime markedly diminished hemolytic activity, biofilm formation, and adhesive capacity, with swarming motility exhibiting almost complete inhibition. Moreover, quantitative real-time PCR (qRT-PCR) analysis revealed variable transcriptional responses of BarA and UvrY in the tested isolates, with the recovery of BarA expression noted after prolonged exposure to antibiotics. These findings emphasize the dynamic expression of UPEC's stress response mechanisms and outline the central role of Bar/UvrY in modulating pathoadaptive mechanisms under antibiotic stress.

Keywords: Uropathogenic *Escherichia coli*, Virulence factors, BarA/UvrY system, Ceftazidime, Sub-MIC effects.

تعديل تعبير النظام ثنائي المكون BarA/UvrY المكون من مكونين في الإشريكية القولونية الناتج عن علاج سيفتازيديم

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قسم علوم الحياة، كلية العلوم، جامعة بغداد، الجادرية، بغداد, العراق.

الخلاصة

تُعد الإشريكية القولونية الممرضة للجهاز البولي العامل المسبب الأكثر شيوعًا لعدوى المسالك البولية المكتسبة مجتمعيًا، ويُعزى ذلك إلى ترسانتها الواسعة من عوامل الفوعة وآلياتها التنظيمية التكيفية. في هذه الدراسة، جرى تحليل التنظيم الظاهري لعوامل الفوعة الرئيسة فعالية تحلل الدم، تكوين الأغشية الحيوية، حركة العج، والقدرة على الالتصاق عند تعرضها لتراكيز تحت المثبطة من السفتازديم، وهو مضاد حيوي من عائلة

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البيتا–لاكتام واسع الطيف. كما تم تحليل الاستجابة النسخية لنظام التنظيم الثنائي المكون BarA/UvrY للكشف عن دوره في الاستجابة للإجهاد المضادي. وأظهرت النتائج، من خلال سلسلة متكاملة من الفحوصات الميكروبيولوجية والجزيئية والمظهرية، ان التعرض للتراكيز تحت المثبطة من السفتازديم ادى إلى انخفاض ملحوظ في فعالية تحلل الدم وتكوين الأغشية الحيوية والقدرة على الالتصاق، بينما ظهرت حركة العج شبه معدومة. وأظهرت تحاليل qRT–PCR استجابات نسخية متباينة لجيني BarA و UvrY بين العزلات المدروسة، مع ملاحظة تعافٍ في التعبير الجيني لـ BarA بعد التعرض المطوّل للمضاد الحيوي. وتؤكد هذه النتائج الطبيعة الديناميكية لاستجابات الإشريكية القولونية الممرضة للجهاز البولي للإجهاد، كما تسلط الضوء على الدور المحوري لنظام BarA/UvrY في تعديل آليات الفوعة تحت الضغط المضادي.

Introduction

Escherichia coli (*E. coli*) is a commensal bacterium found mainly in the human gut, although some pathogenic strains encode virulence determinants that may lead to a variety of intestinal and extraintestinal disorders. Most of these are urinary tract infections (UTIs), which constitute a considerable clinical burden, with their severity intimately linked to the disparate virulence mechanisms expressed by *E. coli* [1]. The past ten years have witnessed increased focus on the clinical management of *E. coli*-associated UTIs, given rising antimicrobial resistance and pathogenic variability [2]. The ability of *E. coli* to adhere to epithelial surfaces, forming a biofilm, is key to its pathogenicity and is attributed to the expression of fimbriae that colonize host tissues. Such biofilms not only enable bacterial persistence but also promote chronic infection, especially in the case of non-removable medical devices, leading to considerable morbidity and mortality [3]. Biofilms are structured microbial communities encapsulated in an extracellular polymeric matrix that are produced by microorganisms and may serve to attach to biotic and abiotic surfaces. In addition to adherence factors, *E. coli* also secretes a variety of toxins, including hemolysins and enzymes, that damage host tissues and help evade the immune response [4]. Moreover, *E. coli* has a flagella-mediated surface motility, known as swarming, which is associated with high expression of virulence genes, increased resistance to the host immune response, and reduced sensitivity to antimicrobials [5]. The high prevalence of β-lactamase–producing strains, such as extended-spectrum β-lactamases (ESBLs) is one of the major therapeutic challenges in the management of *E. coli* infections. This resistance dramatically constrains therapy and exacerbates clinical outcomes in infections caused by Gram-negative bacilli [6]. As recently reviewed, there are many control networks have been proposed for *E. coli*, and several studies have identified the BarA-UvrY two-component system (TCS) as important in regulating the genes of this microorganism. For example, one tripartite sensor kinase, BarA, can engage with the FixJ family response regulator UvrY to regulate gene expression in response to environmental stimuli [7]. UvrY is co-transcribed with *uvrC*, which encodes part of the UvrABC nucleotide excision repair complex; UvrY function has not yet been directly linked to UV-cassistance of DNA repair [8]. The BarA-UvrY TCS is a major controller of the carbon storage regulatory (Csr) network; through activating small RNAs (sRNAs) CsrB and CsrC, it antagonizes the global post-transcriptional regulator CsrA by sequestration. This system controls key cellular processes, including carbon flux, motility, and biofilm formation through modulation of CsrA activity, highlighting its involvement in bacterial adaptation and homeostasis [9,10]. This study further elucidates the transcriptomic responses of the BarA-UvrY TCS in *E. coli* upon exposure to the third-generation cephalosporin antibiotic, Ceftazidime. It concurrently seeks to assess the impact of Ceftazidime on significant virulence-associated phenotypes, including hemolysin production, bacterial adhesion, biofilm formation, and swarming movement. This study aims to elucidate how antibiotic stress affects regulatory circuits and virulence expression in *E. coli*.

Material and methods

Bacterial Isolates

Nine UPEC isolates were isolated from women with UTIs at the Teaching Laboratories in the Medical City during November 2024. The diagnosis of the isolates was confirmed by growing them on selective and differential culture media. The VITEK® 2 Compact system was used to identify the *E. coli* isolates and to test antibiotic susceptibility with the AST-N419 card. The nine isolates were subsequently designated as follows: 2M, 16, 18N, 21, 24, 24M, 24W, 26M, and ab.

Selection of ESBL E. coli

The isolated organism was subcultured onto HiCrome™ ESBL Agar Base with the AC3F selective supplement containing Ceftazidime, Cefotaxime, Ceftriaxone, Aztreonam, and Fluconazole selective media to determine ESBL production [11].

Phenotypic detection of some virulence factors

Hemolysis assay

Hemolytic activity of UPEC was measured by modifying a previously described method [12] after UPEC isolates grew on blood agar to identify hemolysin-producing strains. Erythrocytes were isolated from fresh rabbit blood by centrifugation at 2000 rpm for 5 minutes and then washed three times with phosphate-buffered saline (PBS) to remove residual plasma components. The cells were resuspended in PBS and adjusted to a final concentration of 2%. Bacterial cultures were adjusted to a 0.5 McFarland turbidity standard, and a single bacterial dilution was used instead of the 10-fold serial dilutions described in the original method. 100 µl of the bacterial suspension was transferred to individual wells of a 96-well plate. An equal volume (100 µl) of the 2% erythrocyte suspension was then added to each well. Negative and positive control wells contained only RBCs in PBS and 1% Triton X-100, respectively. The plate was incubated at 4°C for 3 hours, and hemolytic activity was assessed by identifying the last well showing clear hemolysis. UPEC hemolytic activity was then measured using the following formula for calculating the percentage of hemolysis produced by hemolysin:

$$Hemolysis\ (\%) = \frac{A450nm(sample\ with\ hemolysin) - A450nm(control\ without\ hemolysin)}{A450nm(total\ lysis\ by\ Triton\ X{-}100) - A450nm(control\ without\ hemolysin)} \times 100$$

The absorbance of the sample at 450 nm containing hemolysin-producing bacteria is normalized by the negative control (non-hemolytic) and the positive control (complete lysis caused by Triton X–100 treatment)[13].

Swarming assay

The swarming assay agar, prepared according to the procedure described by Kaittan and Flayyih [14], was autoclaved at 121°C for 15 minutes. The agar was transferred into Petri dishes after it was cooled to 50°C. *E. coli* overnight cultures were grown in 5 mL of brain-heart infusion broth, and the bacterial suspension was adjusted to a turbidity equivalent to 0.5 McFarland. 5 µL of this suspension was spot-inoculated onto two swarming agar plates. The plates were incubated at 37°C for 18–24 h to determine the motility of the bacteria and the spreading of the colonies.

Biofilm assay

Biofilm formation by the bacterial isolates was evaluated using a 96-well microtiter plate assay. Bacteria were grown at 37°C in tryptic soy broth (TSB) for 24 hours, and 200 µL of standardized inoculum (0.5 McFarland) 1% sucrose-supplemented TSB was added to three duplicate wells for each. After a 24 h incubation at 37 °C, non-adherent cells were discarded and the wells were washed, fixed with methanol, and stained with 0.1% crystal violet. Bound dye was solubilized with ethanol, and the absorbance was measured at 570 nm [15]. Biofilm formation was defined as shown in Table 1.

Table 1: Quantitative interpretation of biofilm formation based on optical density measurements [16].

Interpretation	Optical Density (OD) Range
Non-biofilm producer	**OD ≤ ODc**
Weak biofilm producer	**ODc < OD ≤ 2 × ODc**
Moderate biofilm producer	**2 × ODc < OD ≤ 4 × ODc**
Strong biofilm producer	**OD > 4 × ODc**

Note: OD = optical density; ODc = cutoff optical density value.

Adhesion ability assay

Following the method mentioned in the previous study [12], after minor protocol optimization, to assess the adhesion potential of UPEC isolates, the assay was performed. After overnight cultivation of the bacterial isolates in Luria Bertani (LB) broth at 37 °C for 24 h, 100 µL of the broth was aseptically transferred into 10 mL of LB broth containing an addition of 1% (w/v) glucose to enhance adherence formation. Subsequently, the bacterial inoculum was distributed into separate wells of a sterile 6-well microtiter plate (10 mL suspension per well). Plates were statically grown at 37°C for 6 hours to allow bacterial adhesion to the plate surface. After incubation, non-adherent cells were removed by washing the wells twice with sterile PBS. The adhered cells were scraped off under sterile conditions by a sterile cell scraper after adding 1 mL of sterile PBS into each well to recover the surface-adherent bacteria. These dislodged bacteria were serially diluted in PBS, and 100 µL fractions were plated on LB agar. The adhesive capacity of each isolate was assessed by enumerating colony-forming units (CFUs) after incubation at 37°C for 24 hours.

Determination of the minimum inhibitory concentration (MIC) of Ceftazidime against E. coli

The MIC of Ceftazidime on *E. coli* was determined using the broth microdilution method in a 96-well microtiter plate, as outlined in the CLSI 2024 guideline. 100 µl of double-strength Mueller-Hinton broth was first added to each well. A volume of 100 µl of Ceftazidime was added to the wells of column 1 and mixed thoroughly, after which 100 µl of the drug was transferred (in duplicate) in a twofold serial dilution to the adjacent wells of columns 2 to 10, with mixing in each step. To ensure uniform well volumes, 100 µl from the last dilution column was excluded. Then 10 µl of the standardized bacterial suspension (0.5 McFarland turbidity) was added to all wells, except the negative control wells, which contained only broth and antibiotics. Column 11 was the positive control and included broth and bacterial inoculum only, with no antibiotic. The plate was incubated at 37 °C for 24 h, after which 15 µL of resazurin (an indicator of metabolism) was added per well, and the plate was incubated for an additional 3 h to detect metabolic activity through a colorimetric change [17].

The impact of Ceftazidime on the phenotypic production of some virulence factors

To shed light on the influence of sub-inhibitory concentrations of antibiotics on the production of virulence factors, phenotypically, sub-MICs and specified lower serial dilutions of Ceftazidime were added to the bacterial isolates. Their ability to swarm, adhere, form biofilms, and produce hemolysin was then assayed using the methodologies mentioned earlier.

RNA extraction and gene expression analysis in E. coli under Ceftazidime treatment

Total RNA was extracted from three *E. coli* isolates treated with or without Ceftazidime, respectively, using the GENEzol TriRNA Pure Kit (Geneaid, Thailand) according to the manufacturer's instructions. Selection of isolates was based on hemolysin production, biofilm

formation, and swarming motility. Quantitative Real-Time PCR (qRT-PCR) was used to determine expression levels of barA and uvrY before and after Ceftazidime exposure. The primers listed in Table 2 were utilized in this study, where GAPDH was used as the housekeeping gene. The qRT-PCR reaction components are listed in Table 3. The ΔΔCT method (gene expression analysis) was used for the calculation of expression fold change as 2^-ΔΔCT [18].

Table 2: Nucleotide Sequences of the Primers Employed in the Present Study.

Target gene	Primer name	Forward 5' ► 3'	Reverse 5' ► 3'
barA		GTGCCAGCATTATTGAGCCG	CAGAAATAATCGGCGTGCGG
urvY		AAAAGTCGTCGGTGAGGCAT	TTTTACGCGTCGCCTCAAGA
GAPDH	HKG	ACTTACGAGCAGATCAAAGC	AGTTTCACGAAGTTGTCGTT

Table 3: Constituents of the qRT-PCR Reaction Mixture.

Component	Volume (µl)	Final concentration
Luna Universal One-Step Reaction Mix (2X)	10	1X
Luna WarmStart® RT Enzyme Mix (20X)	1	1X
Forward primer (10 pmol/µL)	0.8	0.4 µM
Reverse primer (10 pmol/µL)	0.8	0.4 µM
Template RNA	Variable	20 ng/ul
Nuclease-free water	to 20	

Statistical Analysis

The analysis was conducted using the Statistical Package for the Social Sciences (SPSS, 2019) to evaluate the influence of various factors on the study parameters. Comparisons between group means were performed employing the Least Significant Difference (LSD) test to determine statistical significance.

Results and Discussion

Detection of ESBL Production

Nine UPEC isolates were cultured on HiCrome™ ESBL Agar Base with AC3F selective supplement. All of the isolates produced typical pink to purple colonies due to the activity of β-lactamase enzymes, further confirming their ability to produce ESBL, as illustrated in Figure 1. This finding aligns with previous research [19], which demonstrated that extraintestinal pathogenic *E. coli* strains are particularly capable of producing β-lactamase enzymes. These enzymes confer resistance by hydrolyzing a wide range of β-lactams, leading to a significant reduction in the effectiveness of several antimicrobial agents. The enzymatic activity reflects the evolutionary adaptation of UPEC to selective antibiotic pressure and highlights its potential as a pathogen in clinical settings [20]

Figure 1: Characteristic pink to purple colonies of UPEC isolates grown on HiCrome™ ESBL Agar Base supplemented with AC3F, indicating β-lactamase activity and presumptive ESBL production.

Phenotypic detection of selected virulence factors

Hemolytic activity, swarming motility, bacterial adhesion, and biofilm formation of nine UPEC isolates were evaluated as virulence-associated phenotypes. As shown in Table 4, the isolates displayed a variety of phenotypic characteristics, which aligns with the diverse virulence potential of these bacteria. The UPEC isolates exhibited a wide range of virulence profiles. Some isolates demonstrated multiple virulence traits simultaneously, including hemolytic activity, swarming motility, bacterial adhesion, and biofilm formation. In contrast, other isolates displayed only a single trait. That variability indicates that the pathogenic potential of UPEC strains is directly associated with their repertoire of virulence factors [21]. Co-expression of various determinants increases the ability of the bacterium to both colonize and persist at infection foci, thereby enhancing its invasive potential and overall [22].

Table 4: Phenotypic production of virulence-associated traits in nine UPEC isolates.

Isolate	Hemolytic activity	Biofilm intensity	Adhesion (10^5 CFU/mL)	Swarming (mm)
2M	-	No producer	-	11*15
16	30.3%	Moderate	276	13*14
18N	53%	No producer	-	9*9
21	-	Moderate	245	-
24	19.4%	Weak	188	7*7
24M	-	Moderate	290	11*9
24W	36%	No producer	-	10*14
26M	28.7%	Moderate	233	11*10
ab	-	Weak	127	-

Determination of Ceftazidime MIC

Minimum inhibitory concentration testing and susceptibility of UPEC to Ceftazidime showed a wide range of susceptibility patterns. Some isolates (2M and 21) exhibited high MIC values (500 µg/mL and 125 µg/mL), as shown in Table 5, confirming a high level of resistance to Ceftazidime. In contrast, other isolates (24, ab, and 26M) had MIC values that were

significantly lower (15.62 µg/mL), indicating marked susceptibility. These results indicate heterogeneity in the antibiotic response among the tested isolates. The MIC values in these UPEC isolates varied notably, indicating a diversity of intrinsic and acquired resistance mechanisms. Isolates with elevated MICs likely have modes of resistance (most likely ESBLs or alterations in penicillin-binding proteins) that alter Ceftazidime activity. Alternatively, as reported by Knoush *et al.*, [23] and Ehsan *et al.*, [19], low MIC isolates may not harbor these resistance mechanisms or have β-lactamases with restricted hydrolytic activity to Ceftazidime. Moreover, some isolates have ESBL-encoding genes but exhibit low gene expression levels [24] or are grown under laboratory conditions that do not sufficiently induce the production of the enzyme, resulting in observed phenotypic susceptibility [25].

Table 5: Variability in MIC values and Ceftazidime susceptibility patterns among UPEC isolates.

Isolate	MIC of Ceftazidime (µg/mL)	MIC of Ceftazidime (µg/mL) is based on the CLSI, 34th edition (2024) [26]
2M	500	≤ 8 susceptible, 16 intermediates, ≥ 32 Resistant
16	31.25	
18N	62.5	
21	125	
24	15.62	
24M	62.5	
24W	31.25	
26M	15.62	
ab	15.62	

Effects of Ceftazidime on the production of some virulence factors

The results demonstrated that sub-MICs of Ceftazidime (1/2 MIC, 1/4 MIC, 1/8 MIC, 1/16 MIC, and 1/32 MIC) significantly altered the production of major virulence factors, including swarming motility, hemolytic activity, bacterial adhesion, and biofilm formation, as illustrated in Figures 2, 3, and 4. Under antibiotic pressure, hemolysis, adhesion, and biofilm formation were significantly reduced ($P \leq 0.05$). These findings are consistent with a previous study [12]. Remarkably, when the antibiotic concentration was decreased, these phenotypes gradually recovered almost to the baseline, as shown in Figures 5, 6, and 7. By contrast, swarming motility was inhibited at all tested concentrations (1/2 MIC to 1/16 MIC), with only minimal recovery of motility observed at 1/32 MIC (Figure 8), confirming previous findings. The re-establishment of biofilm and adherence at lower ceftazidime concentrations in biofilm could suggest the activation of alternative genetic programs, as UPEC contains numerous gene families that modulate these survival mechanisms. This adaptive response suggests that UPEC may preferentially express adhesin and biofilm-associated genes under antibiotic stress conditions, enabling it to establish persistence in host environments [27,28]. On the other hand, the robust and persistent inhibition of hemolysis and swarming is probably due to a very narrow genetic pool encoding for these traits, making the bacteria less proficient at reacquiring these properties even at lower concentrations of the antibiotic [29-31].

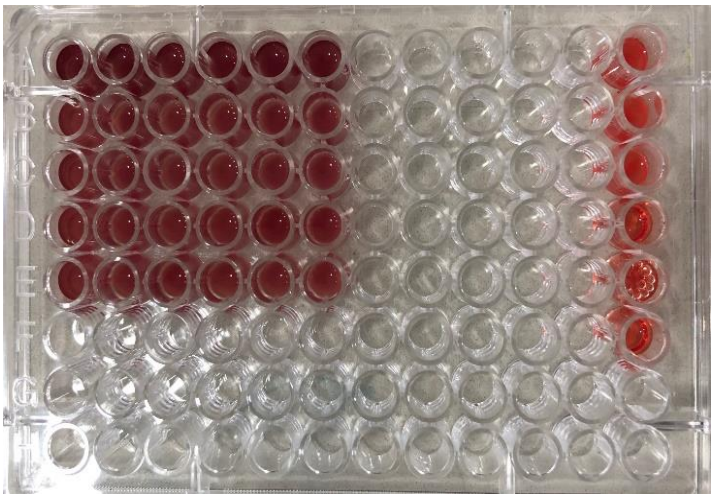

Figure 2: Effect of sub-MIC levels of Ceftazidime (1/2 to 1/32 MIC) on hemolytic activity in *E. coli* assessed by hemolysis assay.

Figure 3: Effect of sub-MIC levels of Ceftazidime (1/2 to 1/32 MIC) on biofilm formation by *E. coli* in the biofilm assay.

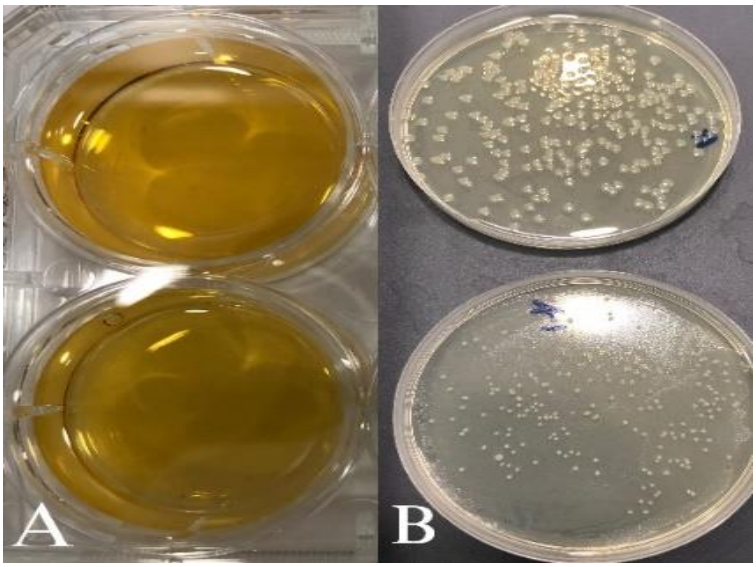

Figure 4: Effect of sub-MIC concentrations of Ceftazidime (1/2–1/32 MIC) on bacterial adhesion in *E. coli*. A) Adhesion ability assay conducted in 6-well microtiter plates (10 mL bacterial suspension per well). B) Representative single *E. coli* colony grown on LB agar.

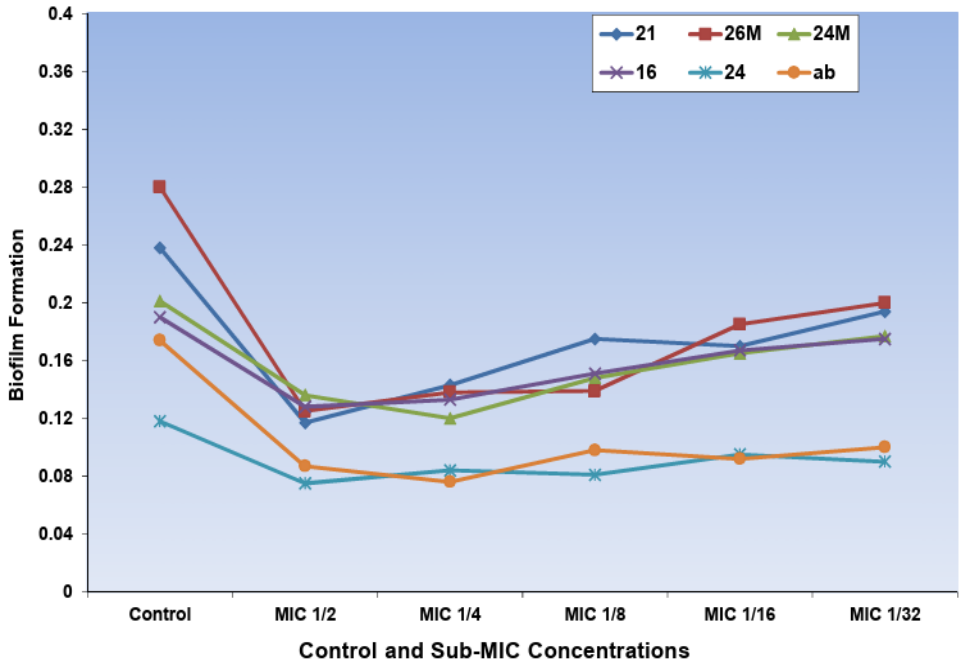

Figure 5: Effect of sub-MIC Ceftazidime concentrations (1/2–1/32 MIC) on *E. coli* isolates biofilm formation revealed statistically significant differences ($P \leq 0.05$) between treated and untreated groups.

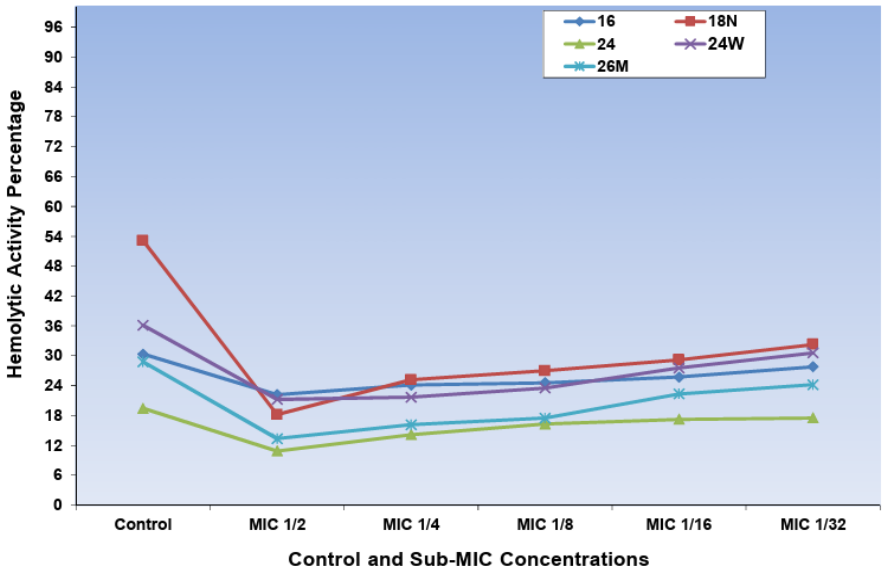

Figure 6: Effect of sub-MIC concentrations of Ceftazidime (1/2–1/32 MIC) on the hemolytic activity of *E. coli* isolates, showing statistically significant differences ($P \leq 0.05$) among the tested concentrations.

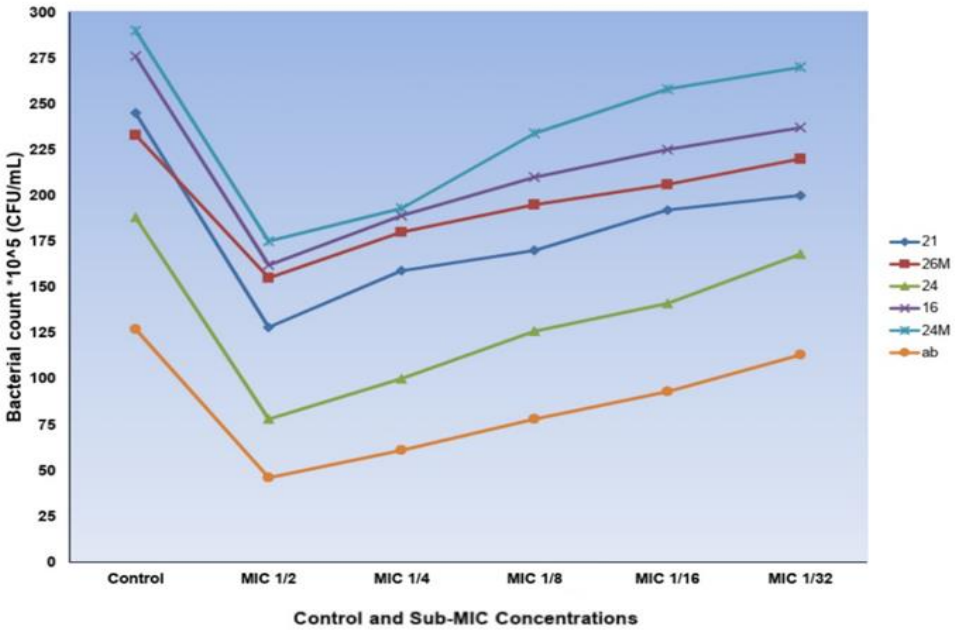

Figure 7: Effect of sub-MIC concentrations of Ceftazidime (1/2–1/32 MIC) on *E. coli* isolates adhesion capacity revealed statistically significant differences ($P \leq 0.05$) across the tested groups

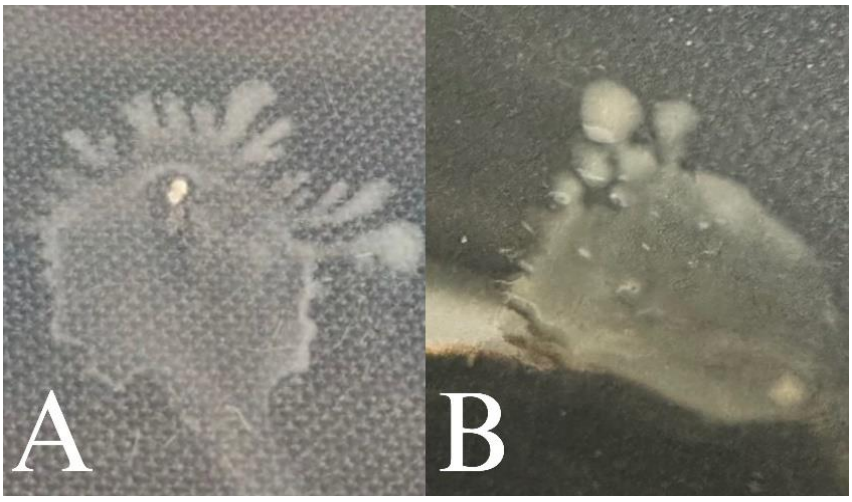

Figure 8: Swarming motility of *E. coli* on swarming agar in the presence and absence of Ceftazidime. A) Swarming motility on swarming agar without Ceftazidime (control condition). B) Minimal recovery of swarming observed at 1/32 MIC Ceftazidime

This study provides insight into the complex and dynamic interaction between antibiotic stress and virulence regulation in UPEC. The findings clearly show that sub-MICs of Ceftazidime not only reduce various classical virulence phenotypes, such as hemolysis, adherence, and biofilm formation, but also markedly suppress swarming motility in UPEC, implying a limit for genetic bandwidth related to motility-driven regrouping in UPEC. Most interestingly, the partial restoration of virulence functions in some isolates at lower antibiotic concentrations suggests an ability, which can be reprogrammed to allow increased resistance, to rebalance gene expression networks, possibly involving post-transcriptional regulators such as CsrA and its antagonistic sRNAs.

Modulation of barA and uvrY gene expression in E. coli induced by Ceftazidime treatment

The results showed that *E. coli* isolates displayed differential expression of *barA* and uvrY genes after exposure to sub-MIC levels of Ceftazidime for 24 hours in vitro. GAPDH was used as an internal control (housekeeping gene), and changes in expression are reported in Table 6. Interestingly, isolates numbered 16 and 24 were maintaining their expression of the *barA* gene while upregulation of UvrY was observed; isolate 26M was downregulating both essential components, *barA* and *uvrY*, versus GAPDH. In order to examine this aberrant response in isolate 26M, a further 8-hour incubation under the same sub-MIC conditions was conducted. The results showed that *barA* expression had recovered, while *uvrY* expression was still suppressed, as detailed in Table 7. This temporal expression profile correlates with the activity of the TCS BarA/UvrY, an environmental stress response regulatory system, with *barA*, which encodes a sensor histidine kinase, sensing environmental stressors like antibiotic pressure [32]. BarA is a sensor kinase that phosphorylates and activates the response regulator UvrY, which forms part of the BarA/UvrY regulatory axis and increases the levels of CsrB and CsrC, two antagonistic small RNAs that help regulate some virulence factors [33].

The sRNAs bind to the global regulator CsrA and thereby influence virulence and metabolic genes that are essential for the adaptation and survival of the bacteria [34]. Inferring similarities to other known systems, the induction of *barA* within 8 h and its stabilization by 24h are consistent with its general environmental sensor function. In contrast, while *uvrY* is a downstream transcriptional effector, it had a delayed transcriptional response and was highly expressed at the 24-hour timepoint, as would be expected given that it is regulated downstream from *barA*, once the signal has percolated through the environment. In fact, isolate 26M, which

had phenotypic production of hemolysis, adhesion, biofilm formation, and swarming motility, did not display classical resistance but rather intermediate susceptibility to Ceftazidime. This may account for an impaired induction of the BarA/UvrY pathway under antibiotic stress, which would imply that these isolates exhibiting intermediate susceptibility also leak regulatory robustness, allowing adaptation to a sub-inhibitory antibiotic concentration. These findings illustrate the responsiveness of two-component regulatory systems to antibiotic stress and their critical function in regulating bacterial virulence in response to environmental signals. Attenuation of BarA/UvrY signaling reduces CsrB/C levels, thereby releasing active CsrA This shift enhances the stability of flagellar transcriptional regulator D/C mRNA, promoting flagellar biosynthesis and swarming motility. Simultaneously, CsrA represses biofilm formation by downregulating the *pgaABCD* operon involved in polysaccharide adhesin synthesis. Regarding hemolytic factors, CsrA exerts dual control by repressing plasmid-encoded enterohemolysin while activating the expression of the chromosomal *hlyE* gene. Additionally, UvrY positively regulates type 1 fimbriae via CsrB; thus, its suppression impairs fimbrial adhesion [32,35,36].

Table 6: Regulation of *barA* and *uvrY* gene expression in *E. coli* following 24-hour incubation at 37 °C with sub-MIC concentrations of Ceftazidime.

Isolate code	Control			Sub-MIC Ceftazidime			ΔΔCt	Fold change	Result
	hkg	*barA*	ΔCt	*hkg*	*barA*	ΔCt			
16	10.3	13.18	2.88	12.42	15.3	2.88	0	1	No change
24	13.38	14.24	0.86	14.21	15.08	0.87	0.01	0.9930925	No change
26M	13.15	13.86	0.71	11.81	13.3	1.49	0.78	0.5823668	Decrease

Isolate code	Control			Sub-MIC Ceftazidime			ΔΔCt	Fold change	Result
	hkg	*urvY*	ΔCt	*hkg*	*urvY*	ΔCt			
16	10.3	15.41	5.11	12.42	14.22	1.8	-3.31	9.9176616	Increase
24	13.38	13.67	0.29	14.21	13.36	-0.85	-1.14	2.2038102	Increase
26M	13.15	12.93	-0.22	11.81	14.33	2.52	2.74	0.1496848	Decrease

Table 7: Expression regulation of *barA* and *uvrY* genes in *E. coli* isolate 26M following 8-hour incubation at 37 °C with sub-MIC concentration of Ceftazidime.

Isolate code	Control			Sub-MIC Ceftazidime			ΔΔCt	Fold change	Result
	hkg	*barA*	ΔCt	*hkg*	*barA*	ΔCt			
26M	13.15	13.86	0.71	13.24	13.62	0.38	-0.33	1.2570134	Increase

Isolate code	Control			Sub-MIC Ceftazidime			ΔΔCt	Fold change	Result
	hkg	*urvY*	ΔCt	*hkg*	*urvY*	ΔCt			
26M	13.15	12.93	-0.22	13.24	15.83	2.59	2.81	0.1425955	Decrease

It is recommended to investigate the functional implications of strain-specific transcriptional flexibility in the BarA/UvrY two-component regulatory system. The observed time gap between the expression of *barA* and *uvrY* along with the delayed and partial induction of *uvrY* suggests a coordinated regulatory cascade that responds to environmental changes. Further research should examine how varying expression levels of *barA* and *uvrY* influence downstream gene regulation, particularly under stress conditions such as antibiotic exposure.

Conclusion

This study demonstrates how antibiotic stress impacts some virulence factors of UPEC. Low concentrations of Ceftazidime decrease hemolysis, adherence, biofilm formation, and swarming motility. The BarA/UvrY system plays a key role, adapting to environmental signals differently in each strain. These results underscore the importance of considering sub-MIC antibiotic effects on bacterial virulence and resistance. These findings not only have implications for our understanding of UPEC pathogenesis under therapeutic pressure but also suggest the need for greater attention to sub-MIC antibiotic effects in the trajectory of bacterial virulence and resistance.

Ethical approval

The study protocol received ethical approval from the Scientific Committee of the College of Science, University of Baghdad, under the reference number CSEC/1124/0111.

Conflict of interest

The authors declare that they have no conflicts of interest.

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